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# Bacteriological Analysis of Mullet (*Mugil cephalus*) in Makoko, Lagos, Nigeria

[1]Moro, D.D.* [2]Aladetohun, N.F., and [1]Sansa, T.L.
[1]Department of Microbiology, Lagos State University, P.M.B. 001, Ojo, Lagos.
[2]Department of Fisheries Technology, Federal College of Fisheries and Marine Technology, Victoria Island, Lagos.
*Corresponding Author
E-mail:dauphin.moro@lasu.edu.ng; drddmoro@gmail.com

**Abstract**

A total of 60 fresh *Mugil cephalus* samples were bought from Makoko market in Lagos and were analysed bacteriologically. Cultures of the gills and intestinal tissues of the 60 fish were made on selective media using standard microbiological methods. The weights and sizes of the fish were measured using Mettler's balance and a ruler. Antibiotics susceptibility patterns of the isolates were carried out using disc diffusion technique. Majority, 30 (50%) of the fish studied weighed between 51 and 100g, 18(45%), weighed between 201 and 250g, while 6(15%) weighed between 101 and 200g. The sizes of the fish were 24(40%) within 16 and 20cm, 24(40%) were between 21 and 25cm, while 12(20%) were within 21and 25cm range in length. A total of 312 bacterial isolates which belong to six different bacterial species were recovered from the fish. One hundred and sixty two (52%) were recovered from the intestine, while 150 (48%) were isolated from the gills. *Klebsiella* spp was the most recovered with 108 (34.6%), *Enterobacter* spp. 60(19.2%), *Proteus* spp. 48(15.4%), *Escherichia coli,* 36 (11.5%) while both *Staphylococcus aureus* and coagulase negative Staphylococci had 30 (9.6%) each, being the least isolated. *E coli* was only recovered from the intestine while none was isolated from the gills. The bacterial isolates showed a high susceptibility to the antibiotics tested, thereby suggesting that the bacteria may not have been exposed to such drugs.

**Keywords:** Mugilidae, *Mugil cephalus, Klebsiella* spp., *Staphylococcus aureus,* Antibiotics, Sensitivity.

## Introduction

Fishery products constitute an important part of international trade with increasing consumer interest in the commodity (Samakupa, 2003).

Unlike other animal products, the quality of fish is often difficult to control due to variations in species, sex, age, habitats and actions of microorganisms producing both autolytic and hydrolytic enzymes on fish (Venugopal, 2002).

*Mugil cephalus* and *Liza falcippinus* in the family Mugilidae constitute an important proportion of the catches by artisanal fishermen and are of high economic importance in Nigeria (Soyinka, 2008).

Mugilids are found in tropical and sub-tropical areas both in seawater and freshwater. Their culture is mainly practiced in Israel and is usually based on polyculture in earth ponds either in freshwater or in seawater together with carp *Cyprinus carpio* and tilapia, *Oreochromis niloticus* ( Paperna, 1975).

The mullets have a worldwide distribution and have been used as a significant source of food in different parts of the world. *M.cephalus* has great economic importance as well as rich nutritional content and potential for aquaculture (Isangedighi *et al.,* 2009). *Mugil cephalus* is a diurnal and opportunistic feeder which feeds almost continuously throughout the day on abundance of resources, both from autochthonous and allochthonous sources.

Mugilids are very salt-tolerant with optimum growth in salinity of 17-21 ppm and temperature of 25°C ( Peterson *et al.,*1999). *Mugil cephalus* swims in large shoal especially when young over muddy bottoms and dense vegetations (Edwards *et al.,* 2001). This species belongs to the group of fish known as Mugilids, which are common faunal components of the West African coastal water. In Nigeria, they are widespread in the Niger Delta area where they constitute a significant proportion of the canoe landing by artisanal fishermen (Akpan and Ubak, 2004). *Mugil cephalus* has rich nutritional content and potential for aquaculture but is faced with the challenge of information on its trophic level which is lacking.

