Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Detection of Staphylococcal cassette chromosome mec -SCC (mec A) gene in Staphylococcus isolated from *Oreochromis niloticus* fish

Eman, M. A. El-Rais[1], Ashraf, A. Abd El Tawab[1], Ahmed A. A. Maarouf [2], Amira M. Rizk[1]

[1] *Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2] *Bacteriology, Immunology and Mycology Department Animal Health Research Institute "Benha Branch, Egypt*

ARTICLE INFO

Keywords

Oreochromus niloticus

Staphylococcus aureus

mecA gene

Received 29/04/2025
Accepted 31/05/2025
Available On-Line
xx/xx/2025

ABSTRACT

Seventy-five fresh Nile tilapia (*Oreochromis niloticus*) were obtained from various fish markets in Qalyubia Governorate, Egypt, between September 2023 and February 2024. These fish samples underwent bacteriological examination to isolate and identify Staphylococcus aureus, with a particular focus on methicillin-resistant Staphylococcus aureus (MRSA). Resistance to methicillin in the strains was verified by amplifying the staphylococcal cassette chromosome mec -SCC (mecA) gene via polymerase chain reaction. A total of 12% (9/75) of the examined fish samples yielded *S. aureus*. Notably, all of these nine bacterial isolates harbored the mecA determinant by PCR. These results imply that aquatic fauna, specifically Nile tilapia, could serve as a reservoir for resistant staphylococci, thereby representing a possible risk to both piscine health and human populations. Further studies are needed to elucidate transmission routes of MRSA in relation to fresh fish and to provide tools for preventing the spread of MRSA. Finally, this study concluded that fish may serve as a reservoir of methicillin-resistant *S. aureus* (MRSA) in aquatic environment that can be a potential risk for both Nile tilapia and human health. We need more research to understand how MRSA spreads in fresh fish and to develop strategies to stop its spread. So,this study also, sheds light on the public health concerns of MRSA for consumers.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*), notably its methicillin-resistant (MRSA) form, remains among the most critical antibiotic-defiant microorganisms impacting humans and diverse animal hosts. This resistant variant demonstrates broad insensitivity to nearly all β-lactam drugs, which are crucial for clinical therapy, and frequently shows resistance to aminoglycosides and macrolides, complicating management of infections (Wulf and Voss, 2008; Duran et al., 2012). Resistance spanning three or more antimicrobial categories is classified as multidrug resistance (MDR) (Magiorakos et al., 2012; Khosravi et al.,2017). Prior investigations reported that the incidenceof resistant *S. aureus* strains in aquatic species ranges between 30% and 60% (Obaidat et al., 2015; Kumar et al., 2016; Vaiyapuri et al.,2019).

Globally, methicillin-resistant strains are prominent culprits of healthcare-associated infections and toxin-mediated foodborne illnesses (Khan et al., 2015; Ossa et al,, 2018; Carvalho et al., 2019). These pathogens are highly resistance compared to other non-spore formers, exhibiting exceptional resistance to elevated salinity, wide pH variations, and extreme temperatures (Talaro and Talaro, 2002; Mohammed et al., 2020).

According to the Clinical and Laboratory Standards Institute (CLSI, 2018) criteria, isolates with the mecA gene must be categorized as oxacillin-resistant . The acquisition of this resistance trait is primarily facilitated through horizontal gene transfer mechanisms (Hiramatsu et al., 2013). The mecA sequence resides within a mobile genetic unit termed Staphylococcal Cassette Chromosome mec (SCCmec) (Wielders et al., 2002). It encodes penicillin-binding protein 2a (PBP2a), a specialized enzyme crucial for the polymerization of peptidoglycan, ensuring the robustness and structural integrity of the bacterial envelope. In non-resistant forms, intrinsic PBPs orchestrate dense peptidoglycan networking, providing mechanical resilience to the cell wall. β-lactam agents inhibit bacterial growth by targeting these native enzymes, preventing D-alanyl-D-alanine cross-linking through pentaglycine interconnections (Hiramatsu et al., 2002, and Kumar et al., 2016). However, alterations within PBP2a's structure markedly diminish β-lactam binding, fostering resistance (Sauvage et al., 2008). The worldwide rise of drug-resistant *S. aureus*, particularly following the emergence of methicillin-resistant strains, is acknowledged as a significant threat to public health, given its link to elevated mortality and severe clinical outcomes (Kong et al., 2016). Typically, *S. aureus* is absent from the indigenous microbiome of fish species; thus, its detection often points to environmental contamination during harvest or lapses in hygienic handling practices by workers (Kumar et al., 2016).

