International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 14-01-2022, Accepted: 29-01-2022, Published: 14-02-2022
Volume 7, Issue 2, 2022, Page No. 68-71

Isolation and identification of staphylococcus bacteria from fresh water fish and its antibiotics sensitivity

Sameer Ahmad Ganaie, G D Sharma*
Department of Zoology, P.M.B. Gujarati Science College, (Affiliated to DAVV), Madhya Pradesh, India

Abstract
During the months of September 2018 and September 2019, a bacteriological test was carried out on 60 local healthy freshwater fish, including 30 goldfish (Carasius auratus) and 30 koi carps (Cyprinus carpio) of various weights purchased from local retail fish markets in Indore. Swabs from the skin and gills were inoculated on blood agar for 24 hours at 37 degrees Celsius (aerobic culture), and then chosen colonies were subjected to gramme staining, morphological features and biochemical assays for Staphylococcus. For all of the fish samples analysed, the proportion of Staphylococcus isolation was maximum. A total of 90 isolates from both examined fish species (48 from Carasius auratus and 42 from Cyprinus carpio) were identified, with different numbers and percentages for Isolated species were included: S. aureus (33.3%, 35.17%), S. epidermidis (25%, 28.57%), S. hyicus (22.91%, 16.66%), S. saprophyticus (12.5%, 11.90%), S. intermedius (6.25%, 7.14%) from total isolates. The results of six antibiotic sensitivity tests (Ampicillin 10 g (AM), Ofloxacin 10 g (OF), Ciprofloxacin 5 g (CIP), chloramphenicol (30 g) (CL), polymaxin-300 (Poly), and CO-Trimaxazol-25g (CO- T)) were variable. Most Staphylococcus isolates were resistant to Ampicillin but susceptible to Ciprofloxacin and Ofloxacin.

Keywords: *staphylococcus, Carasius auratus, Cyprinus carpio*, antibiotics sensitivity

Introduction
Fish is abundant in proteins, vitamins and unsaturated fatty acids. It is also one of the most important feed components in recent years since it is the cheapest source of animal protein. Fish get contaminated due to their harsh environment, which includes sewage, polluted water, harvesting places, and contamination by employees, utensils, equipment, and unclean handling, resulting in the presence of a huge number of bacteria in the fish. Several studies on the bacterial flora of fish have been done. Despite some inconclusive findings, some of these studies demonstrate that the flesh and internal organs of healthy newly caught fish are microbiologically sterile.
Bacterial disease kills a lot of fish, both wild and domesticated. Pathogenic bacteria are naturally occurring saprophytes, which are essentially opportunistic pathogens that infiltrate and cause diseases in the tissue of a fish.
A few bacterial species seem to be necessary fish parasites and they may live in the environment for varying lengths of time. *Aeromonas*, *Pseudomonas*, *Mycobacterium*, *Edwardsiella*, *Streptococcus*, *Staphylococcus* and *Closteridium* are among the more than hundred bacterial species discovered and classified as pathogens of freshwater and marine fish. One of the most prevalent food-borne opportunistic bacteria found from fish samples is *Staphylococcus.* A large population of these bacteria indicates the overall quality of the fish as well as the degree of spoilage. Multidrug-resistant *Staphylococcus* species have grown increasingly widespread in recent years, acting as an etiological infection agent, producing food poisoning and presenting a health risk when enterotoxogenic strains cause significant morbidity and mortality in people. The purpose of this research is to isolate and identify *Staphylococcus* species from the skin and gills of two ornamental fish species, *Carasius auratus* and *Cyprinus carpio* and to check for antibiotic resistance in *Staphylococcus* isolates.

Materials and Methods
The microbiological quality of two freshwater fish species obtained from local retail fish shops in Indore city between September 2018 and September 2019 was recorded. A total of 60 healthy indigenous fish, including 30 goldfish (*Carasius auratus*) and 30 koi carp (*Cyprinus carpio*), ranging in size from 50 to 250 grams. Cotton swaps from fish surfaces were transferred and cultured on nutrient agar plates for microbiological detection for 24 hours at 37º C under sterile circumstances (aerobic culture). Gram staining was performed on selected colonies, and those having *Staphylococcus* morphological features were subjected to a biochemical test. Standard antibiotic discs of *(Ampicillin 10 g (AM), Ofloxacin 10 g (OF), Ciprofloxacin 5 g (CIP), chloramphenicol (30 g) (CL), polymaxin-300 (Poly), and CO-Trimaxazol-25g (CO- T))* (BIOANALYS) were placed on top of the plates and incubated for 4 hours at 37°C using sterile swap (NCCLS procedures) A ruler was used to measure the diameter of the inhibitory zone.