The management and growth are not well studied despite the fact that Mugilids adapt well to artificial feeding (Wright and Eastcott, 1982). In Nigeria, there is still paucity of information on fish diseases and control (Aladetohun and Sogbesan, 2010). Bacterial disease is an illness of fish body caused by bacterial organisms thus creating infection or internal disorder. It is an expression of a complex interaction between a susceptible host, a pathogen and the environment. In the presence of an infective agent in an infective dose, a susceptible host suffers an infection in diverse conditions. Bacterial diseases manifest in various ways thereby impairing the normal physiology of the host (Bassey, 2011). Bacterial pathogens are transmitted by fish having contact with other diseased fish. Bacterial diseased are very common and one of the most difficult health problem to deal with (Bassey, 2011). Bacterial can enter fish's body through the gills or skin or it can stay on the surface of the fish's body (Douglas Duhamel, 2007). Dong (2018) reported that bacterial infections account for about 54.9% of fish diseases, thus are of economic importance to the fishermen as well as the consumers. Such bacterial pathogens cause diseases in the consumers if not properly cooked and result in high economic loss in aquaculture with high mortality.

Studies on bacterial diseases especially *M. cephalus* in Nigeria are very scanty so there is paucity of information on fish diseases and control particularly on the Mugilids (Aladetohun and Sogbesan 2010). In order to bridge the research gap stated above, particularly analysed the bacterial profile of mulets, their prevalence and the antibacterial susceptibility pattern of the bacteria encountered.

## Materials and Methods

### *Study area*

This study was carried out at the Asejere Market, Makoko area of Lagos, by random collection of fish from fishermen who caught the fish from Lagos lagoon. The fish samples were taken to the Department of Microbiology laboratory of the Lagos State University located in Ojo Local Government Area for bacteriological analysis within two hours. Its geographical coordinates are $6^{0}28^{'}$N $3^{0}11^{'}$E, Ojo, Lagos, Nigeria.

### Collection and preparation of samples

A total of 60 fish samples of mullet (*Mugil cephalus*) were collected from the Lagos Lagoon directly from fishermen between January and February 2018 at Asejere Market, Makoko, Lagos. Ten fish samples were collected by week for six weeks. The fish were transported in ice boxes to the laboratory for microbial examination. The specimens were subjected to low temperature storage for slow and effective rigor mortis as well as better preservations.

### Morphometric analysis

Some morphometric parameters of the fish samples such as weight(g), measuring length(cm), standard length(SL) and total length (TL) were measured with the aid of Mettler's floating balance and a metre rule respectively.

### Preparation of culture media

All culture media used were prepared according to the manufacturer's instruction. Buffered peptone water(BPW), MacConkey agar(MCC), mannitol salt agar (MSA) and eosin methylene blue agar (EMB) were sterilized by autoclaving at $121^{0}$C. After sterilization, the agars were allowed to cool to $55^{0}$C before disposing into Petri dishes.

**Preparation of samples for analysis**

Sections of the gills and intestine of 60 fresh mullet fishes were carefully removed by means of sterile scalpels and pair of scissors and razor blade and kept in sterile Petri dishes. Quantitative bacteriological analysis of the fish samples were carried out using total plate count on nutrient agar. Total viable bacteria were detected using MCC agar and the counts were exposed in cfu/ml.

**Bacteriological analysis**

Ten grammes of the gill and intestinal contents were separately added to 10ml of 0.1% peptone water and homogenized in a blender. 1ml of the homogenate was transferred into a test tube containing 9ml of peptone water to obtain a dilution of $10^{-1}$. In a similar manner, 1ml was transferred from this dilution to a test tube containing 9ml diluents and the process was repeated until a dilution of $10^{-9}$ was obtained according to the method of Oramadike *et al,* (2010). Distinct colonies from each plate were then placed by means of a sterile loop and sub-cultured unto a freshly poured nutrient agar medium, MCC, MSA and EMB contained in sterile plates. This was done with a view to obtaining pure growth and the plate was incubated at $37^0$C for 24 h.

Characterization of pure isolates was performed and colonial characters, cell micromorphology and biochemical test to identify isolates to generic and species level as contained in Cheesbrough,( 2000).

**Antibiotic susceptibility testing of isolates**

Isolates were selected for antimicrobial susceptibility testing according to Kirby-Bauer disc diffusion technique on Mueller-Hinton agar using the following antibiotic disc (Oxoid) in microgrammes: gentamycin (10µ), penicillin G (10µ), tetracycline (10µ), ampicilin (10µ), chloramphenicol (30µ), rifampicin (25µ), streptomycin (5µ), and cefotaxime (15µ). The zone of inhibition was interpreted according to CLSI, (2010).