MRSA are recognized as zoonotic agents exhibiting resistance to multiple antimicrobial classes, implicated in both healthcare-associated and community-onset infections in humans, as well as various diseases affecting animals (Dahms etal., 2014; Visnuvinayagam et al., 2015). Nevertheless, investigations focusing on the incidenceof *S. aureus* and its methicillin-resistant counterparts in fish and related products remain relatively scarce (Obaidat et al., 2015; Kumar et al., 2016; Sivaraman et al.,2016; Murugadas et al.,2020 and Sivaraman et al. ,2022). Detection of these organisms in aquatic species may reflect either

* Correspondence to: emanrlrais@gmail.com

contamination during post-harvest handling due to poor hygienic practices among workers or the presence of active infections within the aquatic hosts themselves (Austin and Austin, 2016; Saklani et al.,2020). Additionally, cross-transmission between raw fish and handlers is likely facilitated through contact with contaminated environmental surfaces (Albuquerque et al., 2007).

Recognizing the pathogenic potential of *S. aureus* causing disease in fish, the current research aimed to determine the isolation frequency and undertake genotypic profiling of this bacterium, including methicillin-resistant variants, from Nile tilapia (*Oreochromis niloticus*) sourced from fish markets within Qalyubia Governorate, Egypt.in both aquatic organisms Comment i7, the current research aimed to determine the isolation frequency and undertake genotypic profiling of this bacterium, including methicillin-resistant variants, from Nile tilapia (*Oreochromis niloticus*) sourced from fish markets within Qalyubia Governorate, Egypt.

2. MATERIAL AND METHODS

Ethical approval

The collection and handling of fish specimens, along with all related methodologies, were reviewed and authorized by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (Approval No. BUFVTM03-03-24).

2.1. Samples collection

Seventy-five (n = 75) fresh Nile tilapia(200 gm- 1.00 kg) were systematically obtained from multiple retail fish outlets across Qalyubia Governorate, Egypt, during the interval from September 2023 to February 2024. Each single specimen was collected in a sterile polyethylene bag immediately upon acquisition to safeguard against contamination between samples. The collected fish were promptly transported to the diagnostic laboratory in insulated containers packed with ice, thereby preserving bacterial viability. Once at the laboratory, bacteriological investigations were initiated to isolate phenotypic assessments of *S. aureus* and phenotypic characterization of *S. aureus* and MRSA strains and genotypic detection of *mec*A virulence gene in them. In addition, molecular analyses targeting the mecA genetic marker were carried out to confirm methicillin resistance.

2.2.Preparation of samples

Prior to microbiological processing, the outer surfaces of the specimens were decontaminated using 70% ethanol to eliminate residual surface microbes. Subsequently, aseptic techniques were employed to collect tissue biopsies from dermal lesions and internal organs, which were then transferred into sterile vessels. In a sterile environment, small tissue pieces (0.5-1.0 cm each) from affected areas were inoculated into nutrient broth (Oxoid, UK) to facilitate bacterial growth. These cultures were incubated at 37°C for 24 hours to enrich bacterial populations, thereby optimizing conditions for subsequent isolation and characterization(APHA, 2001).

2.3. Isolation and identification of Staphylococcus aureus:

According to the protocols outlined by APHA, (2001) and Markey et al.(2013), a loopful from each enriched nutrient broth culture was streaked onto nutrient agar plates supplemented with 7% NaCl and incubated at 37°C for an additional 24 hours positive colony showed golden yellow convex colonies . Preliminary differentiation of presumptive colonies was based on standard biochemical assays, including oxidase, catalase, and coagulase tests, focusing on yellow, convex colonies indicative of *S. aureus*. Colonies demonstrating oxidase negativity and catalase positivity were further subcultured onto selective and differential media—namely Baird-Parker agar, Mannitol Salt agar, and Blood agar (all obtained from Oxoid). These plates were incubated for 48 hours at 37°C suspected colonies (black colonies with yellow halo around them on Baird-Parker agar; yellow colonies surrounded by halo zone on Mannitol salt agar; yellow colonies and turned media to colourless on 7% salted milk agar and white or yellow, smooth round and shiny colonies on blood agar). Colonies exhibiting typical morphology were preserved in semi-solid transport media for subsequent confirmatory assessments. Morphological examination was conducted via Gram staining and motility testing. Then, they were identified biochemically by Indole; Methyl red; Voges-Proskauer; urease; Triple sugar iron (H2S production); Sugar fermentation and Coagulase tests.

