



## Antibiogram of Bacterial Isolates from the Skin of Catfish from Lake Kainji Area, Nigeria

<sup>1</sup>Kolndadacha, O.D., <sup>2</sup>Ezeokoli, C. D., <sup>3\*</sup>Mshelia, M.B., <sup>2</sup>Adikwu, I. A and <sup>4</sup>Atiribom, R.Y.

<sup>1</sup>National Institute for Freshwater Fisheries Research, P.M.B. 6006, New Bussa, Niger State.

<sup>2</sup>Department of Biological Science, Benue State University, Makurdi, Benue State.

<sup>3</sup>Department of Fisheries, University of Maiduguri, Nigeria

<sup>4</sup>Department of Veterinary Medicine, University of Agriculture, Makurdi

---

Received January 8, 2014

Accepted May 16, 2014

---

### ABSTRACT

A study on the antibiogram of Catfish from the Lake Kainji area of Nigeria was conducted. A Total of forty (40) fish samples were used for this study. Bacterial cultures obtained from the skin of twenty *Clarias anguillaris* and twenty *Heterobranchus bidorsalis* were subjected to microbial analysis. The antibiotic sensitivity tests using seven antibiotics revealed that all the five isolates viz: *Pseudomonas aeruginosa*, *Aeromonas aureus*, *Bacillus firmus*, *Klebsiella aerogines* and *Streptococcus iniae* were only sensitive to clofuraxine and conflax. *Aeromonas aureus* and *Pseudomonas aeruginosa* were sensitive to streptomycin, while *Streptococcus iniae* was less sensitive to streptomycin, but sensitive to conflax and neocloxin. *Streptococcus faciaus* and *Bacillusfirmus* were sensitive to neocloxin while all were resistant to oxytetracycline. Hence clofuraxin and conflax could be recommended for the treatment of fish diseases associated with these microorganisms due to their antimicrobial activity in this study.

**Keywords:** Antibiogram, Bacteria, skin, catfish, Nigeria

---

### INTRODUCTION

Bacteria constitute the most economically significant group of pathogenic agents to fish (Muniruzzaman and Chowdhury, 2004) because of their ubiquity. Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes worldwide (Muniruzzaman and Chowdhury, 2004) and most causative microorganisms are naturally occurring opportunistic pathogens which invade the tissue of a fish host and rendered it susceptible to infection. Bacterial infection of fish and fish products may influence human health either directly by inducing disease or indirectly through the residues of antimicrobial agents used to treat such infections in fishes when consumed raw or half done in which the heat is not enough to kill them (Inglis *et al.*, 1993).

To clinicians, the susceptibility of organisms to antibiotics is very important. Habitual use of antibiotics can lead to bacterial resistance and with unacceptable residues in aquaculture products and/or environment (Ogbondeminu and Olayemi, 1993). The resistant bacterial strains could have a negative impact on the therapy of fish or human diseases and spread in the environment of fish (Smith *et al.*, 1994). The predominant public concerns on microbial resistance due to the use of antibiotics are the possible impacts on human health resulting from the emergence of drug-resistant strains in fish caused by prolonged use of low doses of antibiotics.

Currently the increasing use of antibiotics in treating farm and aquatic animals have resulted in development of resistance among the microbes that could transfer the resistance factor (R-plasmid) to

---

\*Corresponding Author: Email – [badawimbilari@yahoo.com](mailto:badawimbilari@yahoo.com), Tel: +234 (0) 8065432018

another bacteria, making consumers vulnerable to the resistant bacteria. The problem is a growing global concern as a result of indiscriminate use of antimicrobial agents without proper diagnosis and sensitivity tests. Even the world health organization (WHO) had raised alarm on the rapid development of resistance to drugs that were once highly effective against microbial infections (Anonymous, 1997).

