Advance Journal of Food Science and Technology 4(3): 121-125, 2012
ISSN: 2042-4876

Submitted: October 05, 2011 Accepted: November 23, 2011 Published: June 25, 2012

Bacterial Flora from Healthy *Clarias Gariepinus* and their Antimicrobial Resistance Pattern

[1]M.O. Efuntoye, [2]K.B. Olurin and [1]G.C. Jegede
[1]Department of Microbiology,
[2]Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Nigeria

Abstract: The antibiotic resistance of bacteria isolated from *Clarias gariepinus* from 3 farms in Ago-Iwoye, Nigeria was investigated. Morphological and biochemical characteristics of isolates revealed that majority of the bacteria belonged to the family *Enterobacteriaceae*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were also recovered. *E. coli* strains were highly resistant to ampicillin, chloramphenicol and oxytetracycline (82.4%). Majority of the *Pseudomonas aeruginosa* were resistant to ampicillin (63.6%), amoxycillin (54.5%), nalidixic acid (63.6%) and oxytetracycline (72.7%), whereas most of the *Salmonella* spp. were resistant to erythromycin (85.7%), gentamycin (71.4%), amoxicillin (57.1%), chloramphenicol (57.1%) and sulphamethoxazole (57.1%). All isolates were highly sensitive to ciprofloxacin, novobiocin and ofloxacin. While the presence of potentially pathogenic bacterial species as observed in the study may not present a serious human health hazard because of heat treatment accorded fish before consumption, the presence of antibiotic resistant strains should not be ignored because of the potential for horizontal gene transfer in the food chain.

Keywords: Antibiotic resistance, bacterial flora, *Clarias gariepinus*

INTRODUCTION

Fish culture is a relatively new enterprise in the developing world, especially in Africa. However there are a number of constraints to its growth/expansion, which include among others, infection by bacteria. Aquatic bacteria that infect fish belong to three groups: the Gram-negative bacteria (most common), Gram-positive bacteria and acid-fast bacteria, which are obtained from food or from the environment. Gram-negative bacteria cause most diseases in tropical fish. Several workers have conducted investigations on these bacteria (Ducenci and Candan, 2003; Kar and Ghosh, 2008); some of which are opportunistic pathogens (Schmidt *et al*., 2000) while others are obligatory pathogens (Tendencic, 2004). In recent studies, some of the bacteria inhabiting the intestine of fish have been isolated and cultured and used as probionts (Vines *et al*., 2006; Marzouk *et al*., 2008; Verschuere *et al*., 2000). This novel method has replaced the use of chemotherapy in many fish diseases. Bacteria tend to develop resistance to these chemotherapeutics, which at times is transferred to related species, rendering these drugs impotent. Seafood is becoming an important avenue for the evolution of antibiotic-resistant bacteria and their dissemination.

Clarias gariepinus is a popular fish for aquaculture because of its hardiness, ease of larval production in captivity and good market price. Investigations have been conducted on the bacteria infecting different catfish species including *Pangasius hypophthalmus* (Crumlish *et al*., 2002; Dung *et al*., 2004; El-Yazeed and Ibraheem, 2009; Ferguson *et al*., 2001; Sarter *et al*., 2007). Whereas studies have been conducted on the bacteriology of fish samples purchased from fish markets, there is limited data on the prevalence of bacteria and on the antibiotic resistance of bacteria isolated from *Clarias gariepinus*, a very common species, directly obtained from fish farms in Nigeria. The present study was aimed at determining the intestinal bacteria of *Clarias gariepinus* and their susceptibility to antibiotics commonly used in aquaculture in southwestern Nigeria.