Expanded biochemical characterization included assays for indole production, methyl red and Voges-Proskauer reactions, urease activity, triple sugar iron (TSI) testing (focusing on hydrogen sulfide production), sugar fermentation profiling, and definitive coagulase testing to confirm the identity of *S. aureus*.

2.4. Phenotypic detection of methicillin-resistant S. aureus (MRSA):

The phenotypic identification of MRSA was carried out following the method described by Becker et al., (2002). Four to five well-isolated colonies from each confirmed *S. aureus* culture were inoculated into Brain Heart Infusion (BHI) broth (Oxoid) and incubated at 37°C for 24 hours. After incubation, an aliquot from each broth culture was streaked onto Oxacillin Resistance Screening Agar Base (ORSAB) supplemented with ORSAB Selective Supplement (SR195E, Oxoid Limited, Basingstoke, UK). Following a 24-hour incubation at 37°C, colonies displaying the characteristic morphology associated with methicillin-resistant strains on ORSAB were recorded.

2.5. Genotypic detection of methicillin-resistant S. aureus (MRSA) by PCR

All nine bacterial isolates previously confirmed as *S. aureus* were subjected to molecular characterization aimed at detecting the presence of the *mec*A gene, a well-established genetic determinant of methicillin resistance. Genomic DNA was meticulously extracted utilizing the QIAamp® DNA Mini Kit (Qiagen, Germany), strictly adhering to the manufacturer's standardized protocol to ensure high-quality and contaminant-free DNA suitable for downstream applications.

The PCR assay was performed employing EmeraldAmp GT PCR Master Mix (Takara, Japan), in conjunction with primers specifically designed to target the mecA gene (primer sequences and details are outlined in Table 1). The amplification process was carried out under a precise thermal cycling regime, using conventional PCR(Sambrook et al., 1989; McClure et al., 2006) beginning with an initial denaturation phase at 94°C for 5 minutes to ensure complete separation of DNA strands. This was followed by 35 consecutive amplification cycles, each consisting of a denaturation step at 94°C for 30 seconds, a primer annealing phase at 50°C for 30 seconds to facilitate specific binding of primers to their complementary DNA sequences, and an extension phase at 72°C for 30 seconds, during which the DNA polymerase enzyme synthesized new DNA strands. Subsequently, a final extension step was conducted at 72°C for 7 minutes to ensure complete elongation of all PCR products. The amplified DNA fragments were then

subjected to electrophoretic separation on 1.5% agarose gels prepared with an appropriate nucleic acid staining dye, thereby allowing visualization of the PCR amplicons. Electrophoresis was conducted in accordance with the method described by (Sambrook et al., 1989). Visualization of the amplified DNA fragments was accomplished using a gel documentation system.

Table (1): Primer used for detection of *mec*A gene in *S. aureus* isolates.

Target gene		Primer sequence (5'-3')	Amplified product	References
*S. aureus mec*A	F	GTA GAA ATG ACT GAA CGT CCG ATA A	310 bp.	McClure et al.,(2006)
	R	CCA ATT CCA CAT TGT TTC GGT CTA A		

3. RESULTS

3.1 .Phenotypic identification Staphylococcus aureus

All recovered isolates demonstrated characteristic colony morphology across different culture media. On nutrient agar supplemented with 7% sodium chloride, the isolates formed yellow, convex colonies. When cultured on Baird-Parker agar, the isolates produced distinctive black, shiny colonies resulting from tellurite reduction, accompanied by yellow halos. In several strains, an additional clear zone surrounding the colonies was observed, indicative of proteolytic activity. Growth on Mannitol Salt Agar resulted in yellow colonies due to mannitol fermentation, each surrounded by a clear halo zone. On blood agar, the isolates exhibited shiny colonies with evidence of hemolysis, specifically beta and alpha hemolysis patterns.

