

Food safety and Public health

Prevalence of *Staph aureus*, *Pseudomonas species* and *E. coli* in Some Fish Flesh.

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ABSTRACT

Fish provide a great source of protein. Fish is one of the main vectors of bacterial pathogen that is related to consumers and public health. Control of food poisoning outbreaks depends on the detection of the causative agents in food (fish and fishery products) and elimination of them to produce safe food free from food poisoning microorganisms as possible. The examined samples of *Tilapia nilotica*, Mackerel and Salted sardines (50 samples of each) collected from various localities in El-Menoufia governorate for isolation of *Staphylococcus aureus*, *Pseudomonas species* and *E. coli*. The examined fish samples showed that the prevalence of *S. aureus* was 36%, 22 % and 14% of the examined *Tilapia nilotica*, Mackerel and Salted sardine, respectively, while the prevalence of *pseudomonas species* was 18%, 26 % and 14% of the examined samples, respectively, and *E. coli* prevalence was 32%, 26 % and 12% of the examined samples, respectively.

Keywords: *Staphylococcus aureus*, *Pseudomonas species*, *E. coli*, *Tilapia nilotica*, Mackerel and Salted sardines.

INTRODUCTION

Fish is esteemed as a comestible of elevated nutritive worth and a pivotal constituent of the human alimentation, furnishing proteins of eminent biological merit, unsaturated lipids, vitamins, and minerals (Fuertes Vicente et al., 2014). Fish and ichthyic commodities persist as among the most extensively bartered alimentary items globally. The ingestion of fish has amplified markedly across all continents, escalating from 5.2 kilograms per capita in 1961 to 19.4 kilograms in 2017, with an average

annual augmentation rate of 2.4%. Fish consumption constituted 17% of the intake of animal protein in the world and 7% of total protein consumption (FAO, 2020). Ingesting seafood tainted with pathogenic microorganisms and/or their deleterious metabolites can precipitate alimentary maladies ranging from mild gastroenteritis to fatal syndromes, manifesting as infection, intoxication, or a combination thereof (Iwamoto et al., 2010).

E. coli is the most famous bug that can live with or without oxygen in guts of

people and animals, part of Enterobacteriaceae family. Finding it in food means poor contamination (Mohamed, 2014).

S. aureus can grow in food if temps and times get messed up, pumping out strong, heat-stable toxins that mess you up good (Hennekinne et al., 2012). *S. aureus* is one of the main food germs causing sickness, living on the skin and mucus of food handlers. These folks can spread the bugs and dirty up food, while handling it (Bencardino and Vitali, 2019). In fish, *Pseudomonas* bacteria cause nasty diseases like bacterial hemorrhagic septicemia, fin rot, gill rot, skin darkening, scale loss, belly swelling, bulging eyes, red fever, red spots on pike and carp, and trout red mouth (Thomas et al., 2014).

Consequently, the purpose of this research was to investigate the occurrence and presence of *S. aureus*, *Pseudomonas* species and *E. coli* in some fish flesh.

2. MATERIAL AND METHODS

2.1. Collection of fish samples:

A total of 150 samples of Tilapia nilotica, Mackerel and Salted sardines (50 of each) were collected from random areas in El-Menoufia governorate for isolation of *S. aureus*, *Pseudomonas species* and *E. coli*. Each sample was kept in a separated clean sterile bag and put in an ice box, then send to Animal Health Research Institute lab in Shebin Elkom, under complete aseptic conditions.

2.2. Preparation of fish samples:

According to APHA (2001): 25 g from each fish flesh sample, mashed it up in 225 ml of buffered peptone water (BPW) using a stomacher blender for 2 minute. then ready for bacteriological examinations.

2.3. Isolation of *S. aureus*, *Pseudomonas spp* and *E. coli*

2.3.1. Isolation of *S. aureus* (APHA, 2001; Quinn et al., 2002):

One ml of homogenized sample was inoculated into nutrient broth and incubate at 37 °C for 24 hours. Then grabbed a loop, smeared onto Baird Parker agar plates, and incubated for 24-48 hours. Looked for shiny black colonies surrounded by the halo zone of inhibition. These colonies were picked up and kept in semisolid agar for further biochemical identification.

2.3.1.1. Identification of suspected *S. aureus*:

The suspected colonies were examined for their colonial morphology following Quinn et al. (2002).

2.3.1.2. Morphological identification of *S. aureus*:

Made slides from pure cultures, stained with Gram stain, and looked under the microscope - should see purple grape-like cocci.