MATERIALS AND METHODS

Collection of samples and bacteria isolation: Catfish (*Clarias gariepinus*) samples were collected randomly from 3 fish farms located in Ago-Iwoye, Southwestern Nigeria. The samples were collected between June and August, 2010. Five live fishes were each collected from the farms at 2 weeks interval, making a total of 30 samples per farm. The intestines and gills of fishes collected from each farm were aseptically removed, pooled and chopped with sterile knife. Twenty five grams of a mixture from intestines and gills was blended using

Corresponding Author: M.O. Efuntoye, Department of Microbiology, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Nigeria

a stomacher with 225 mL Buffered Peptone Water (BPW). The BPW-enriched mixture was incubated at 30º C for 24 h after which direct plating was done on Eosin Methylene Blue agar, Mannitol Salt Agar, Glutamate Starch Pseudomonas agar, Baird Parker agar and Blood agar. Water samples from each farm was diluted up to 10^4 times in sterile physiological saline. One ml of the dilution was inoculated onto Plate Count Agar (PCA) in addition to the solid media used above using the spread plate method. The plates were incubated at 30ºC for 24 h. After incubation, the numbers of colony forming unit (cfu) were recorded by regarding only the plates containing cfu between 30-300 and the bacterial densities expressed in cfu/mL of water sample. The bacterial colonies that grew on the media were selected, purified and subjected to morphological and biochemical tests for identification. Tests carried out for identification included Gram's stain, catalase, oxidase, coagulase, motility, O-F, indole, gelatin hydrolysis, methyl-red, Voges-Proskauer, ONPG, lysine decarboxylase, citrate utilization, Triple Sugar Iron (TSI) agar, nitrate reduction and fermentation of sugars such as glucose, sucrose, lactose, mannitol, arabinose, sorbitol, inositol, fructose, mannose and rhamnose. The identities of some enterobacteria were confirmed using commercial identification system kit (API 20E, Biomerieux, France).

Antibiotic susceptibility testing: The antibiotic susceptibility test was performed according to Kirby-Bauer disk diffusion method (Bauer *et al*., 1966) using Mueller-Hinton (MH) agar (Oxoid, Basingstoke, U.K.). The inoculums was prepared in tryptone soy broth and the concentration of the bacterial cells were adjusted to a 10^6 colony forming unit using sterile physiological saline to correspond to 0.5 MacFarland standard. The inoculums was swabbed on the prepared MH agar. The antibiotics tested and their concentrations were, ampicillin 10µg, amoxicillin 25 µg, tetracycline 30 µg, chloramphenicol 30 µg, nalidixic acid 30 µg, novobiocin 30 µg, erythromycin 15 µg, streptomycin 30 µg, nitrofurantoin 300 µg, sulphamethoxazole 25 µg, gentamicin 10µg, ofloxacin 5 µg and ciprofloxacin 5 µg. E. coli ATCC 25922 was included as control for the series of antibiotic susceptibility determinations. After incubation for 24 h, the zones of inhibition were interpreted according to the criteria recommended by CLSI (2006).

RESULTS

Isolation and identification of bacterial species: Bacteria were isolated from the intestines and gills of *Clarias gariepinus* from three farms in Ago-Iwoye, Nigeria. A total of 56 bacterial isolates belonging to 8 genera namely, *Edwardsiella, Escherichia, Morganela, Proteus, Pseudomonas, Salmonella, Shigella* and *Staphylococcus* and representing 11 bacterial species were recovered from 90 fish samples from the 3 farms studied. *Escherichia coli* was the most prevalent species identified and the species occurred in all the farms visited. This was followed by *Staphylococcus aureus* which was also recovered from the 3 farms. *Edwardsiella tarda, Morganella morganii*, *Proteue vulgaris*, *Salmonella enteritidis* and *Shigella* sp. were recovered from one farm each and at low frequencies (Table 1).

Table 1: Bacteria isolated from intestine and gills of *Clarias gariepinus*

	Fish farm (No. of isolates)			
Isolate identified	Farm A	Farm B	Farm C	Total
Edwardsiella tarda	2	-	-	2
Escherichia coli	5	6	6	17
Morganella morganii	-	1	-	1
Proteus vulgaris	2	-	-	2
Pseudomonas aeruginosa	3	-	5	8
Pseudomonas fluorescens	2	1	-	3
Salmonella typhimurium	1	4	-	5
Salmonella enteritidis	2	-	-	2
Shigella sp.	-	1	-	1
Staphylococcus aureus	4	2	5	11
Staphylococcus saprophyticus	1	3	-	4
	22	18	16	56