Microscopic examination revealed that all isolates were Gram-positive cocci arranged in irregular, grape-like clusters and exhibited non-motility. Biochemical testing confirmed the identity of the isolates as S.aureus; all nine isolates tested positive for catalase, methyl red, Voges-Proskauer, urease, coagulase, triple sugar iron (TSI) production, and the fermentation of multiple sugars, including glucose, lactose, mannitol, and sucrose. Additionally, all isolates produced negative results for the indole and oxidase tests, supporting their classification as *S. aureus*.

Table(2)The biochemical characters of the isolated members of Staphylococcus spp.

Biochemical tests	*S.aureus*
Indole	-ve
Voges-Proskauer	+ve
Catalase test	+ ve
Oxidase test	- ve
Coagulase test	+ve
Haemolysis	+ ve
Pigment production	+ve
Glucose F.	Ag
Lactose F.	+ve
Mannitol salt agar	+ ve
Sucrose F.	+/-ve

+ve =Positive - ve = Negative +/-ve =Most of isolates gave positive results Ag =Acid and gases d= Mostly positive F. = Fermentation

3.2 .Prevalence of Staphylococcus aureus isolates in examined samples .

Out of the 75 fresh Nile tilapia (*Oreochromis niloticus*) samples examined, nine isolates of *S. aureus* were successfully recovered, corresponding to an overall prevalence rate of 12%.

3.3 .Identification of methicillin-resistant S. aureus (MRSA):

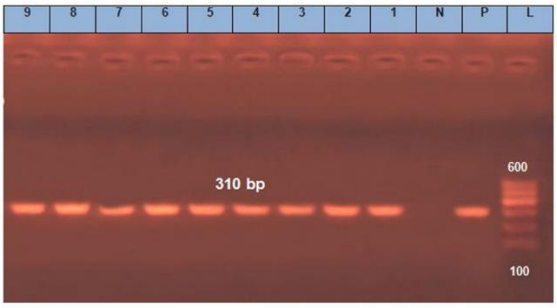
3.3.1 .Phenotypic identification of MRSA.

Phenotypic screening for methicillin resistance was conducted by culturing the nine confirmed *S. aureus* isolates on ORSAB medium. All isolates demonstrated robust growth and developed deep blue-colored colonies on ORSAB, a distinctive feature associated with methicillin resistance.

3.3.2 .Genotypic identification of MRSA:

Molecular characterization of MRSA was carried out by PCR targeting the mecA gene. All nine *S. aureus* isolates were exhibited to be positive for the mecA gene, as evidenced by the amplification of specific bands at approximately 310 base pairs (bp) (Fig. 1).

Fig. 1. Agarose Gel electrophoresis of methicillin resistant (mecA) gene



Lane L: 100-600 bp. DNA Ladder. N.: Negative control (Negative control. (methicillin- susceptible *S. aureus* ATCC 25923), P.: Positive control (methicillin- resistant *S. aureus* ATCC BAA-1683 at 310 bp.). Lanes (1 - 9): Positive methicillin- resistant *S. aureus* for mecA gene at 310 bp.

4.DISCUSSION

MRSA is recognized as a highly adaptable pathogen responsible for a wide range of clinical infections in animals and humans and is increasingly implicated in foodborne disease outbreaks. Recently, MRSA has also been considered an emerging bacterial pathogen within aquaculture environments, likely linked to the contamination of aquatic systems through contact with animal or human waste products (Murugadas et al., 2020). In the present investigation, the isolation rates of *S. aureus* from fresh Nile tilapia (*Oreochromis niloticus*) was found to be 12%(9/ 75 samples). This prevalence is consistent with the findings reported by Grema et al.(2015), Correia et al.(2019), Mohammed et al.(2020),and Sivaraman et al.(2022). However, it differs from the higher prevalence rates reported by Costa et al.(2015),and Obaidat et al. (2015) who recorded higher incidences of *S. aureus* at 30%, 47%, respectively and with Vázquez-Sánchez et al.(2012) who did not find any MRSA in fish. These variations may be linked to differences in the methodology or the collected samples sources. The observed discrepancies among different studies may be attributed to several factors, including variations in sampling methods, differences in the health status of fish populations, geographical location, environmental conditions, and differences in laboratory diagnostic protocols. Moreover, the sources of the fish samples, whether wild-caught or farm-raised, and post-harvest handling practices might also contribute to these variations.

