PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 10 (10): 982-986, 2011
ISSN 1680-5194

Isolation and Antibiotic Profile of *Aeromonas* Species from Tilapia Fish (*Tilapia nilotica*) and Catfish (*Clarias betrachus*)

A.W. Ashiru[1], P.O. Uaboi-Egbeni[1], J.E. Oguntowo[1] and C.N. Idika[2]
[1]Department of Biological Science, Yaba College of Technology, Yaba, Lagos, Nigeria
[2]Nigerian Institute of Medical Research, Lagos, Nigeria

Abstract: In this study, the surface and the intestinal tract of catfish and tilapia fish purchased from Makoko market, Lagos metropolis in Nigeria were analyzed for the presence of *Aeromonas* species and their susceptibility to antibiotics was determined. The surface and intestinal tract of the fishes were found to be contaminated with *Aeromonas* species (*Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas sobria*) and this is a potential risk for public health. *Aeromonas caviae* was predominant in tilapia fish while *Aeromonas hydrophila* and *Aeromonas sobria* were predominant in catfish. The *Aeromonas* species exhibited different level of antibiotics susceptibility based on the zone of inhibition observed around the antibiotics disc. *Aeromonas caviae*, *Aeromonas sobria* and *Aeromonas hydrophila* were all resistant to tetracycline, nitrofurantoin and augmentin with an average zone of inhibition of 9mm, 10mm and 8mm respectively but highly susceptible to pefloxacin, ofloxacin and ciprofloxacin with an average zone of inhibition of 17 mm, 21 mm and 24 mm respectively while they were randomly susceptible to ceftriazone, gentamycin, cotrimozazole and amoxycillin. Hence, pefloxacin, ofloxacin and ciprofloxacin are suitable drugs that can be use in the treatment of *Aeromonas* associated infections. There is need for antibiotic susceptibility test before treatment of *Aeromonas* associated infection since some strains of the *Aeromonas* species were randomly susceptible to the some of the antibiotics.

Key words: *Aeromonas*, catfish, intestinal tract, antibiotics

INTRODUCTION

Fish are abundant in the sea and in fresh water and it is an important food source for human. The microflora of fish depends on degree of contamination of the water in which they live over the years. Contaminations of water with pathogenic microorganisms are mostly caused by human beings and animals. The seas are constantly being deposited with sewage and industrial wastes which are the results of man's various activities. When sea foods are harvested from water, water microbes can contaminate the surface gills and intestinal tract of fresh shell fish and other sea foods (Morita *et al*., 1994). *Aeromonas* has been associated with diseases of fish (Hayes, 2003).

The genus *Aeromonas* consists of ubiquitous Gram-negative rods that are widely distributed in freshwater, estuarine and marine environments worldwide (Holmes *et al*., 1996). The aeromonads are bacteria with aquatic habitat and belong to the autochthonous flora of fishes and amphibians.

Aeromonas sp. are pathogens that cause foodborne gastroenteritis in human and extraintestinal symptoms such as septicemia, meningitis, endocarditis and osteomyelitis (Gold and Salit, 1993) with a high mortality rate in immunocompromised person. The main virulence factors that have effect on pathogenicity are; extacellular toxins (enterotoxin, hemolysin and protease), structural features (pilli, S-layer, lipopolysaccharide), adhesion and invasion (Cahill, 1990; Janda and Abbott, 1998). *Aeromonas* species can grow and produce toxins in refrigerated conditions (Eley *et al*., 1993) indicating that refrigeration can not be effective enough to control the pathogen (Kirov, 1993). As *Aeromonas* species are frequently isolated from food due to their pyscrotrophy and existence of pathogen in water, faeces of human and animals, the risk of foodborne *Aeromonas* infections are increased. Pathogen are mostly isolated from; rivers, lakes, sewers, chlorinated drinking water, retail fresh vegetables, sea foods, red and minced meat, raw and pasteurized milk, unpasteurized cheese (Gavriel *et al*., 1998; Hanninen and Siitonen, 1995; Krovacek *et al*., 1991; Singh, 1997). It is also widespread in freshwater fishes (Gonzalez *et al*., 1999; Wang and Silva, 1999).