Result

30 goldfish (Carasius auratus) and 30 carp fish (Cyprinus carpio) were tested from two different freshwater fish species.

For both fish species, the proportion of Staphylococcus isolation was 100% for all tested samples. A total of 130 isolates were obtained from goldfish (Carasius auratus) (48 isolates) and Cyprinus carpio (60 isolates) (42 isolates).

According to morphological traits, Gram stain, and biochemical test, five species of Staphylococcus were identified with varying quantities and percentages (table 1).

Table 1: Biochemical reactions and other characteristics of Staphylococcus isolates from fish.

Species	Pigmentation production	Haemolysis	Coagulase	Uearse	Oxidase	Catalase	MR	Glucose	Lactose	Mannitol	Sucrose	Motility
S. saprophyticus	-	-	-	+	-	+	+	+	+	+	+	-
S.epidermidis	-	v	-	+	-	+	+	+	+	+	+	-
S.hyicus	-	-	v	v	-	+	+	+	+	+	+	-
S.intermedius	-	+	+	+	-	+	+	+	+	+	+	-
S.aureus	+	+	+	v	-	+	+	+	+	+	+	-

(-): Negative, (+): positive, V: Variable

Isolated species were included: *S. aureus* (33.3%, 35.17%), *S. epidermidis* (25%, 28.57%), *S. hyicus* (22.91%, 16.66%), *S. saprophyticus* (12.5%, 11.90%), *S. intermedius* (6.25%, 7.14%) from total isolates (tables 2, 3).

Table 2: Number and percentage of Staphylococcus species isolated from skin, and gills of *gold fishes (Carasius auratus).*

Species	Number and percentage of isolates			
	30/Gills	30/skin	Total	%
S. aureus	8	8	16	33.3
S. epidermidis	6	6	12	25
S. hyicus	5	6	11	22.91
S.saprophyticus	5	1	6	12.5
S. intermedius	2	1	3	6.25
Total	26	22	48	
%	54.16	45.83	100	

Table 3: Number and percentage of Staphylococcus species isolated from skin and gills of *Cyprinus carpio*.

Species	Number and percentage of isolates			
	30/Gills	30/skin	Total	%
S. aureus	9	6	15	35.17
S. epidermidis	7	5	12	28.57
S. hyicus	4	3	7	16.66
S.saprophyticus	3	2	5	11.90
S. intermedius	2	1	3	7.14
Total	25	17	42	
%	59.52	40.47	100	

The results of the Antibiotic Sensitivity test were mixed. Most *Staphylococcus* species were resistant to Ampicillin (AM), but responsive to Ciprofloxacin 5g (CIP) and Ofloxacin (OF), with varying susceptibility to other antibiotics such as chloramphenicol (30 g) (CL), polymaxin-300 (Poly), and CO-Trimaxazol-25g (CO- T) (table 4).

Table 4: Antibiotic sensitivity test for Staphylococcus species isolates

Antibiotic Species	Number of I Isolates Solate	Poly		CL		CO-T		AM		Cip		OF	
		S	R	S	R	S	R	S	R	S	R	S	R
S.aureus	31	0	31	14	17	21	10	0	31	27	4	28	3
S.epidermidis	24	0	24	13	11	4	20	4	20	21	3	22	2
S.hyicus	18	6	12	12	6	12	6	5	13	15	3	18	0
S.saprophyticus	11	0	11	0	11	2	9	1	10	11	0	11	0
S.intermedius	6	5	1	0	6	2	4	0	6	6	0	6	0

S: sensitive, R: resistant.