The increased interest in fish farming has prompted the awareness of problems associated with their health. Bacterial species have been known to cause major mortality in fish farms especially those caused by gram negative organisms (Subasinghe *et al.*, 2001)

This study is therefore aimed at identifying the effective antibiotics from eight (8) selected drugs that could be employed in the treatment of bacterial fish diseases in Nigeria

## MATERIALS AND METHODS

### Experimental fish

Twenty each of apparently healthy *Clarias anguillaris* and *Heterobranchus bidorsalis* weighing between 100 and 250 grams were collected from homestead ponds at Monai, Borgu local government area, Niger State, Nigeria using a dragging net measuring 15 by 6 meters. 10 fish each caught randomly from 4 earthen ponds with average size of 10 x 6 meters with stocking density of 100 fish as earthen pond receiving water supply from river Niger. The live fish were transported to the laboratory using buckets containing same pond water were identified as described by Olaosebikan and Raji (2004) and then sacrificed in a humane manner to obtain culture samples from the skin of each fish sample one after the other.

### Identification of bacterial isolates:

Culture samples for bacterial isolates were obtained from the skin of each of the fish species. One gram (1g) of skin was cut using a sterile dissecting scissors and homogenized into 9milliliters of sterilized distilled water for serial dilution in the following order: 1/10, 1/10<sup>2</sup>, 1/10<sup>3</sup>, 1/10<sup>4</sup> and 1/10<sup>5</sup> (Mette *et al.*, 2004) . Then, 0.1ml of each dilution were inoculated on Nutrient Agar, and incubated for 18-24 hrs at 37<sup>o</sup>C. Pure colonies obtained were sub-cultured on the brain heart infusion agar (BHIA) and incubated at 37<sup>o</sup>C. They were then characterized and identified using standard bacteriological methods at the Fish Health Diagnostic Laboratory, National Institute for Freshwater Fisheries Research, New-Bussa. The bacterial isolates were further re-confirmed as documented by Cowan and Steel (1993) and Mette *et al.* (2004) at the Diagnostic Laboratory of National Veterinary Research Institute, Vom, Nigeria.

### Antibiotic testing

Bacteria isolates from this stud were tested for their susceptibility to 7 broad spectrum antibiotics. This was because, the antibiotic disc (100GBMTS-UR EK01/P of Abtek Biological limited) used contain only the 7 antibiotics. The Kirby Bauer disc diffusion method (Kirby *et al.*, 1996) was used in this study. Sensitivity tests were performed on all the bacteria isolated from the skin to determine their sensitivity patterns to seven antibiotics using multidisc product of 100GBMTS-UR EK01/P of Abtek Biological limited. The multidisc products of varied concentration were used as follows: 25ug/disc (clofuraxine and streptomycin), 30ug/disc (conflux, fural, vitox and oxytetracycline) and 100ug/disc (neocloxin). Fresh colonies of bacterial culture were inoculated individually onto Brain Heart Infusion Agar and incubated for 18- 24 hrs at 37<sup>o</sup>C.

Inoculums previously grown in Nutrient Broth of 4.5x10<sup>7</sup> CFU ml<sup>-1</sup> were seeded by spreading evenly on Nutrient Agar plates using wire loop and bent glass rod. Antibiotic sensitivity test discs were pressed on the seeded Nutrient Agar using sterilized forceps and allowed to stand on the laboratory bench for 10 minutes followed by incubation for 18-24hours at 37<sup>o</sup>C.

The zone of inhibition diameter that were measured in millimetre (mm) using standard method (McCasland, and True, 2001) and compared with the NWFHS laboratory manual (MacCasland and True, 2001) to classify the seven antibiotics used into resistant (-), low (+), intermediate (++) and sensitive (+++) respectively.

## RESULTS AND DISCUSSION

Twenty seven bacterial isolates categorized into 5 genera from 40 fish samples were identified using biochemical tests in this study, with 9 isolates as *Aeromonas hydrophila*, 12 as *Pseudomonas aeruginosa*, 2 as *Streptococcus facies*, 3 as *Klebsiella aerogins* and 1 was *Bacillus cereus* (table 1). The presence of these bacterial species in fish has been reported to be of public health significance, because of their primary role as occupational hazard to fish handlers (Ibiwoye *et al.*, 2001). More so the use of apparently healthy fish, with *P. aeruginosa* and *A. hydrophila* isolates from skin and gills might encourage bacterial disease condition in polyculture or high stocking density under unfavorable condition. The five bacterial genera harboured by the skin of fish in this work are among the common ones isolated by other workers (Ogbondeinu, 1990; Ibiwoye *et al.*, 2001) in both fresh and marine waters on and or inside aquatic animals including fish.