Although infections due to *Aeromonas* may be self-limiting, treatment with antibiotics is generally necessary to curb the progressing and persistence of the disease, particularly in vulnerable groups, such as the young, elderly and immunocompromised individuals (Albert *et al*., 2000; Palu *et al*., 2006). The growing antibiotic resistance of pathogenic bacteria worldwide is compounding factor for the effective management of

Corresponding Author: A.W. Ashiru, Department of Biological Science, Yaba College of Technology, Yaba, Lagos, Nigeria

bacterial infections. An increase in antibiotic resistance of the genus *Aeromonas*, particularly to antibiotics, has been reported (Albert *et al*., 2000; Palu *et al*., 2006). Waters received human and animal waste water discharges, which are expected to contain antimicrobial agents likely to exert a selective pressure and commensal resistant bacteria, capable of transferring their resistances to autochthonous bacteria. Consequently, the freshwater indigenous flora may become a reservoir for antimicrobial genes and the reuse of these waters for humans and animals may contribute to the limitation of antimicrobial's efficiency (Morita *et al*., 1994).

Ko *et al*. (1996) found increasing resistance to tetracyclines, trimethoprim-sulamethoxazole, some extended spectrum cephalosporins (Ceftriaxone, cefotaxime and cefoxime) and tobramycin in strains from Taiwan. The molecular epidemiology of tetracycline-resistant aeromonads, especially *Aeromonas salmonicida* has been well documented. These studies indicate that tetracycline resistance is plamid-encoded (Abbott *et al*., 1998). Goni-Urriza *et al*. (2000) in the study of antimicrobial resistance of mesophilic *Aeromonas* species isolated from two European rivers found as many as 49% tetracycline-resistant *Aeromonas* species. Neither sulphamethoxazole nor trimethoprim alone is very active against *Aeromonas* species but cotrimoxazole is generally efficient due to the strong synergy between the drugs (Jones and Wilcox, 1995). Most of the isolate in the study performed by Ko *et al*. (1996) were resistant to first generation quinolones (pipemidic acid and oxolinic acid) but clinically susceptible to fluoroquinolones (from pefloxacin (54%) to ciprofloxacin (98%). Antibiotic resistance frequencies and profiles varied according to the source of the strains (Ko *et al*., 1996). Indeed, for several decades, tetracycline has been widely used in clinical medicine, veterinary and agriculture contributing to higher levels of microbial resistance especially among the genus *Aeromonas* (Goni-Urriza *et al*., 2000). The aim of this study was to isolate and identify *Aeromonas* species from the surface and intestinal tract of Catfish (*Clarias betrachus*) and Tilapia fish (*Tilapia nilotica*) as well as to determine the antibiotic profile of genus *Aeromonas* and hence proffer suitable antibiotics for the treatment of *Aeromonas* infection.

MATERIALS AND METHODS

In this study, catfish and tilapia fish were purchased from the local market in Makoko in the Lagos metropolis. A total of twenty fish samples were purchased; ten samples of tilapia fish and ten samples of catfish. The surface and intestinal tract of these fishes were investigated for the presence of the bacteria genus *Aeromonas*.

Isolation of *Aeromonas* species: Enrichment; sample was taken from the surface and intestinal tract of tilapia fish and catfish respectively. 5 g of each sample was weighed, macerated in sterile mortar and pestle and transferred into separate conical flasks. 45 ml of alkaline peptone water was transferred into each sample in the conical flask and then incubated at 37°C for 24 h.

Plating and the evaluation of the suspected colonies; after incubation, serial dilution of the enrichment culture was prepared using peptone water for each sample. 0.1 ml aliquot was aseptically transferred from the dilution of each sample unto the sterile petridishes containing *Aeromonas* agar to which *Aeromonas* selective supplement (AES80004) was added and plated out using the spread plate method. The plates were incubated at 37°C for 24 h. At the end of the incubation period of 24 h, the plates were observed for bacterial growth and representative colonies (green colonies with opaque centre and yellow colonies were randomly selected). Isolates were sub-cultured on nutrient agar and incubated at 37°C for 24 h. The colonies were then tested for Gram stain, oxidase, catalase, motility, growth without sodium chloride, growth in 6% sodium chloride and growth at 37°C.