2.3.1.3. Detection of bacterial motility (Cruickshank et al., 1975):

Stabbed suspected colonies into soft agar tubes (0.4%), incubated at 37°C for 24-48 hours. *S. aureus* is non motile.

2.3.1.4. Biochemical identification of suspected *S. aureus* isolates:

Using Oxidase, Catalase activity, Indole production, Methyl red, Sugar fermentation, and Coagulase production tests.

2.3.2. Isolation of pseudomonas species (ISO, 2004):

One ml of mix was inoculated into nutrient broth and incubate at 37 °C for 24 hrs. A loopful from incubated nutrient broth was streaked on *Pseudomonas* selective agar medium supplemented with glycerol, positive colonies (greenish-yellow colonies) after the inoculation at 25°C for 48 hours. These colonies were picked up and kept in semisolid agar for further biochemical identification.

2.3.2.1. Identification of suspected pseudomonas species:

The suspected colonies were examined for their colonial morphology following Quinn *et al.* (2002).

2.3.2.2. Morphological Identification of pseudomonas species:

Films were prepared from a pure culture of isolate stained with Gram's stain and examined microscopically, appear gram negative straight or slightly curved rods, single, non-sporulated.

2.3.2.3. Detection of bacterial motility (Cruickshank *et al.*, 1975):

As mentioned before, *Pseudomonas* species is motile by polar flagella.

2.3.2.4. Biochemical tests of suspected pseudomonas species isolates

were performed using Oxidase, Catalase activity, Citrate utilization, Methyl red, Voges Proskauer, Indole production, Urease, Pigment formation and Hydrogen Sulfide production tests.

2.3.3. Isolation of E. coli (ISO, 2004):

One ml of mix was dropped into MacConkey broth tubes supplemented with inverted Durham's tubes and incubated aerobically at 37 °C for 24 hrs. A loopful from incubated MacConkey broth was smeared on Eosin methylene blue agar medium (EMB) and incubated aerobically again at 37 °C for another day. Looked for shiny metallic green colonies. These colonies were picked up and kept in soft agar form further biochemical identification. The suspected colonies let it grow onto nutrient agar slopes and incubated at 37°C for 24 hrs. Then subjected for further morphological or biochemical identification following (MacFaddin, 2000; Quinn *et al.*, 2002; Arora, 2003).

2.3.3.1. Identification of suspected isolates:

The suspected colonies were examined for their colonial morphology according to Quinn *et al.* (2002).

2.3.3.2. Morphological Identification:

Films were prepared from a pure culture of isolated organism stained with Gram's stain and examined microscopically; *E. coli* appears as medium-sized, evenly stained, Gram-negative coccobacilli.

2.3.3.3. Detection of bacterial motility (Cruickshank *et al.*, 1975):

As mentioned before, *E. coli* is motile.

2.3.3.4. Biochemical tests of suspected isolates of E. coli:

Using Oxidase, Citrate utilization, Methyl red, Voges Proskauer, Urease and Hydrogen Sulfide production tests.

2.3.3.5. Identification of E. coli serologically.

According to (Cruickshank et al., 1975).

S. aureus was 18 (36%) in Tilapia, 11 (22 %) in Mackerel and 7 (14%) in Salted sardine, respectively, as shown in Table (1), Figure (1).

3. RESULTS

3.1. Isolation of *S. aureus*:

The bacteriological examination of fish samples revealed that the prevalence of

Table 1. Incidence of *S. aureus* species isolated from examined samples. (n=50 of each)

Samples	No. of isolates	Incidence %	X2	p-value
Tilapia	18	36%	6.7982	.033
Mackerel	11	22%		
Salted sardine	7	14%		
	36	24%		

X2 =chi-square the result is significant at $p < .05$.