Table 2: Total colony forming unit of bacteria isolated from water samples collected at 2 weeks interval for 3 months

Farm	*E. coli*	*Pseudomonas* spp.	*Salmonella* spp	*Edwardsiella* tarda	*Staphy-lococcus* spp.
Farm A					
1	10^5	10^4	10^4	10^5	10^5
2	10^5	10^4	10^4	1.2×10^6	10^5
3	10^4	10^2	10^4	3.2×10^6	10^5
4	10^5	10^3	10^4	-	10^5
5	10^5	10^4	10^4	-	10^5
6	10^5	10^4	- -		10^5
Farm B					
1	10^6	-	10^5	-	10^5
2	10^5	-	1.2×10^7	-	10^5
3	10^5	1.2×10^4	10^5	-	10^4
4	10^4	-	10^5	-	10^4
5	10^5	1.8×10^7	10^6	-	10^4
6	10^5	-	10^5	-	10^4
Farm C					
1	10^5	-	-	-	1.8×10^7
2	1.2×10^7	-	-	-	1.3×10^6
3	3.4×10^6	-	-	-	10^5
4	1.8×10^7	-	-	-	10^6
5	1.3×10^6	-	-	-	105
6	10^{50}	-	-	-	10^5

The total colony forming unit of representative bacterial isolates from water samples collected in the present investigation ranged from 10^2 to 10^7 (Table 2).

Antibiotic resistance patterns of bacterial species: All isolates showed high sensitivity to ciprofloxacin and novobiocin. Resistance to amoxycillin and streptomycin was highest for *Staphylococcus* spp. than other organisms whereas *Salmonella* spp. showed the highest resistance for erythromycin, gentamicin, chloramphenicol and sulphamethoxazole when compared with the other organisms. The highest percentage resistance for nalidixic acid was recorded for *Pseudomonas* spp. The two isolates of *Edwardsiella tarda* were 100% resistant to erythromycin and sulphamethoxazole whereas they were

Table 3: Antibiotic resistance (%) in bacteria isolated from *Clarias gariepinus*

Antibiotic	*E. coli*	*Pseudomonas* spp.	*Salmonella* spp.	*Staphylococcus* spp	*Edwardsiella* tarda
Ampicillin	82.4	63.6	42.9	73.3	0
Amoxycillin	41.2	54.5	57.1	66.7	0
Chloramphenicol	82.4	36.4	57.1	53.3	0
Ciprofloxacin	0	9.1	0	0	0
Erythromycin	47.1	9.1	85.7	66.7	100
Gentamicin1	7.6	18.2	71.4	40.0	0
Nalidixic acid	5.9	63.6	14.3	6.7	0
Novobiocin	11.8	9.1	0	6.7	0
Nitrofurantoin	17.6	27.3	28.6	6.7	0
Streptomycin	29.4	36.4	42.9	46.7	0
Sulphamethoxazole	0	9.1	57.1	20	100
Tetracycline	82.4	72.7	28.6	40	0
Ofloxacin	0	0	0	0	0

sensitive to all the other antibiotics tested. Majority of the *E. coli* strains were sensitive to most antibiotics used except ampicillin, chloramphenicol and tetracycline (Table 3).