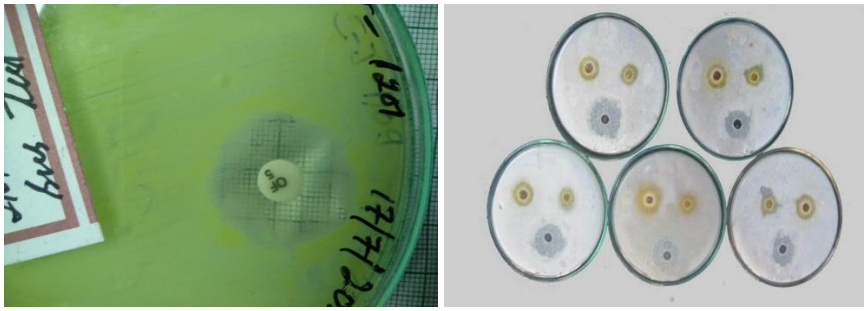

Fig 1: Photographs showing antibiotic sensitivity tests for various antibiotics

Discussion

Many key species of food-poisoning bacteria, such as Salmonella, Staphylococcus, Cl. Botulinum, Bacillus cereus etc. might be found in fish and fisheries products. Five species of Staphylococci were isolated from *Carasius auratus* and *Cyprinus carpio* in this investigation. Different percentages of both fish species were found in the isolated species from skins and gills. *S. aureus (33.3%, 35.17%), S. epidermidis (25%, 28.57%), S. hyicus (22.91%, 16.66%), S. saprophyticus (12.5%, 11.90%), S. intermedius (6.25%, 7.14%)*. These findings are consistent with those of Shah and Tyagi (1986) [28], Lin *et al*., (2007) [19], Ahmeed *et al*. (2008) [2]. Different predominant bacterial species infect the fish and enter the body through skin and gills that later spread to muscle, blood and different visceral organs during storage. The fish gut was found to be heavily laden with Staphylococcus species in general. The increased number of bacterial isolates found in fish (*Carasius auratus*) (48 isolates) and *Cyprinus carpio* in our study (42 isolates). The presence of Staphylococcus in fish samples indicates the unhygienic handling of fish and that Staphylococcus associated with aquatic environments, as well as contamination during post-harvest handling, hastens fish degradation. Several investigations have found these opportunistic and dangerous Staphylococcus from fish. Hamad *et al.,* (2008) discovered *S. aureus* in his studies produce enterotoxins, which are major causes of gastroenteritis caused by consuming fish. Because Staphylococci are present as commensals on the skin and mucous membranes, as well as environmental contaminants, infection may be endogenous or exogenous Quinn *et al*., (2004) [26]. Many infections are opportunistic, although certain low-virulence types are pathogenic. Most isolated Staphylococci were resistant to Ampicillin, and some of them were multidrug resistant to more than two or three antibiotics; the widespread presence of antibiotic resistance in microorganisms emphasises the importance of good hygienic practise against antibiotic resistant infectious agents; however, most species were sensitive to Ciprofloxacin, which agrees with Sugita *et al.,* (1996), Al-Obaidy and Dabahg (2011) [4]

Conclusion

All contaminating organisms, such as those found in water, post-harvesting, marketing, dealers and handlers, are dangerous to fish. Fish flesh quality would suffer as a result of this processing, and fundamental guidelines for food borne diseases prevention and sanitation must be followed to safeguard the public from any health dangers, as well as the widespread existence of antibiotic resistant microbes.

References

1. Abdulla RK. Nutritional value of fish. Arabian Scientific Research Journal,2003:1:16-20.
2. Ahmeed, H Al - Harbi, Naim Uddin M. Aerobic bacterial flora of common carp *Cyprinus carpio* cultured in Earthen ponds in Saudi Arabia. Journal of Appl. Aquaculture,2008:20:108-119.
3. Albuquerque WF, Macrae A, Sousa OV, Vierira GHF, Vieira RHF. Multiple drug resistant *Staphylococcus aureus* strain isolated from a fish market and from fish handlers. Brazilian Journal of Microbiology,2007:38:131-134.
4. Al-Obaidy, NK Al - Dabahg SY. Isolated and identification of pathogenic bacteria from intestine of *Cyprinus caprio* fish from Tigris river in Mosul city. J. Edu. and Sci. 3 (25): (in Arabic), 2011.
5. Ames GR. the kinds and levels of post-harvest losses in African inland fisheries. Food Agriculture Organization of the United Nation. Rome. Italy. CIFA Technical paper, 1992, 19.
6. Austin B, Allen ADA. Bacterial fish pathology 2 nd, Ellis Her wood London, 1993, 384-398.
7. Ayulo AMR, Machado RA, Scussel VM. Enterotoxigenic *E. coli* and Staphylococcus aureus in fish and sea food the southern region of Brazil. Int. J. Food Microbiol,1994:14:687-695.
8. Banning, P. Long - term reading of some fish disease using general fishery research surveys in the south east part of the north sea. Disease of aquatic organisms,1987:3:1-110.
9. Baron JE, Feingold SM. Diagnostic Microbiology.8 thed. Philadelphian. ST. Louis: C.V. Mosby Company, 1990, 95-107.
10. Benson HJ. Microbiological Applications laboratory Manual in General Microbiology. 8 th Edition. London: McGraw - Hill publishing, 2002, 39-45.
11. Burrows GE. Therapeutic consideration in the use of Antibacterials. Bor.Prac,1980:15:99-102.
12. Cahill MM. Bacterial flora of fishes: a review. Microb. Ecol,1990:19:21-41.