MRSA strains, characterized by the mecA gene, have been reported to exhibit resistance to both methicillin and oxacillin, as outlined by the CLSI (CLSI, 2018). In the present investigation, phenotypic characterization confirmed that all nine *S. aureus* isolates demonstrated strong growth on ORSAB, a selective chromogenic medium

for MRSA detection. Each isolate produced deep blue colonies, attributed to the presence of an acid-sensitive chromogenic compound (aniline blue) in the medium. These results are consistent with those reported by Grema et al. (2015); Hafsat et al.(2015) ;Mohammed et al. (2020); Saklani et al.(2020) and Sivaraman et al. (2022) and such findings highlight the reliability of chromogenic media for preliminary identification of MRSA strains.

Regarding genotypic confirmation, PCR analysis revealed successful mecA gene amplification in all nine *S. aureus* isolates. This complete concordance between phenotypic identification on ORSAB medium and the molecular detection of the mecA gene strongly validates the identification of these strains as MRSA. These results came in agreement with those of Soliman et al. (2014); Suleiman et al.(2015); Correia et al.(2019); Mohammed et al.(2020) and Sivaraman et al.(2022).

Staphylococcus aureus mainly MRSA is not a common microflora of fish, but now it is considered as new attitude fish pathogen with high zoonotic consideration (Murugadas et al., 2020) and its presence indicates contamination from water or ice and due to anthropogenic activity indicating the poor sanitary condition. Even though protocol is developed for the screening of MRSA from clinical samples (CDC, 2017), for surveillance programme (Weese, 2007; Bortolami et al., 2017), there is no approved protocol from international bodies for the isolation of MRSA from fishes. Incidence of MRSA in fish and fish products varied significantly because of the variation in the methods, sample collection scheme, sample types, enrichment protocol used (Correia et al., 2019 and Murugadas et al., 2020).

5. CONCLUSIONS

Finally, the recorded results concluded that, fish may serve as reservoir of methicillin-resistant *S. aureus* (MRSA) in aquatic environment that can be a potential risk for both Nile tilapia and human health. Further studies are needed to elucidate transmission routes of MRSA in relation to fresh fish and to provide tools for preventing the spread of MRSA.