**Results**

The bacterial count of *Mugil cephalus* showed that the count varied between the gills and the intestine. While the intestine showed the mean count of 5.6 x $10^6$ cfug-, gills revealed a mean count of 3.0 x $10^5$ cfug-.

From the bacteriological analysis of the fish, a total of five bacterial species and six bacterial species were isolated from the gills and intestine respectively. *Klebsiella.*spp., had the highest occurrence (34.6%) followed by *Enterobacter* spp. (19.2%), *Proteus* spp. (15.4%) while the Staphylococci (*S aureus*) and coagulate negative staphylococci had the least occurrence (9.6%). (Table 2).

The morphometric study revealed that 50% weighed between 51 and 100g, 10% each weighed between 101-150g and 151-200g, while 30% weighed between 201-250g. Majority, 40% of the

**Table 1:** Count and mean count or total viable bacteria of the mullets

| Parameter | Total Viable Bacteria | | |
|---|---|---|---|
| Organ | Minimum(cfug-.) | Maximum (cfug-.) | Mean (cfug-.) |
| Gills | $1.8 \times 10^5$ | $2.4 \times 10^6$ | $3.0 \times 10^5 \pm 2.8 \times 10^5$ |
| Intestine | $2.2 \times 10^6$ | $8.6 \times 10^6$ | $5.6 \times 10^6 \pm 3.2 \times 10^6$ |

**Table 2:** Distribution of bacteria in mullet

| Bacterium | Number | | Total | Percentage |
|---|---|---|---|---|
| | Gills | Intestine | | |
| *Klebsiella* spp. | 54 | 54 | 108 | 34.6 |
| *Enterobacter* spp. | 36 | 24 | 60 | 19.2 |
| *Proteus* spp. | 36 | 12 | 48 | 15.4 |
| *E coli* | - | 36 | 36 | 11.5 |
| *S aureus* | 18 | 12 | 30 | 9.6 |
| *Coagulase negative staphylococci* | 18 | 12 | 30 | 9.6 |
| TOTAL | 162 | 150 | 312 | 100 |

**Table 3:** Distribution of *Mugil cephalus* by weight and length

| Weight (G) range frequency | | Total length (CM) range frequency | | Standard lengght (CM) range frequency |
|---|---|---|---|---|
| 1-50 | | 6-10 | - | - |
| 51-100 | 30 | 11-15 | - | 12 |
| 101-150 | 6 | 16-20 | 24 | 24 |
| 151-200 | 6 | 21-25 | 12 | 24 |
| 201-250 | 18 | 26-30 | 24 | - |

**Table 4:** Antibiotic susceptibility pattern (%)

| Antibiotic/ Species (No) | Cefotaxime | Streptomycin | Rifampicin | Ampicillin | Tetracycline | Pen G | Gentamycin |
|---|---|---|---|---|---|---|---|
| *Klebsiella* spp. *(100)* | 96 | 95 | 95 | 92 | 92 | 90 | 92 |
| *Enterobacter* spp. *(50)* | 88 | 72 | 74 | 70 | 88 | 74 | 88 |
| *Proteus* spp. *(45)* | 93.3 | 88.9 | 97.8 | 88.9 | 95.6 | 95.6 | 95.6 |
| *E . coli (32)* | 93.8 | 90.6 | 93.8 | 90.6 | 93.8 | 87.5 | 93.8 |
| *S aureus (26)* | 88.5 | 79.3 | 84.6 | 79.3 | 79.3 | 84.6 | 88.5 |
| *CONS (26)* | 100 | 100 | 92.3 | 92.3 | 100 | 100 | 92.3 |

mullets had length ranging from 16-20cm and 20-30cm respectively.

The antibiotics susceptibility testing of the bacterial isolates showed high susceptibility to all the antibiotics tested. The degree of susceptibility of the bacteria tested ranged between72% and 100%. (Table 4).

