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Prevalence of Multidrug-resistant Enterotoxigenic *Staphylococcus aureus* in Pagrus and Saurus Fish Intended for Human Consumption

Wageh S. Darwish[1*], Ahdab Othman[1], Ahmed E. Tharwat[1], Karima M. Eissa[1], Ehab E. Nabawy[2], Amany M. Abd Elmoaty[3], Samar E. El-Wehedy[4]

[1]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

[2]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

[3]Bacterioloy, Mycology, Immunology Department, Faculty of Veterinary, Medicine, Mansoura University, Egypt.

[4]Zagazig University Hospital, Zagazig University, Zagazig, Egypt.

***Correspondence**
Corresponding author: Wageh S. Darwish
E-mail address: wagehdarwish@gmail.com

Abstract

Staphylococcus aureus (*S. aureus*) is considered the most important cause of food borne intoxication, which occurs due to ingesting of food contaminated with enterotoxin of *S. aureus*. In this study, *S. aureus* from saurus, and pagrus fish species were isolated, then identified by morphological and biochemical examination. *S. aureus* coagulase, and D-Nase +ve were detected in 16 from 25 (64%), and 17 from 25(68%) of the examined samples of saurus, and pagrus, respectively. Total *S. aureus* counts were 5.14±0.06 in saurus, and 5.02±0.03 log 10 cfu/g in pagrus fish, respectively. Detection and typing of enterotoxin by Reverse Passive Latex Agglutination technique "RPLA" revealed the detection of staphylococcal enterotoxins (SEs) SEA, SEC, and SED at 18%, 6%, 0% in saurus and 5%, 0%, and 11%, at pagrus, respectively. *S. aureus* was tested for antimicrobial susceptibility. The recovered *S. aureus* coagulase, and D-Nase +ve in the current study showed resistance to kanamycin, clindamycin, nalidixic acid, and sulphamethoxazole at 100%, 87.9%, 84.8%, and 81.8%, respectively. The isolates showed sensitivity to amikacin, imipenem, meropenem, oxacillin at 90.9%, 87.9%, 84.8%, and 81.8% respectively. It could be concluded that multidrug resistant enterotoxigenic strains of *S. aureus* could be isolated from *Saurus* and *Pagrus* spp. Therefore, it is highly recommended to adopt strict hygienic measures and efficient cooking before consumption of such fish species.

KEYWORDS
S. aureus, saurus, pagrus, Drug resistance.

INTRODUCTION

Currently, 30% of the world's population suffers from malnutrition and other ailments, and since the oceans, seas, and rivers cover 70% of the planet's surface, seafood is regarded as the primary source of global food, which is crucial for human health. Fish are a good source of lipids, vitamins, minerals, and proteins. Fish provides our bodies with essential nutrients that are crucial for optimal health, provide energy, and aid in the control of bodily processes. Proteins make up 15–20% of fish meat, whereas lipids make up 5–20% and ash makes up 0.5–2% (Morshdy *et al*., 2013; 2019). High biological value protein can be found in fish meat. The best nourishment for the impoverished is therefore that. High-quality proteins, vitamins such as vitamins A, B, and D, lipids, and minerals like magnesium, calcium, and phosphorus are all produced by fish and are all beneficial to humans. Due to the minimal amount of connective tissue present, fish meat is more easily digestible than other animal proteins and contains practically all of the essential amino acids (FAO, 2013; Morshdy *et al*., 2021). Additionally, fish is a good source of iodine, selenium, fluorine, iron, zinc, and potassium, all of which are necessary for thyroid gland and teeth development in humans as well as hormone synthesis (Mishra, 2020). The health of kids depends on omega-3 fatty acids (Chavan-Gautam *et al*., 2018). Infants that consume large amounts of omega-3 fatty acids had better attention, social skills, and eye-hand coordination, as well as higher IQ test scores (Uauy and Dangour, 2009).