Identification of *Aeromonas* species: From the colonies detected as *Aeromonas*, hydrogen sulphide formation, glucose and lactose fermentation, gas from glucose, acid formation from sucrose and inositol, indole test, citrate as carbon source, arginine hydrolysis and urease test were one for the identification of *Aeromonas* of species.

The biochemical reactions of *Aeromonas* species were given in Table 1 (Using Cowan and Steel"s Manual for the Identification of Medical Bacteria (3rd Edition).

Table 1: Identification tests applied for *Aeromonas* species

Biochemical tests	*Aeromonas hydrophila*	*Aeromonas caviae*	*Aeromonas sobria*
Indole production	+	+	+
Glucose fermentation	+	+	+
Lactose fermentation	+	-	-
Acid from sucrose	+	-	-
Acid from inositol	+	-	-
Gas production from glucose	+	-	+
Citrate as C source	+	+	+
Arginine hydrolysis	+	+	+

Antibiotic susceptibility profile: Nutrient agar was prepared and allowed to gel. Discrete colonies of the isolate were transferred into 5 ml physiological saline in a bijoux bottle. This was mixed and poured unto nutrient agar plate. It was allowed to cover the entire surface of the nutrient agar, left for few seconds after which it was drained off the nutrient agar plate. Antibiotic discs were positioned on the agar surface. This was incubated at 37°C for 24 h. Diameters of inhibition zones was observed and measured in milimeters. A sensitive reaction to the antibiotics gives a zone of inhibition of 12 mm and above and a resistant reaction gives a zone of inhibition below 12 mm.

RESULTS AND DISCUSSION

Bacteria of the genus *Aeromonas* were isolated from the surface and intestinal tract of Tilapia fish (*Tilapia nilotica*) and catfish (*Clarias betrachus*) and tested for their susceptibility to antibiotics. Three probable species of *Aeromonas* namely: *Aeromonas caviae*, *Aeromonas sobria* and *Aeromonas hydrophila* were identified. They were all Gram-negative rod, catalase positive, oxidase positive, motile, indole positive, ferment glucose with or without gas production, citrate positive, positive for arginine hydrolysis, grow without sodium chloride and grow when incubated at 37°C. From the total plate count of the isolates, *Aeromonas caviae* had a colony forming unit (cfu/ml) of 3.4×10^7 and 2.2×10^7 on the surface and in the intestinal tract of tilapia fish respectively, *Aeromonas sobria* had a cfu/ml of 2.8×10^7 on the surface of catfish and *Aeromonas hydrophila* had a cfu/ml of 3.8×10^7 and 4.0×10^6 in the intestinal tract and on the surface of catfish respectively.

Aeromonas caviae was isolated from the surface and intestinal tract of tilapia fish while *Aeromonas hydrophila*; the most well known of the *Aeromonas* species which causes diseases in fish, amphibians as well as human was not isolated. This could be because tilapia fish tolerate adverse water quality and other stressors better than most other commercial aquaculture species. Since stress is a major factor that predisposes fish to *Aeromonas* infection, tilapia fish is labeled as being very disease resistant and in the presence of pathogens, tilapia fish is the last to break with disease (Helfman *et al*., 1997).

Also according to other authors, *Aeromonas caviae* is the most common species in waters with considerable faecal contamination and it is the species most frequently isolated in human faeces (Araujo *et al*., 1991) while *Aeromonas hydrophila* have the best survival and replication attitude in low polluted environment. Hence the water from which the tilapia fish was caught could have faecal contaminant bringing about the presence of *Aeromonas caviae* in the tilapia fish. *Aeromonaas sobria* and *Aeromonas hydrophila* were isolated from the surface and intestinal tract of catfish respectively with *Aeromonas hydrophila* showing a higher density with a total colony count of 3.80×10^7 cfu/ml than *Aeromonas sobria* which has a total count of 2.80×10^7 cfu/ml on the surface of catfish. This could be because *Aeromonas hydrophila* is the most well known of the species belonging to the genus *Aeromonas* that inhabits aquatic environments (Hayes, 2003). Wang and Silva (1999) isolated *Aeromonas hydrophila* from channel catfish.