## Discussion

The results from this study showed that the bacterial load varied in the two segments of the fish samples analysed namely; gills and intestine. The bacterial load in all samples were high but the intestine had a higher load ($5.6x\ 10^6$ cfug-) than the gills ($3.0 \times 10^5$ cfug-). This may be attributed to the high ambient temperature as well as availability of nutrients in the lagoon. The high bacterial load on both gills and intestine may also be due to contamination by genuinely aquatic species from the sewage pollution or those handling the fish. The gills had a lower bacterial population compared to the intestine. According to Ezeri *et al.* (2001), the number of bacteria in the gills are actively maintained at low level, thus it keeps the bacterial number low and therefore afford it some degree of protection against opportunistic bacterial invasion by the gills micro-flora. This study agrees with the report of Ezeri, (2001).

The bacterial load which fell within the $10^6$ bacterial count in one grami is not suitable for human consumption according to ICMSF (1986). However, the gills and intestine are usually removed and discarded, so may not constitute a health threat. Mullets from the lagoon in this study should be processed carefully and properly cooked in order to prevent outbreaks of food-borne infections. The microbial load in, or on such fish will be reduced, thus are made safe for human consumption (Olugbojo and Ayoola, 2015).

It was observed that there were no significant differences in the morphometric properties of the mullets studied. The high microbial populations may be due to the fish environment and the extent of contamination or pollution around where the fish lives as reported by Cahill (1990). The high bacterial population from the mullets can lead to an increase in biological oxygen demand (BOD), thus reduce the quality of dissolved oxygen (DO) available to the fish in the water; which in turn imposes stress on fish and make them susceptible to infection by bacterial pathogens. This may account for the preponderance of bacterial diseases among fishes in the Lagos Lagoon (Olugbojo and Ayoola, 2015; Zaky and Ibrahim, 2017).

The six bacterial species recovered from the study of the Mullets were *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Escherichia coli, S. aureus* and

coagulase negative Staphylocci. The high concentration of these bacteria in fish from the Lagos lagoon is an evidence of heavy pollution. The results of this study correspond with Saleh *et al.* (2013) who reported that all the tilapia from the samples studied were contaminated with very high amount of pathogenic bacteria, so the Lagos Lagoon is likely to be highly polluted and fishes from such a lagoon are dangerous for human health. Majority of the bacteria found are members of the *Enterobacteriaceae*, thus suggesting environmental faecal pollution, which is a public health hazard and of concern, especially the isolation of pathogens and opportunistic pathogens. It is interesting that *Enterobacter* spp., and *E. coli* which are indicator bacteria were isolated from the fish. This further confirmed that the lagoon from which the mullets were caught was contaminated with human and animal faeces (Ovcharenko, 2015).

Multi-drug resistance by strains is defined as being resistant to four or more antimicrobial agents but sometimes as low as two antibiotics from different classes (CLSI, 2010; Da Costa *et al.,* 2013). The results of this study revealed that almost all the bacterial isolates from the mullets had high susceptibility to all the antibiotics tested as against a high resistance reported by Mauffret *et al.*, (2012). It is likely that the bacteria recovered from the mullets may not constitute a source of health hazard because they may not have been exposed to such drugs. This may be responsible for the high susceptibility of the bacterial isolates tested among the bacteria recovered in this study.

Therefore, only multidisciplinary studies involving the characteristics of potential pathogenic microorganism for fish, aspects of the biology of the fish-host as well as a better understanding of the environmental factor affecting such fish culture, will allow application of adequate measures to prevent and control the main disease limiting the production of fishes.

## Conclusion

The presence of diverse enteric bacteria in fish which are likely to be from human, animal or environmental origin may arise from the Lagos Lagoon and human handlers. This may be a potential hazard to human health, especially those who are debilitated, immunocompromised or are on immunosuppressive drugs. Food safety training of fish vendors (fishermen and traders) as well as stringent regulation and monitoring measures with good food hygiene and good manufacturing practices are recommended. Fishes must be carefully handled and properly cooked prior to consumption to avert the likely adverse health consequences.

## References

Aladetohun, N.F. and Sogbesan, S. (2010). Ectoparasitic investigation on catfish, *Clarias gariepinus* reared in Epe area of Lagos State, Nigeria. *Agriculture and biology journal of North America*.94:75-78.

Akpan, A.W. and Ubak, R. (2004). Seasonality in diet spectrum and foraging activity in *Liza grandisquamus* (Mugilidae) in the Cross River estuary, Nigeria. *Living System sustainable Development* 1 (3): 6-14.