Bacteria are generally classified as non-indigenous and indigenous. The first group is a part of the aquatic flora that exists naturally in the environment, while the second group contaminates fish when it is handled in a commercial or residential setting. The majority of bacteria are found in the digestive system, gills, and skin. Normally, fish meat is sterile when it is caught. The environment and the bacteriological quality of the water have an impact on the level of bacterial contamination of fish at the time of catch. Some of the diseases that fish can spread to humans include bacterial infections. They arise either from the product being directly contaminated by contaminated water or from secondary contamination occurring during the product's acquisition, handling, processing, storage, distribution, or preparation for consumption. When the products are eaten fresh or barely cooked, direct contamination is very crucial. In unhygienic settings, secondary contamination is extremely common. Fish becomes spoiled as a result of microbial development, physical harm, and chemical response. The growth and metabolism of microorganisms that result in the generation of amines, sulphides, alcohols, aldehydes, ketones, and organic acids that cause off-flavors and ammonia odor in fresh seafood are the primary causes of food deterioration. There are numerous factors that might cause fish to spoil, including temperature, nutrition, pH,

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osmotic pressure, and redox potential (Atia *et al*., 2018; Darwish and Thompson, 2023). Fish skin and gut contain microorganisms that can ruin food. Human allergies to fish, food-borne illnesses, bacterial and parasite infections, and histamine fish poisoning are all caused by fish (El-Ghareeb *et al*., 2020; Hafez *et al*., 2022). The oxidation of polyunsaturated fatty acids, which results in the appearance of unpleasant flavor, colors, the loss of nutritional content, and changes in texture, causes physical deterioration in fish flesh (Abdelhamid *et al*., 2018). Diseases in people are brought on by contaminated fish handling, filthy transit, poor preparation, and storage issues (Gram and Dalgaard, 2002).

S. aureus is a gram-positive, facultative anaerobic, non-motile, catalase-positive, non-spore-forming bacteria (Darwish *et al*., 2018). *S. aureus* is seen on the workers' skin, hair, nails, and noses. *S. aureus* can survive in dryness for a long time and tolerate high levels of salts. Some *S. aureus* strains produce staphylococcal enterotoxins (SEs) which are responsible for food poisonings in human (Morshdy *et al*., 2022). Nausea, vomiting, diarrhoea, stomach discomfort, and headache are signs of staphylococcal poisoning. These symptoms may appear for up to eight hours. Food poisoning from *Staphylococcus* is fatal in the very young, the very old, and individuals with impaired immune systems (Darwish *et al*., 2022).

In sight of the previous facts, this study was designed to examine the prevalence of *S. aureus* in two major fish species retailed in the fish markets in Egypt, namely *Saurus*, and *Pagrus* spp. Moreover, the ability of the recovered isolates to produce enterotoxins and their antimicrobial susceptibilities were further examined using the disk diffusion method.

MATERIALS AND METHODS

Sample collection

A total of 50 marine water fish samples; 25 of pagrus fish and 25 of saurus spp., were purchased randomly from various fishery retail shops at Sharkia governorate, Egypt. The samples were transported in an ice box to the laboratory of Meat Hygiene, Safety and Technology at Faculty of Veterinary Medicine, Zagazig University, Egypt, and immediately tested for *S. aureus* isolation, identification, enterotoxin detection and antimicrobial susceptibility. Sensory evaluation of the examined fish samples was done according to Morshdy *et al*. (2019), where fish samples were washed with potable water and examined physically for general appearance of the skin, consistency of flesh, odor and color of gills, color and condition of eyes, and slime formation.

Preparation of fish samples

Under aseptic condition and using a sterile material, 5 g from each fish sample were homogenized in 45 ml of 0.1% sterile buffered peptone water and represented 10-1 dilution factor. This mixture stands for 5 minutes in room temperature and then serial dilution was followed according to APHA (2001).

S. aureus isolation and identification

Isolation of *S. aureus* was done on Baird parker agar according to Splittstoesser (1992). In brief, under aseptic condition, 0.1 ml was taken from each dilution and cultured by the surface spreading method directly to sterile prepared Baird parker plates containing egg yolk tellurite emulsion and was incubated at 37°C for 24 to 48 hours. Dark colonies surrounded by a clear hallow zone were regarded as *S. aureus* colonies. Separate colonies were picked up and incubated in brain heart broth to obtain a pure culture that was preserved in 0.5 ml glycerol and used for further identification via morphological and biochemical examination (MacFaddin, 2000; ISO, 2013).

Detection and typing of enterotoxin (Shingaki, 1981)

The clear culture supernatant fluid was tested serologically by Reverse Passive Latex Agglutination technique "RPLA" using kits for the detection of staphylococcal enterotoxins A, B, C and D (SET-RPLA, Denka Sekeu LTD, Japan).