DISCUSSION

The concentration of bacteria associated with the water samples in the farms visited was high. This high level of bacteria recovered in the water samples calls for concern and provides an early warning since the fishes reared in them stand the potential risk of being devastated by disease outbreak with time if the level is not monitored. Fishes could be contaminated by the water in which they are grown (Alcaide *et al*., 2005). Although the bacterial species found in the present study did not cause mortality to the fishes in the studied farms probably because the fishes have strong host defence response yet the species are both opportunistic and pathogenic species which could be involved in causing fish diseases. In addition, these organisms could also be involved in the transmission of diseases to human beings. Fish and their products have been reported as vehicles of foodborne bacterial infections in humans (Novotyn *et al*., 2004; Hastein *et al*., 2006). Some of the bacterial species recovered in this study were also identified from catfish, *Pangasius* sp. in Vietnam (Sarter *et al*., 2007). A number of investigators have reported the occurrence of *Edwardsiella* spp. in fishes (especially *Pangasius* and *Pangasionodon* spp.) from freshwater aquaculture environment (Ferguson *et al*., 2001; Crumlish *et al*., 2002). *Edwardsiella ictaluri* infection was first reported from channel catfish in 1979 (Hawke, 1979) and has been attributed to cause 50-90% mortality for Tra catfish in Vietnam (Dung *et al*., 2004). Ho *et al*. (2008) also isolated the species along with *Aeromonas hydrophila* from infected *Pangasionodon hypophthalmus* (Sauvage, 1879). In the present investigation, *Edwardsiella tarda* was recovered in low frequency and from only one farm. The isolates were isolated from apparently healthy fishes, indicating that the organism does not constitute a serious threat to fishes in the environment studied. However the organism is of public health significance in that human liver infections caused by it has been reported in literature (Manchanda *et al*., 2006). The organism has also been associated with a number of other human infections such as gastroenteritis, osteomyelitis, cellulitis and meningitis (Janda and Abbott, 1993). It is not surprising that *E. coli* was recovered in high frequency from the 3 farms. Although it is not known whether the fish farms use livestock manures, it is pertinent to say that most isolates recovered may have been introduced as a result of contamination with animal faecal matter. In this environment, it is not uncommon to find fish grown in aquaculture fed with faecal matter of livestock origin. This practice poses a serious danger to human health since strains of *E. coli* that are pathogenic and enterotoxigenic are known to cause life-threatening foodborne diseases (Temelli, 2002; Manna *et al*., 2008).

Salmonella spp. have been recovered from gills, intestine and whole body of catfish, *Clarias gariepinus* and seafood in Malaysia and elsewhere (Budiati *et al*., 2011; Bremer *et al*., 2003; Kumar *et al*., 2009; Heinitz *et al*., 2000; Ponce *et al*., 2008). The present study recovered two species, *Salmonella typhimurium* and *Salm. Enteritidis*. This constitutes a food safety problem because catfish could be a potential agent of transfer of these species to unsuspecting consumers. *Aeromonas hydrophila* and *Vibrio* spp. which are ubiquitous in the aquatic environment and which are common fish pathogens (Hatha *et al*., 2005; Ottaviani *et al*., 2001; Vivekanandhan *et al*., 2002) were not recovered in this study.

Out of the 17 isolates of *E. coli* recovered from the 3 farms 14 were resistant to tetracycline, chloramphenicol and ampicillin. It is not known whether the resistance to tetracycline and other antibiotics was as a result of the practice of incorporation of drugs into feeds used in aquaculture. Although the fish farmers claimed to use industrial feed meals, it is not clear whether the meals contain antibiotics. The use of antibiotics may select for antibiotic resistant bacteria in aquaculture ecosystem (Nawaz *et al*., 2001; Carbello, 2006). High level resistance to chloramphenicol has been reported by Michel *et al*. (2003) although the % resistance reported in the present investigation was a little higher and the fishes examined by them were different from the current study.

The results of the resistance rate of *E. coli* to nitrofurantoin compares favourably with that of Teophilo *et al*. (2002). In the present study, resistance of the bacterial isolates to nitrofurantoin and sulphamethoxazole were lower than those reported by Phuong *et al*. (2005) and Sarter *et al*. (2007) from *Pangasius* sp. in Vietnam.

In conclusion, the widespread use of antibiotics in fish farming for therapeutic, prophylactic purposes and as growth promoters can promote the emergence of resistance in bacteria. Many of the antibiotics investigated in the present study are known to be used in veterinary practice (Michael, 2001). It is therefore necessary to monitor the usage of antibiotics in aquaculture practices to prevent the frequency of development of resistant fish pathogens and bacteria. Moreover the transferability of fish pathogens that are multi-drug resistant through horizontal gene transfer in the food chain has human health consequences (Nawaz *et al*., 2001; Heuer *et al*., 2009).