13. Cruiskshank RC, Dugnid JP, Marmion BP, Swain RH. Medical Microbiology 12 th edition. London: Churchill Livingstone, 1975, 77-86.
14. Elmossalami MK. Safety and quality of fresh water cry fish *Procambarus clarkia* in the Nile river. Journal of Tropical Microbiology,1997:43:126-128.
15. Hamad MA, Eshak FA. Detection the bacterial types in *Albornus capito* fish in water of Khazer River. Alkofa Jour. For Vet. Sci,2012:3(1):37-46.
16. Hefnawy Y, Refal RS, Moutafa S. Prevalence of some potential pathogens in Nile fishes in upper. Egypt. Assiut Vet. Med. J,1989:21(42):101-108.
17. Koletar S, Mahon CR, Manuselis G. Jr. Textbook of Diagnostic Microbiology. Editor Saunders company. Philadelphia cap,1995:3:50-96.
18. Leung CK, Huang YW, Pancorbo OC. Bacterial pathogens and indicators in catfish and pond environment. Journal of food protection,1992:91:1-11.
19. Lin B, Chen S, Cao Z, Lin Y, Mo D, Zhang H *et al*. Acute phase response in zebrafish upon Aeromonas salmonicida and Staphylococcus aureus infection: striking similarities and obvious differences with mammals. *Molecular immunology*,2007:44(4):295-301.
20. Lotfi ZZ, Shehata AA, Mohamoud MS, Farid AF, Nada SM. Bacterial flora in Nile and sea fishes in Egypt. Presented to the 10th Arab Vet. Cong, 1972, 589-600.
21. National committee for clinical standards. (NCCLS). Performance standards for Antimicrobial susceptibility Testing, fifteenth information and supplement v25.n.l. M, 2005, 100-514.
22. Novotny L, Dvorska L, Lorencova A, Berna V, Pavlik I. Fish: A potential source of bacterial pathogens for human beings. Vet. Med – Czech,2004:49(9):343-358.
23. Oghondeminus FS. The occurrence and distribution of enteric bacteria in fish and water of tropical aquaculture ponds in Nigeria. Journal of Aquaculture in the Tropics,1993:8(1):61-66.
24. Purvis J. Post - harvest fishers on the eastern food plains. Research Discussion paper,2002:51:29-32.
25. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. Veterinary Microbiology and Microbial disease. Blackwell publishing company, 2002, 43-48.
26. Quinn PJ Carter, ME Markey, B Garter GR. Clinical Veterinary Microbiology, USA. Mosby an imprint of Elsevier Limited, 2004, 43-49.
27. Roberts RJ. Fish pathology 3 rd Ed WB Saunders Elsevier science limited, 2001, 297-366.
28. Shah KL, Tyagi BC. An eye disease in silver carp, Hypophthalmichthys molitrix, held in tropical ponds, associated with the bacterium Staphylococcus aureus. *Aquaculture*,1986:55(1):1-4.
29. Shewan JM. Microbiology of fish and fishery product. Appl. Bact,1962:61:212-223.
30. Sugita H, Miyajma C, Kobayashi H, Deguchi Y. Distribution of microflora in the intestinal tract of crap *Cyprinus caprio*. Nippon suisan Gakkaishi,1996:56(7):113-138.
31. Toranzo, Combarro AE, Lemos PML, Barja JL. Plasmid coding for transferable drug resistance in bacteria isolated from cultured rainbow trout. Appl. Environ. Microb,1984:48:872-877.
32. Wyatt LE, Nickelson R, Vanderzant C. *Edwardisella tarada* in fresh water cat fish and their environment. Appl. Environ. Microbiol,1979:38:716-714.