Singh (1997) isolated motile *Aeromonas* species in ground meat samples from different animal species (19 ground beef, 4 ground turkey, 4 ground chicken and 4 ground pork) and he reported that all the ground turkey meat samples were contaminated with *Aeromonas* species. He found that 56% of the isolates from ground turkey meat samples were *Aeromonas hydrophila*, 16% *Aeromonas caviae*, 8% *Aeromonas sobria* and 24% *Aeromonas species*. The isolation rate was higher than this study but similar in that all the three species isolated by Singh (1997) were isolated in this study.

The main reason of differences between the studies is due to lesser samples being used. Also, it could be as a result of the different sample used. In this study fish was used while Singh (1997) used turkey meat which is of animal origin.

Based on the Antibiotic profile, the *Aeromonas* species encountered in this study (*Aeromonas caviae* *Aeromonas sobria* and *Aeromonas hydrophila*) were all susceptible to Pefloxacin, Ofloxacin and Ciprofloxacin. They all show a highs sensitivity to ciprofloxacin (26mm, 26 mm and 22 mm respectively), *Aeromonas sobria* showed the highest sensitivity to pefloxacin (24 mm) and *Aeromonas caviae* showed the highest sensitivity to Ofloxacin (26 mm). All the species (*A. caviae*, *A. sobria* and *A. hydrophila*) were resistant to tetracycline, nitrofurantoin and augmentin and randomly sensitive to ceftriazone, gentamycin, cotrimozazole and amoxicillin. Oxytetracycline resistance frequencies are high among environmental *Aeromonas* isolate (Schmidt *et al*., 2000). Goni-Urriza *et al*. (2000) found as many as 49% tetracycline-resistant *Aeromonas* species in the study, antimicrobial resistance of mesophilic *Aeromonas* species isolated from European rivers. The result conforms to this study which shows that *Aeromonas* species are poorly susceptible to tetracycline with 100% of the *Aeromonas* species showing resistance to tetracycline.

In a study by Ko *et al*. (1996), most of the isolates were resistant to first-generation quinolones (pipemidic acid and oxolinic acid) but clinically susceptible to fluoroqinolones from pefloxacin (54%) to ciprofloxacin (98%). This study confirms the susceptibility of *Aeromonas* species to ciprofloxacin, pefloxacin and ofloxacin. *Aeromonas* species isolated in this study were all 100% susceptible to these antibiotics. Antibiotic resistance frequencies and profile varied according to the source of the strains (Ko *et al*., 1996).

In this study, *Aeromonas caviae* isolated from the intestinal tract of tilapia fish was averagely susceptible to amoxycillin, cotrimozazole and gentamycin while the *Aeromonas caviae* isolated from the surface was not susceptible to these antibiotics. Cotrimozazole resistance was widespread in *Aeromonas hydrophila* while *Aeromonas sobria* was susceptible to cotrimozazole.

Conclusion: The genus *Aeromonas* is widely distributed in aquatic environment and increasingly reported as a primary pathogen of human and lower vertebrates. *Aeromonas* has been reported as an etiological agent in variety of human infections including gastroenteritis and extra intestinal infections. From this study on the isolation and antibiotic profile of *Aeromonas* species in tilapia fish and catfish, it was found that samples were considerably contaminated with *Aeromonas* species. This causes risks for public health, especially for immunocompromised person, children and aged. Hence, there is need for public enlightment, campaign and general education to assist in curtailing the outbreak of diseases in human through ingestion of the bacteria along with fish. Preventive method should be taken during food preparation; fish should be thoroughly cooked before consumption and good personal hygiene and proper sanitation procedure should always be use to prevent human exposure to this disease. Also, the preservation times should be shortened in markets and houses as pathogens are able to survive and grow in refrigerated conditions. The development of drug resistance is of clinical concern, both because this is most probably the consequence of increasing and often indiscriminate use of antibiotics and because this organism may cause human infections. Given the severity of aeromonad infections in both fish and humans, correct identification of the infectious agent is essential for the rapid selection of antibiotic therapy. Ciprofloxacin, pefloxacin and ofloxacin are suitable antibiotics that can be used in the treatment of *Aeromonas* associated infections.

REFERENCES

Abbott, S.L., L.S. Seli, M. Catino, M.A. Hartley and J.M. Janda, 1998. Misidentificatiion of unusual *Aeromonas* species as members of the genus *Vibrio*: A continuinig problem. J. Clin. Microbiol., 36: 1103-1104.