REFERENCES

Alcaide, E., M.D. Blasco and C. Esteve, 2005. Occurrence of drug resistant bacteria in two European eel farms. Appl. Environ. Microbiol., 71: 3348-3350.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Pathol., 45: 493-496.

Bremer, P.J., G.C. Fletcher and C. Osborne, 2003. Salmonella in Seafood. New Zealand Institute for Crop and Food Research Ltd., New Zealand.

Budiati, T., G. Rusul, A.F.M. Alkarkhi, R. Ahmad and Y.T. Arip, 2011. Prevalence of *Salmonella* spp. from catfish (*Clarias gariepinus*) by using improvement isolation methods. International. Conference. Asia Agric. Animal IPCBEE, 13: 71-75.

Carbello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ. Microbiol., 8: 1137-1144.

Clinical and Laboratory Standards Institute (CLSI), 2006. Methods for antimicrobial dilution and disk susceptibility testing. Sixteenth informational supplement (M100-S16), CLSI, Wayne, PA.

Crumlish, M., T. Dung, J. Turnbull, N. Ngoc and H. Ferguson, 2002. Identification of *E. ictaluri* from the diseased frech water catfish *Pangasius hypophthalmus* Sauvage, cultured in the Mekong Delta, Vietnam. J. Fish Dis., 25: 733-736.

Ducenci, S.K. and A. Candan, 2003. Isolation of Aeromonas strains from the intestinal for a of *Atlantic salmon* (*Salmo salar* L., 1758). Turk. J. Vet. Anim. Sci., 27: 1071-1075.

Dung, T, M. Crumlisj, N. Ngoc, N. Thinh and D. Hty, 2004. Investigation of the disease caused by the genus *Edwardsiella* from Tra catfish (*Pangasionodon hypophthalmus*). J. Science Can Tho University.

El-Yazeed, H.A. and M.D. Ibraheem, 2009. Studies on *Edwardsiella tarda* infection in catfish and Tilapia nilotica. Beni-Seuf Veterinary Med. J., 19: 44-59.

Ferguson, H.W., J.F. Turnbull, A.P. Shinn, K. Thompson, T.T. Dung and M. Crumlish, 2001. Baccilary necrosis in farmed *Pangasius hypophthalmus* [Sauvage] from the Mekong Delta. Vietnam. J. Fish Dis., 24: 509-513.

Hastein, T.B., A. Hjeltnes, J. Lillehaug, U. Skare, M. Berntssen and A.K. Lundebye, 2006. Food safety hazards that occur during the production stage: Challenges for fish farming and the fishing industry. Rev. Sci. Technol., 25: 607-625.

Hatha, M., A.A. Vivekanandhan, G.J. Joice and A. Christol, 2005. Antibiotic resistance pattern of motile aeromonads from farm raised freshwater fish. Int. J. Food Microbiol., 98: 131-134.

Hawke, J.P., 1979. A bacterium associated with disease of pond-cultured channel catfish. J. Fish Res. Bd. Can., 36: 1508-1512.

Heinitz, M.L., R.D. Ruble, D.E. Wagner and S.R. Tatini, 2000. Incidence of *Salmonella* in fish and sea food. Int. J. Food Microbiol., 63: 579-592.

Heuer, O.E., H. Kruse, K. Grave, I. Karunasagar and F.J. Angulo, 2009. Human health consequences of use of antimicrobial agents in aquaculture. Clin. Infect. Dis., 49: 1248-1253.

Ho,T.T., N. Areechon, P. Srisapoome and S. Mahasawasde, 2008. Identification and antibiotic sensitivity test of the bacteria isolated from Tra catfish (*Pangasianodon hypophthalmus* [Sauvage, 1879] cultured in pond in Vietnam. Karsetsat J. Nat. Sci., 42: 54-60.

Janda, J.M. and S.L. Abbott, 1993. Infections associated with the genus *Edwardsiella*: The role of *Edwardsiella tarda* in human disease. Clin. Infect. Dis., 17: 742-748.

Kar, N. and K. Ghosh, 2008. Enzyme producing bacteria in the gastrointestinal tracts of *Labe rohita* (Hamilton) and *Channa punctata* (Bloch). Turk. J. Fish Aquat. Sci., 8: 115-120.