S. aureus antibiotic resistance profiling

The single diffusion method used by Daka and Yihdego (2012) for *S. aureus* strains were used to test antimicrobial susceptibility. As a result, antimicrobial susceptibility testing was performed in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2001).

Statistical analysis

Data were expressed as mean value with standard deviation, the significance was detected at $p<0.05$ using SPSS utilizing a one-way variance analysis (ANOVA) (version 20; IBM, Chicago, IL, USA).

RESULTS AND DISCUSSION

In the present study, the obtained results in Figure 1 demonstrated that *S. aureus* coagulase and DNase positive was isolated from *Saurus* spp., at 16 out of 25 (64%), while in *Pagrus* spp., at 17 out of 25 (68%). Such values exceeded the Egyptian standards for *S. aureus* in fish, 13% only (EOS, 2005). Isolation of *S. aureus* from the examined fish samples indicates improper hygienic measures applied during fishing, handling, and use of contaminated water, contaminated ice, soiled surfaces, and improper sanitary conditions during storage and poor personal hygiene. In addition, isolation of *S. aureus* is an important indicator for post harvest contamination of fish (Austin *et al*., 2007; Mhango *et al*., 2010). The mean total *S. aureus* counts in the examined fish species were 5.14±0.06 (4.67-6.29) in saurus, and 5.02±0.03 (4.74-5.29) log10 cfu/g in pagrus fish, respectively. Likely, *S. aureus* was isolated from fishery products and fish processing workers in India (Simon and Sanjeev, 2007). Besides, *S. aureus* was also isolated from fresh fishery products retailed in Galicia, Northwest Spain at 43% (Vázquez-Sánchez *et al*., 2012). In Egypt, *S. aureus* was isolated from 6 fish species retailed at Zagazig city including pagrus and saurus fish species at 4 to 36% with counts ranged from 2 to 4 log 10 cfu/g (Hussein *et al*., 2019).

Foodborne intoxication occurs due to ingestion of food contaminated with staphylococcal enterotoxins (SEs) (Darwish *et al*., 2022). In our study, we observed that only 12% from the recovered *S. aureus* isolated from saurus, and 8 % isolated from pagrus could produce enterotoxins. Classical staphylococcal enterotoxins are classified as SEA to SEE, where SEA is the most common cause related to *Staphylococcus*-related food poisoning (Sokari, 1991). In the current study SEA, C, and D were recovered from the two examined marine fish. SEA, SEC, and SED were recovered from saurus fish at 18%, 6%, and 0% respectively while in pagrus such enterotoxins were recovered at 5%, 0%, and 11%, respectively (Fig. 2). Likely, the most frequent enterotoxin in the two fish species is reported to be SEA (Marin *et al*., 1992). In Egypt, the *SED, SEA*, and *SEB* coding genes were detected in the recovered

S. aureus isolates from fish at 40%, 26.6%, and 20%, respectively (Hussein *et al*., 2019).

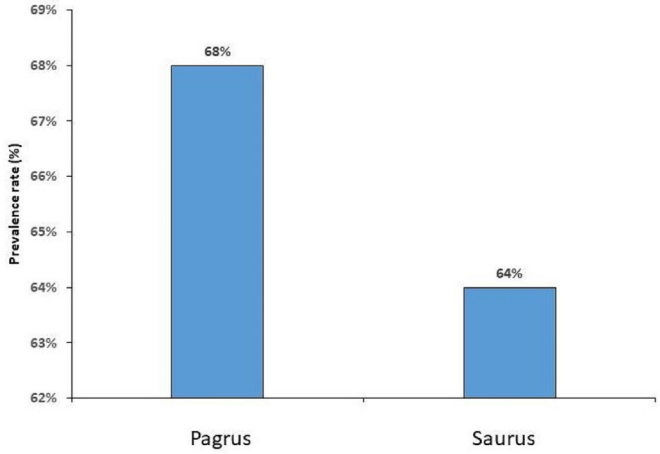

Fig. 1. Prevalence of *S. aureus* isolated from saurus and pagrus fish species.