Bassey, S.E. (2011). *A Concise Dictionary of Parasitology.* 1st Edition, Zetus Concepts, Port Harcourt, pp 115-130.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries Part 2, Cambridge University Press : 13-25.

Cahill, M.M. (1990). Bacterial flora of fishes. *J Microbiol. Ecology* 19 (1): 21-40.

Carbral, P.S. (2010). Water microbiology: Bacterial pathogens and water. *International Journal of Environmental Research and Public Health,* 7: 3658-3663.

Da Costa, P.M.,Loureiro, I., Auguero, J. and Matos, F. (2013). Transfer of multi-drug resistant bacteria between intermingled ecological niches: The interface between humans, animals and environment. *International Journal of environmental research and Public Health:* 278 -294.

Dong, H.T. (2018). Bacterial diseases of tilapia. Suan Sunandha Rajabhat University, Fish health platform, Thailand.

Douglas DuHamel (2007). Identifying fish diseases http://fish.suite101.com/article.cfm.

Edwards, A.J., Gill, A. C. and Abohweyere, P.O. (2001). A revision of irvines marine fishes of tropical West Africa. Darwin Initiative Report 2 Ref 162/7/451,-157.

ICMSF (1986). International Commission on Microbiological Specification for Food, Fish and Fishery products. McMnnville, United States of America.

Ezeri, G.N.O. (2001). Haematological response of *Clarias gariepinus* to bacterial infection and prophylactic treatment with antibiotics. *J. Aquatic Science,* 16:22-24.

Isangbedighi, I.A.,Udo, P.A. and Ekpo, I.E. (2009). Diet composition of Mugil cephalus (Pisces: Mugilidae) in the Cross river estuary, Niger Delta, Nigeria. *Nig. J. Agric. Food and Environment* 5(2-4): 10-15.

Mauffret, A., Capras, M., and Gournelon, M. (2012). Relevance of Bacteroidales and F-Specific RNA bacteriophages for efficient faecal contamination tracking at the levels of a catchment in France. *Applied and Environmental Microbiology,* 78:5143-5152.

Olugbojo, J.A. and Ayoola, S.O. (2015). Comparative studies of bacterial load in fish species of commercial importance at the Aquaculture Unit and Lagoon Front of the University of Lagos. *Int. J. of Fisheries and Aquaculture* 7(4): 37-46.

Oramadike, C.E., Ibrahim, A.O. and Kolade, O.Y. (2016). Biochemical and microbiological quality of frozen fish available in some supermarkets in Lagos State, Nigeria. *Int. J. Life Phys. Sci.* 3(2): 48-51.

Ovchanrenko, M. (2015). Microparasite of worldwide mullets. *Annals of Parasitology,* 61(4): 229-239.

Paperna, I., and Overstreet, R. ( 1981). *Parasites and diseases of mullets (Mugilidae),* Faculty Publications from Harold W. Manter Laboratory of Parasitology 579:411-493.

Peterson, W.T., Keister, J.E. and Feinberg, I.R. (2002). The effects of the 1997-99 El-Nina/ La Nina events on hydrography and zooplankton office Oregon Coast. *Progress in Oceanography.* 54: 381-398.

Saleh, A., Shawky, R., Sabae, Z and Abou-El-Ghei, E.N. (2013). Microbiological water quality and bacterial infections among Tilapia fish in Lake Manzala, Egypt . *Int. J. Environ. Water* 2: 75-85.

Samakupa, A. (2003). Hygiene indicators in a fish processing establishment. *J. Food Production* 61: 280-284.

Soyinka, O.O. (2008). The feeding ecology of *Mugil cephalus* (Lingneus) from a high brackish tropical lagoon In South-West, Nigeria. *African Journal of Biotechnology* 7(2): 41988 pp.

Vanugopal, (2002) Biosensors in fish production and quality control. *Biosensors and Bioelectronics,* 17. 147-157

Wright, D, E. and Eastcott, A., (1982). Operation conditioning of feeding behaviour and patterns of feeding in thick lipped mullet *Grenimugil, Labrosus* and common carp *Cyprinus Carpio. J. of Fish Biology* 20: 625-634

Zaky, M.M.M and Ibrahim, M.E., (2017). Screening of bacteria and fungi biota associated with *Oreochromis niloticus* in Lake Manzala and its impact on human health. *Health,* 9: 697-714.

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