The antibiotic sensitivity in this study found that Clofuraxin was the most effective with a sensitivity pattern of (+++) to all isolates and a zone of inhibition measuring between 15 and 17mm, followed by conflux which was highly effective (+++) against *Streptococcus facies* and intermediately effective (++) to the rest of the 4 isolates. (Table 2).

Table 1: Prevalence and Bacterial isolates from the skin of Catfish examined

Bacterial species isolates	No. of fish examined	No. (%) isolates (n=27)
Overall	40	27(67.5)
<i>Aeromonas hydrophila</i>	40	09(33.3)
<i>Pseudomonas aeruginosa</i>	40	12(44.4)
<i>Streptococcus facies</i>	40	02(7.4)
<i>Klebsiella aeruginosa</i>	40	03(11.1)
<i>Bacillus cereus</i>	40	01(3.7)

Table 2: Sensitivity patterns of bacterial isolates to the seven antibiotics

Bacteria Species	Antibiotic Clofuraxin	Sensitivity patterns Streptomycin	Conflux	Neocloxin	Fural	Vitox	Oxytetracycline
<i>A. hydrophila</i>	+++	+++	++	++	+	+	-
<i>P. aeruginosa</i>	+++	+++	++	-	-	-	-
<i>S. facious</i>	+++	+	+++	+++	-	-	-
<i>K. aerogins</i>	+++	+	++	+	-	-	-
<i>B. cereus</i>	+++	++	++	+++	+	-	-

**Key:** - = No zone (Resistant), + = 5mm (low resistant), ++ =10-14mm (intermediate), +++ =15-17mm (sensitive) (McCasland and True, 2001).

In the case of prevalence of inhibitory effect of the seven antibiotics, clofuraxine was most effective since it inhibited 80% of the bacteria isolated at 25µg/disc. The second most effective was conflux in that 70% of the bacteria were inhibited at 30µg/disc (Figure 1). All the 5 bacteria were found to be resistant to oxytetracycline and negligible susceptibility to vitox (Figure 1).

These could not be far from the indiscriminate use of antimicrobial drugs and other synthetic chemotherapeutic agent to treat fish without accurate diagnosis and sensitivity test most especially the aforesaid in fish farms (Ogbondeminu and Olayemi, 1993).

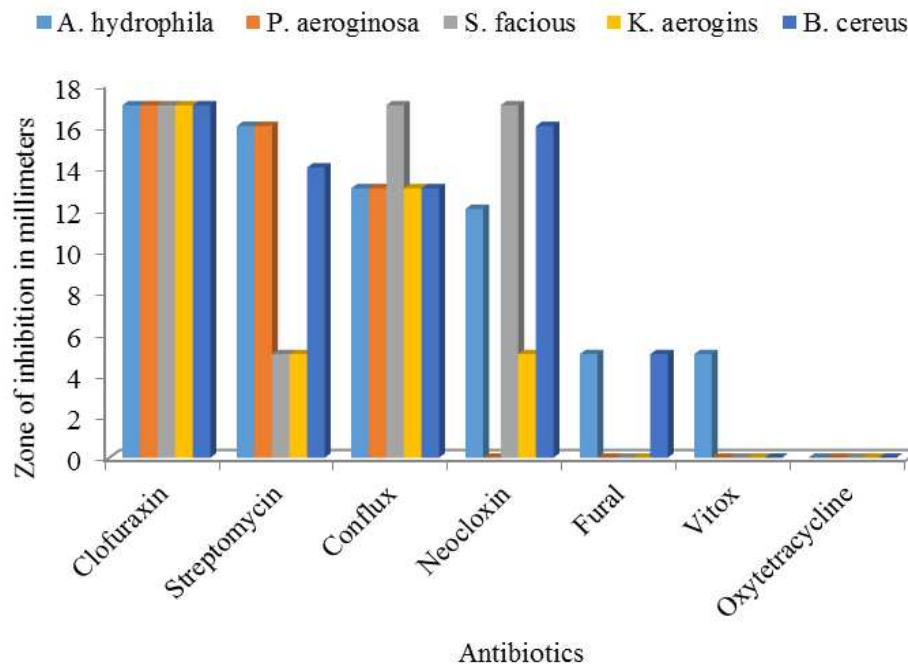


Figure 1: Inhibitory effect of seven antibiotics on bacteria isolates

Antimicrobial agents are widely used by farmers especially in the intensive culture system (Okaeme, 2006). Misuse of drugs and non-compliance with treatment regimens among users can cause treatment to be less effective thereby prolonging the duration of disease (Muniruzzaman and Choudhury, 2004). The data generated from this experiment are essential in the choice of most effective antimicrobial agents against fish pathogenic bacteria of fish.