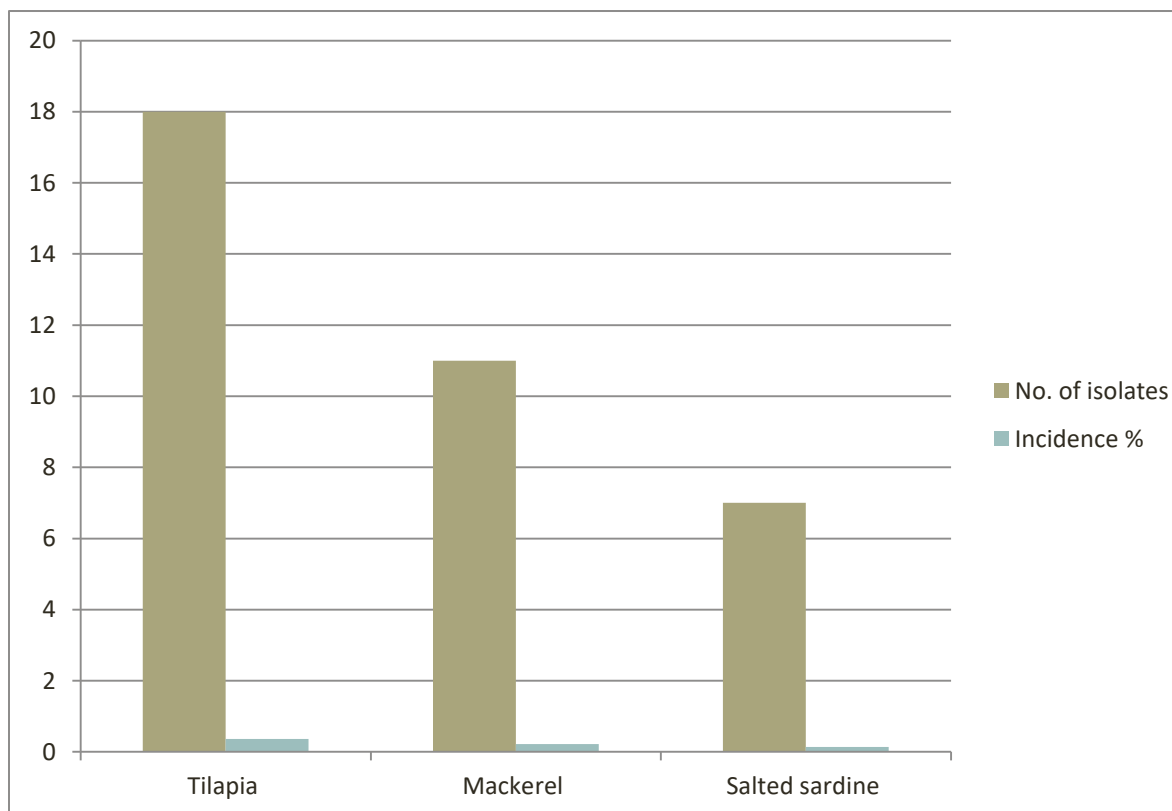


Figure 1. Incidence of *S. aureus* isolated from examined fish samples.

3.2. Isolation of *pseudomonas* species:

The bacteriological examination of fish samples revealed that the prevalence of

pseudomonas species was 9 (18%) in Tilapia, 13 (26 %) in Mackerel and 7 (14%) in Salted sardine, respectively, as shown in Table (2), Figure (2).

Table 2. Incidence of *pseudomonas species* isolated from examined samples (n=50 of each)

Samples	No of isolates	Incidence %	X2	p-value
Tilapia	9	18%	2.3938	.302
Mackerel	13	26%		
Salted sardine	7	14%		
	29	19.3%		

X2 =chi-square the result is not significant at $p < .05$.