6. REFERENCES

1. Albuquerque, W.F., Macrae, A. , Sousa, O.V. , Vieira, G.H.F. and Vieira, R.H.S.F. 2007. Multiple drug resistant Staphylococcus aureus strains isolated from a fish market and from fish handlers. Braz. J. Microbiol., 38 ,1: 131-134.
2. APHA "American Public Health Association" (2001): Compendium of Methods for the Microbiological examination of Foods. 4th Ed. F.P. Downes and K. Ito(editors), APHA. Washington D.C., USA.
3. Austin, B., Austin, D.A., 2016. Bacterial Fish Pathogens, Diseases of Farmed and Wild Fish 6th Ed, Springer International Publishing Switzerland.
4. Becker, A. ;Forster,D.H.and Kniehl,E. 2002 . Oxacillin Resistance Screening Agar Base for Detection of Methicillin-Resistant Staphylococcus aureus. J. Clinical Microbiology, 40, 11: 4400–4401.
5. Carvalho, S. P., Almeida, J. B., Andrade, Y. M. F. S., Silva, L. S. C., Chamon, R. C. C., Santos, K. R. N., Marques, L. M. 2019. Molecular characteristics of methicillin resistant Staphylococcus aureus isolates from hospital and community environments in northeastern Brazil. Brazil J. Infect. Dis., 23, 2, 134–138.
6. CDC "Centers for Disease Control and Prevention". 2017. Laboratory detection of oxacillin/methicillin resistant Staphylococcus aureus. Retrieved October, 26, 2017
7. CLSI" Clinical Lab Standards Institute". 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Wayne, PA.
8. Correia, T. M. A., Oliveira, A. P. D., Miyasato, I. F., Santos, T. M. B., Dias, F. S. 2019. Characterization of Staphylococcus aureus isolated from tilapia and utensils used in the sale of tilapia in the street markets of a semi-arid Brazilian municipality. Acta Sci. Pol. Technol. Aliment., 18,4, 413–425.
9. Costa, W. L. R., Ferreira, J. D. S., Carvalho, J. S., Cerqueira, E. S., Oliveira, L. C. and Almeida, R. C. C. 2015. Methicillin resistant Staphylococcus aureus in raw meats and prepared foods in public hospitals in Salvador, Bahia. Brazil. J. Food Sci., 80:147-150.
10. Dahms, C., Hubner, N. O., Wilke, F. and Kramer, A. 2014. Mini-review: Epidemiology and zoonotic potential of multi resistant bacteria and Clostridium difficile in livestock and food. GMS Hyg. Infect. Control, 9.3: 1-16.
11. Duran, N., Ozer, B., Duran, G. G., Onlen, Y., and Demir, C. 2012. Antibiotic resistance genes and susceptibility patterns in staphylococci. Indian J. Med. Res., 135: 389-396.
12. Grema,H.A., Geidam,Y.A., Gadzama,G.B., Ameh, J.A., Suleiman,A. and Gulani,I.2015. Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MRCoNS) Isolated from Fish and Fish Handlers in Maiduguri, Nigeria. Advance J. Food Science and Technology, 9.7: 494-502.
13. Hafsat, A.G., Yaqub, A.G., Abubakar, S., Isa, A.G., Roy, B.B.2015. Multi drug resistant bacterial isolated from fish and fish handlers in Maiduguri, Nigeria. International J. Animal and Veterinary Advances, 7,3:49-55.
14. Hiramatsu, K., T. Ito, S. Tsubakishita, T. Sasaki, F. Takeuchi, Y. Morimoto, Y. Katayama, M. Matsuo, K. Kuwahara-Arai, T. Hishinuma, and T. Baba. 2013. Genomic basis for methicillin resistance in Staphylococcus aureus. Infect. Chemother. 45:117–136.
15. Hiramatsu, K., Y. Katayama, H. Yuzawa, and T. Ito. 2002. Molecular genetics of methicillin-resistant Staphylococcus aureus. Int. J. Med. Microbiol. 292:67–74.
16. Khan, H. A., Ahmad, A. and Mehboob, R. 2015. Nosocomial infections and their control strategies. Asian Pac. J. Trop. Biomed., 5,7: 509-514.
17. Khosravi, A.D.,Jenabi, A. and Montazeri, E.A.2017.Distribution of genes encoding resistance to aminoglycoside modifying enzymes in methicillin-resistant Staphylococcus aureus (MRSA) strains. Kaohsiung J. Med. Sci., 33.12:587-593.
18. Kong, E. F., Johnson, J. K., Jabra-Rizk, M. A. 2016. Community-associated methicillin-resistant Staphylococcus aureus: an enemy amidst us. PLOS Pathogens, 12,10, e1005837. https://doi.org/10.1371/journal.ppat.1005837.
19. Kumar, L. R. G., Kasim, A. K., Lekshmi, M., Nayak, B. B. and Kumar, S. 2016. Incidence of methicillin resistant Staphylococci in fresh seafood. Advances in Microbiology, 6,6: 399–406.
20. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson Liljequist, B. and Paterson, D. L. 2012. Multidrug resistant, extensively drug resistant and pandrug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect., 18,3: 268-281.
21. Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A. and Maguire, D., 2013. Clinical Veterinary Microbiology, 2nd Ed. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.
22. McClure, J.A., Conly, J.M., Lau, V., Elsayed, S., Louie, T., Hutchins, W., Zhang, K. 2006.Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J. Clin. Microbiol., 44: 1141-114.
23. Mohammed,U.H., Bala,A.M., Malam,M.B. Barde,M.Z.2020. Phenotypic and molecular detection of methicillin resistant Staphylococcus aureus (MRSA) isolated from Clarias gariepinus (Burchel, 1822) and *Oreochromis niloticus* (Linneaus, 1758) IN Maiduguri. International J. Fisheries and Aquatic Studies , 8,4: 365-372.
24. Murugadas,V., Joseph,C., Lalitha,K.V. and Prasad,M.M.2020. A Simplified Scheme for efficient isolation