### Conclusion

Bacterial threat is a major challenge to fish disease control, hence there is the need for detailed drug sensitivity tests of the prevalent bacterial isolates such as *Vibrio*, *staphylococcus*, *Escherichia coli*, *Streptococcus*, *Flexibacteria columnaris* etc. to determine the proper antibiotics to be used in fish production in Nigeria. The antibiotic susceptibility tests using seven antibiotics in this work revealed that all isolates were sensitive to clofuraxin and conflux. These antibiotics can therefore be recommended for treatment of diseases associated with these microorganisms in fish medicine, because of their high efficacy in the study area of Kainji. However, fish farmers should apply the right dose since any over- or under-dose may have negative consequences of drug residue or development of resistant strains to the drugs, respectively which is quite important for product quality. Disease prevention should be carried out by means of immunizations, use of biological control, good culture and proper health management to ensure an optimum yield and best quality of the products.

**REFERENCES**

- Cowan, S. T. and Steel, K. J. (1993). *Manual for Identification of medical bacteria*. Third Edition, edited and revised by G.I. Barrow and R.K.A. Feltham Cambridge, University Press, 1-300.
- Cruickshank, R., Duguid, J.P., Marmion, B.P and Swain, R.H.A. (1975). *Medical Microbiology 2: The practice of Medical Microbiology*, 12<sup>th</sup> edition. Churchill Livingstone, London, 206Pp
- Kirby, W., Bauer, A.W., Sherris, J.C. and Truck, M. (1996). Antibiotic susceptibility testing by a standard single disc method. *American Journal of clinical pathology*, 45:493-496
- Ibiwoye, T.I.I., Okaeme, A.N. and Agbontale, J.J. (2001). Distribution and occurrence of bacteria Fish diseases in different culture facilities of Kainji Lake area. *A paper presented at the 16<sup>th</sup> annual conference and silver jubilee of FISON at Maiduguri Nov. 2001.* 221-226
- McCasland, B. and True, K. (2001). Zone diameter standards. Bacteriology, Chapter 5. In: *NWFHS Laboratory Procedure Manual 10 June 2001. California-Nevada Fish Health Centre, Anderson California*, 1-28
- Mette, H., Bergh, Q., Riaza, A., Nialsen, J., Melchirsen, J., Jensen., Duncan, H., Ahrens, Birkbeck, and Gram, L. (2004). Selection and identification of autochthonous potential probiotic bacteria from *Turbot* Larvae (*Scophltnus maximus*) rearing units. *Systemic and applied microbiology*, 27:360-371
- Ogbondeminu, F.S. (1993). Health significance of Gram-negative bacteria associated with wasted tropical aquaculture system. *International Journal of Environmental Research* 3:10-17.
- Olaosebikan, B.D. and Raji, A. (2004). The Family Claridae. In: *Field guide to Nigerian Fresh-Water Fisheries*, second edition. Federal College of Freshwater Fisheries Technology, New-Bussa. 51-55.
- Smith, P., Heny, M.P. and Samuelsen, S.B. (1994). Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. *Annual Review of Fish Diseases* 4:273-313.
- Muniruzzaman, M. and Chowdhury, M.B.R. (2004). Sensitivity of fish pathogenic bacteria to various medical herbs. *Bangladesh Journal of Veterinary Medicine*. 2(1): 75-82.
- Inglis, V., Roberts, R.J., and Bromage, N.R. (1993). *Bacterial Disease of fish*. 1<sup>st</sup> edition Blackwell scientific publications, London. 1-30.
- Ogbondeminu, F.S. and A.B. Olayemi, (1993). Antibiotic Resistance in enteric bacterial isolates from fish and water media. *Journal of Aquaculture in the Tropics*, 8: 207-212.