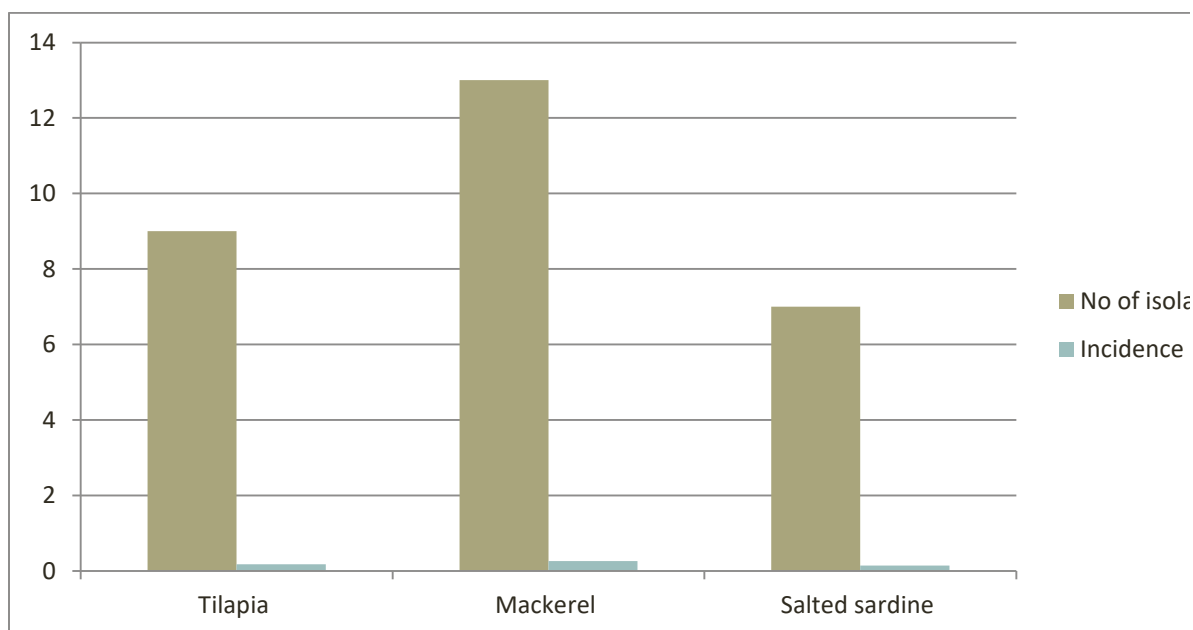


Figure 2. The incidence of *pseudomonas species* isolated from examined fish samples.

3.3. Isolation and serotyping of *E. coli* species:

The bacteriological examination of examined samples showed that the prevalence of *E. coli* was 16 (32%) in Tilapia, 13 (26 %) in Mackerel and 6 (12%) in Salted sardine, respectively, as shown in Table (3), Fig. (3). The serotyping of examined Tilapia samples revealed 4 serotypes of O118: K (25%), 4 serotypes of O111:K58 (25%), 3

serotypes of O78:K80 (18.7%) and 1 serotype of O124 (6.25%). The serotyping of examined Mackerel samples revealed 2 serotypes of O164: K (15.3%), 5 serotypes of O44:K80 (38.4%), 3 serotypes of O78:K80 (23%) and 3 serotype of O114:K90 (23%). The serotyping of examined salted sardine samples revealed 2 serotypes of O78:K80 (33.3%) and 4 serotype of O11:H2 (66.6%), respectively.

Table 3. Incidence and serotyping of *E. coli* species isolated from examined samples. (n=50 of each)

Samples	No of isolates	incidence %	X2	P-value	Serotyping	No	%
Tilapia	16	32%	5.888	.0526	O118:K-	4	25%
					O111:K58	4	25%
					O78:K80	3	18.7%
					O111:H2	4	25%
					O124	1	6.25%
Mackerel	13	26%			O164:K-	2	15.3%
					O44:K80	5	38.4%
					O78:K80	3	23%
					O114:K90	3	23%
Salted sardine	6	12%			O78:K80	2	33.3%
					O11:H2	4	66.6%
Total	29	19.3%					

X2 =chi-square The result is not significant at $p < .05$.