Kumar, R., P.K. Surendran and N. Thampuran, 2009. Distribution and genotypic characteristics of *Salmonellar* serovars isolated from tropical seafood of Cochin, India. J. Appl. Microbiol., 106: 515-524.

Manchanda, V., N.P. Singh, H.K. Eideh, A. Shamweel and S.S.Thukral, 2006. Liver abscess caused by *Edwardsiella tarda* biotype 1 and identification of its epidemiological triad by ribotyping. Indian J. Med. Microbiol., 24: 135-137.

Manna, S.K., R. Das and C. Manna, 2008. Microbiological quality of fish and shellfish from Kolkata, India with reference to *E. coli* 0157. J. Food Sci., 73: 283-286.

Marzouk, M.S., M.M. Moustafa and M. Mohammed, 2008. The influence of some probiotics on the growth performance and intestinal microbial flora of Oreochromis niloticus. The Proceedings of the 8th International Symposium on Tilapia culture.

Michael, T., 2001. Veterinary use and antibiotic resistance. Current Opinion Microbiol., 4: 493-499.

Michel, C., B. Kerouault and C. Martin, 2003. Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: Comparison of minimum inhibitory concentration using recommended provisory standards for fish bacteria. J. Appl. Microbiol., 95: 1008-1015.

Nawaz, M.S., B.D. Erickson, A.A. Khan, S.A. Khan, J.V. Porthuluri, F. Rafii, J.B. Sutherland, R.D. Wagner and C.E. Cerniglia, 2001. Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. Regul. Res. Perspect., 1: 1-8.

Novotyn, L., L. Dvorska, A. Lorencova, B. Beran and I. Pavlik, 2004. Fish: A potential source of bacterial pathogens for human beings. Vet. Med. Czech., 49: 343-358.

Ottaviani, D., I. Bacchiocchi, L. Masini, F. Leoni, A. Carraturo, M. Giammarioli and G. Sbaraglia. 2001. Antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood. Int. J. Antimicrob. Agents, 18: 135-140.

Phuong, N.T., D.T.H. Oanh, T.T. Dung and L.H. Sinh, 2005. Bacterial resistance to antimicrobials used in shrimps and fish farms in the Mekong Delta, Vietnam. Proceedings of the International Workshop on Antibiotic Resistance in Asian Aquaculture Environments. Chiang May, Thailande.

Ponce, E., A.A. Khan, C.M. Cheng, C. Sumage-West and C.E. Cerniglia, 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol., 1: 29-35.

Sarter, S., H.N.K. Nguyen, L.T. Hung, J. Lazard and D. Montet, 2007. Antibiotic resistance in Gram-negative bacteria isolated from farmed catfish. Food Control, 18: 1391-1396.

Schmidt, A.S., M. Bruun, S.I. Daalsgard, K. Pedersen and J. Larsen, 2000. Occurrence of antimicrobial resistance in fish pathogen and environmental bacteria associated with Danish rainbow trout farms. Appl. Environ. Microbiol., 66: 4908-4915.

Temelli, S., 2002. Food poisoning agent Escherichia coli 0157: H7 and its importance. J. Fac. Vet. Med., 21: 133-138.

Tendencic, E.A., 2004. The first report of Vibrio harveyi infection in the sea horse. Hippocampus kuda Bleeker, 1852 in the Phillipines. Aquacult. Res., 35: 1292-1294.

Teophilo, G.N.D., R.H.S. dos Fernandes Vieira, D. dos Prazeres Rodrigues and F.G. Menezes. 2002. *Escherichia coli* isolated from seafood: Toxicity and plasmid profiles. Int. Microbiol., 5: 11-14.

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology Molecular Biol. Rev., 64(4): 655-671.

Vines, N.G., W.D. Leukes and H. Kaiser, 2006. Probiotics in marine larviculture. FEMS Microbiol. Rev., 30: 404-427.

Vivekanandhan, G., K. Savithamani, A.A.M. Hatha and P. Lakshmanaperumalsamy, 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int. J. Food Microbiol., 76: 165-168.