and identification of MRSA from seafood and aquatic environment. Fishery Technology, 57: 141 – 146.
25. Obaidat, M. M., Bani, A. E. and Lafi, S. Q. 2015. Prevalence of Staphylococcus aureus in imported fish and correlations between antibiotic resistance and enterotoxigenicity. J. Food Prot., 78,11:1999– 2005.
26. Ossa, R. P. L., Prado, S. I., Cervi, M. C., Lima, D. A. F. S., Martinez, R., Rodrigues, F. B. 2018. Is communityassociated methicillin-resistant Staphylococcus aureus (CA-MRSA) an emerging pathogen among children in Brazil? Braz. J. Infect. Dis., 22,5: 371–376. https://doi. org/10.1016/j.bjid.2018.10.276.
27. Saklani,P., Lekshmi,M., Nayak,B., Kumar,S.2020. Survival of Methicillin-resistant Staphylococcus aureus in fish and shrimp under different storage conditions. J. Food Protection, 83,5: 844–848.
28. Sambrook, J., Fritsch, E., Montias, T., 1989. Molecular Biology. In: Molecular cloning. Laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory press, USA.
29. Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A. and Charlier, P. 2008. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol. Rev., 32:234–258.
30. Sivaraman, G.K., Deesha, V., Prasad, M.M., Kumar,A.J., Visnuvinayagam, S., Nadella, R.K., Chandni, V., Basha, A.2016. Incidence of community acquired methicillin-resistant Staphylococcus aureus (CA- MRSA) in seafood and its environment, Gujarat, India. International J. Recent Scientifc Research, 7,11:14279–14282.
31. Sivaraman,G.K., Gupta,S.S., Visnuvinayagam,S., Muthulakshmi,T., Elangovan,R. , Perumal,V. , Balasubramanium,G. , Lodha,T. and Yada,A.2022. Prevalence of *S. aureus* and/or MRSA from seafood products from Indian seafood products. BMC Microbiology , 22:233-246.
32. Soliman, M., Ellakany, H., Gaafar, A., Elbialy, A., Zaki, M., and Younes, A. 2014. Epidemiology and antimicrobial activity of methicillin-resistant Staphylococcus aureus (MRSA) isolated from Nile tilapia (*Oreochromis niloticus*) during an outbreak in Egypt. Life Science J.,11,10:1245-1252.
33. Suleiman, A.B., Umoh, V.J., Kwaga, J.K.P., Shaibu, S.J.2015. Prevalence and antibiotic resistance profile of methicillin resistance Staphylococcus aureus isolated from mills in Plateau State. International Research J. Microbiology, 7,3:65-69.
34. Talaro, K.P., Talaro A. (2002). Foundations in Microbiology. 4th Ed. McGraw Hill New York, :544-552.
35. Vaiyapuri, M., Joseph, T. C., Rao, B. M., Lalitha, K. V., Prasad, M. M. 2019. Methicillin-resistant Staphylococcus aureus in seafood: prevalence, laboratory detection, clonal nature, and control in seafood chain. Journal of Food Science, 84,12: 3341–3351.
36. Vázquez-Sánchez, D., López-Cabo, M., Saá-Ibusquiza ,P., Rodríguez-Herrera, J.J.2012. Incidence and characterization of Staphylococcus aureus in fishery products marketed in Galicia (Northwest Spain). Int. J. Food Microbiol., 157,2:286–296.
37. Visnuvinayagam, S., Joseph, T. C., Murugadas, V., Chakrabarti, R. and Lalitha, K. V. 2015. Status on methicillin resistant and multiple drug resistant Staphylococcus aureus in fishes of Cochin and Mumbai coast, India. J. Environ. Biol., 36,3: 571-575.
38. Weese, J. S. 2007.Environmental Surveillance for MRSA. In: Methicillin-Resistant Staphylococcus aureus (MRSA) Protocols (Ji, Y., Ed). Methods Mol. Biol. Vol. 391. Humana Press
39. Wielders, C. L. C., Fluit, A. C. , Brisse, S., Verhoef, J. and Schmitz, F. J. 2002. mecA gene is widely disseminated in Staphylococcus aureus population. J. Clin. Microbiol., 40,11:3970–3975.
40. Wulf, M., and Voss, A. 2008. MRSA in livestock animals-an epidemic waiting to happen. Clin. Microbiol. Infect., 14: 519-521.