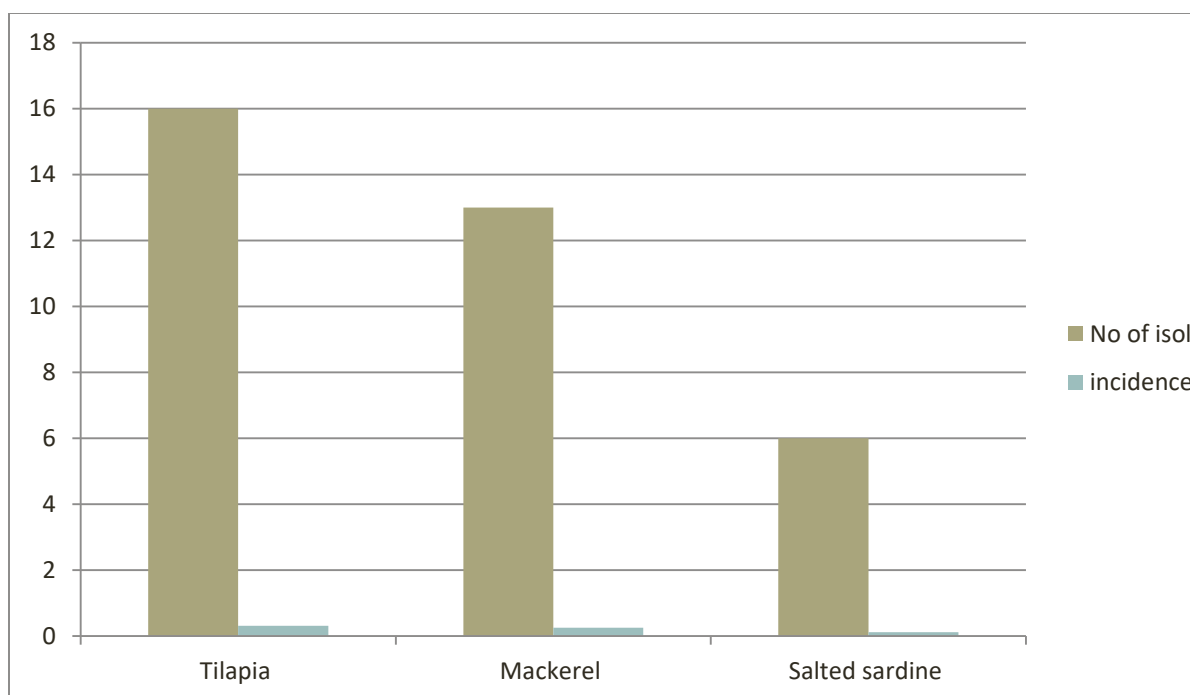


Figure 3. Incidence and serotyping of *E. coli* species isolated from examined fish samples.

4. DISCUSSION

Keeping an eye on the bug levels in fish is a big part of making food safer along the fish's journey from water to plate. Fish can pick up dirt and germs at all sorts of stops-catching, hauling, handling, and processing. The usual culprits are raw fish, sloppy workers, creepy crawlies, dirty gear, and even forklifts. Plus, fish can get yucky after processing during storage, transport, market time, and cooking Sanjee and Karim (2016).

The present study revealed that 36 *S. aureus* could be isolated from 36 (24%) out of 150 examined fish samples. All isolates looked like purple grape-like cocci under the microscope, and their colonies showed up black colonies with halo zone of precipitation on Baird Parker agar. All *S. aureus* isolates give positive to catalase, coagulase while negative results on the oxidase test, matching with Cortés-Sánchez *et al.*

(2020). Also Table (1) showed that *S. aureus* were found in 36% of Tilapia nilotica, 22% of Mackerel and 14% of salted sardine. The results were nearly similar to Edris *et al.* (2017) who found *staph aureus* in Tilapia and frozen fish. and Basti *et al.* (2006) who said that the *S. aureus* was the main pathogen isolated from highly salted fish. So, *S. aureus* came from dirty handlers and bad handling, just like Shena and Sanjeev (2007), These results higher than those recorded by Hassan *et al.* (2020) who reported that that the bacteria isolated from different fish farms at Kafr El-Sheikh Governorate, Egypt, was *S. aureus* by 7.3%.

Regarding the *Pseudomonas* species, 29 *Pseudomonas* isolates were isolated at a rate of 19.3%. *Pseudomonas* species was detected at an incidence of 18%, 26% and 14% in of Tilapia nilotica, Mackerel and salted sardine samples, respectively. Table (2), Figure (2), Our

findings were lower than those obtained by Abd El-Aziz (2015), who reported that all examined fish samples collected from Assiut were contaminated with *Pseudomonas spp.* And also, lower than those obtained by Abd-El-Maogoud et al. (2021) who found that 65% (78/120) samples were contaminated with *Pseudomonas spp.* at Sohag governorate. Some researchers reported that *Pseudomonas spp.* were the causative agents of *Pseudomonas* septicemia in various species of fish (Eissa et al., 2010; EL-Nagar, 2010)

Furthermore, 29 *E. coli* isolates were isolated at rate of 19.3%, So, *E. coli* was detected at an incidence of 32%, 26% and 12% in of Tilapia, Mackerel and salted sardine samples, respectively. And *E. coli* incidence was higher in Tilapia samples and lower in Mackerel and salted sardine samples, respectively. Table (3), Figure (3), The findings from this study are similar to results observed in other studies Mumbo *et al.* (2023), and partial agree with Saqr et al. (2016) and AbdEl-Tawab et al. (2018) who reported incidences of *E. coli* 18.3%, 17.5% respectively. While higher incidences of *E. coli* were recorded by David et al. (2009); Amr et al. (2012); Galal et al. (2013); Gupta et al. (2013) who reported incidences of *E. coli* 50%, 57.1%, 29.34% and 36% respectively.

5. CONCLUSION

- Fish are a great source of food poisoning for humans so preventing the spreading of this infection is a very important key through preventing contamination of the natural habitat of fish as well as avoid contamination of fish during shipping, storage and handling.
- The fish preparation places must be clean and keeping proper sanitation

measures among fish staff.

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