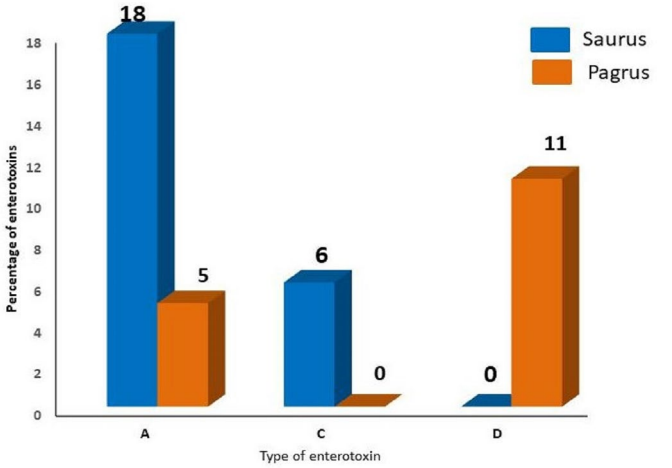

Fig. 2. Percentages of the enterotoxins produced by the recovered *S. aureus* isolates from *Saurus* and *Pagrus* fish species.

In addition to multiple dead birds being dumped into Egypt's

Table 1. Antimicrobial resistance profile of *S. aureus* (n=33).

NO	Key	Strains	Antimicrobial resistance profile	MAR index
1	6	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX, M, IPM, AK	1
2	14	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX, M, IPM	0.94
3	32	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX, M, IPM	0.94
4	11	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX, M	0.88
5	23	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX	0.81
6	30	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G, OX	0.81
7	1	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX, FEP, G	0.75
8	17	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX	0.63
9	28	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP, AMX	0.63
10	3	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP	0.56
11	15	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP	0.56
12	29	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E, CP	0.56
13	20	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E	0.5
14	33	*S. aureus*	K, CL, NA, SXT, T, CZ, AM, E	0.5
15	18	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
16	24	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
17	13	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
18	5	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
19	22	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
20	31	*S. aureus*	K, CL, NA, SXT, T, CZ	0.38
21	8	*S. aureus*	K, CL, NA, SXT, T	0.31
22	26	*S. aureus*	K, CL, NA, SXT	0.25
23	19	*S. aureus*	K, CL, NA, SXT	0.25
24	2	*S. aureus*	K, CL, NA, SXT	0.25
25	7	*S. aureus*	K, CL, NA, SXT	0.25
26	21	*S. aureus*	K, CL, NA	0.19
27	10	*S. aureus*	K, CL, NA	0.19
28	16	*S. aureus*	K, CL, NA	0.19
29	27	*S. aureus*	K, CL	0.13
30	9	*S. aureus*	K	0.06
31	25	*S. aureus*	K	0.06
32	4	*S. aureus*	K	0.06
33	12	*S. aureus*	K	0.06
Average	0.442			

E: Erythromycin; AMX: Amoxicillin; OX: Oxacillin; AM: Ampicillin; G: Gentamicin; AK: Amikacin; T: tetracycline; NA: Nalidixic; acid; CL: Clindamycin; L: Levofloxacin; CP: Ciprofloxacin; SXT: Sulphamethoxazole.

main water streams, antimicrobials are frequently utilized in intensive fish farming (Alsayeqh *et al*., 2021). This work was expanded to examine the recovered *S. aureus* antibiotic susceptibility. The results showed that the recovered *S. aureus* isolates had a significant level of resistance to kanamycin, clindamycin, nalidixic acid and Sulphamethoxazole at 100%, 87.9%, 84.8%, and 75.8%, respectively, while it has a good sensitivity to amikacin, imipenem, meropenem, oxacillin at 90.9%, 87.9%, and 81.8% respectively. The multiple antibiotic resistance (MAR) index for the recovered *S. aureus* isolates ranged from 0.062 to 1 with an average value of 0.442 (Table 1). Such antibiogram agrees with previous reports of Simon and Sanjeev (2007); Vázquez-Sánchez *et al*. (2012) and Hussein *et al*. (2019). As the recovered *S. aureus* isolates showed clear drug resistance towards penicillin, chloramphenicol and ciprofloxacin, and most to tetracycline (82.4%).

CONCLUSION

The current study indicated that saurus and pagrus fish species should be regarded as potential sources of enterotoxigenic multidrug resistant *S. aureus*. Therefore, strict hygienic measures should be adopted during the entire production chain of the fish starting from catching of the fish, to transportation, handling, marketing and storage.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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