Albert, M.J., M. Ansanizzaman, K.A. Talukeler, A.K. Chopra, I. Kuhn and M. Rahman, 2000. Prevalence of enterotoxin genes in *Aeromonas* sp. isolated from children with diarrhoea, healthy controls and the environment. J. Clin. Microbiol., 38: 3785-3790.

Araujo, R.M., R.M. Arribas and R. Pares, 1991. Distribution of *Aeromonas* species in water with different levels of pollution. J. Applied Bacteriol., 71: 182-186.

Cahill, M.M., 1990. Virulence factors in motile *Aeromonas* species. J. Applied Bacteriol., 69: 1-16.

Eley, A., I. Geary and M.H. Wilcox, 1993. Growth of *Aeromonas* species at 4°C and related toxin production. Lett. Applied Microbiol., 16: 36-39.

Gavriel, A.A., J.R.B. Landre and A.J. Lamb, 1998. Incidence of mesophilic *Aeromonas* within a public drinking water supply in North-East Scotland. J. Applied Microbiol., 84: 383-392.

Gold, W.L. and I.E. Salit, 1993. *Aeromonas hydrophila* infections of the skin and soft-tissue: Report of 11 cases and review. Clin. Infect. Dis., 16: 69-74.

Goni-Urriza, M., L. Pineau, M. Capdepuy, C. Roques, P. Caumette and C. Quentin, 2000. Antimicrobial resistance of mesophilic *Aeromonas* species isolated from two European Rivers. J. Antimicrobial Chemotherapy, 46: 297-301.

Gonzalez, C.J., T.M. Lopez-Diaz, M.L. Garcia-Lopez, M. Prieto and A. Otero, 1999. Bacterial microflora of wild brown trout, wild pike and aquaculture rainbow trout. J. Food Prot., 62: 1270-1277.

Hanninen, M.L. and A. Siitonen, 1995. Distribution of *Aeromonas* phenospecies and genospecies among strains isolated from water, food or from human clinical samples. Epidemiol. Infect., 115: 39-42.

Hayes, J., 2003. *Aeromonas hydrophila*: Disease of Fish. Spring 2000 Term Project. Oregon State University, Portland, pp: 7.

Helfman, G., B. Collette and D. Facey, 1997. The Diversity of Fishes (3rd Edn.) Blackwell Publishing, USA., pp: 327.

Holmes, P., L.M. Niccolls and D.P. Sartory, 1996. The Ecology of Mesophilic *Aeromonas* in the Aquatic Environment (1st Edn.). John Wiley and Sons Ltd, Chichester, pp: 127.

Janda, J.M. and S.L. Abbott, 1998. Evolving concepts regarding the genus *Aeromonas*: An expanding panorama of species, disease presentations and unanswered questions. Clin. Infect. Dis., 27: 332-344.

Jones, B.L. and M.H. Wilcox, 1995. *Aeromonas* infections and their treatment. J. Antimicrobial Chemotherapy, 35: 453-461.

Kirov, S.M., 1993. The public health significance of *Aeromonas* species in foods: A review. J. Food Microbiol., 20: 179-198.

Ko, W.C., K.W. Yu, C.Y. Liu, C.T. Huang, H.S. Leu and Y.C. Chuang, 1996. Increasing antibiotic resistance in clinical isolates from clinical and environmental sources. Antimicrobial Agents Chemotherapy, 40: 1260-1262.

Krovacek, K., A. Paris and I. Mansson, 1991. Growth and toxin production by *Aeromonas hydrophila* and *Aeromonas sobria* at low temperatures. Int. J. Food Microbiol., 13: 165-176.

Morita, K., N. Watanabe, S. Kurata and M. Kanamori, 1994. Beta-lactam resistance of motile *Aeromonas* isolates from clinical and environmental sources. Antimicrobial Agents Chemotherapy, 38: 353-355.

Palu, A.P., L.M. Gomes, M.A. Miguel, I.T. Balassiano, M.L. Queiroz and A.C. Freilas-Almeida, 2006. Antimicrobial resistance in food and clinical *Aeromonas* isolates. Food Microbiol., 23: 504-509.

Schmidt, A.S., M.S. Bruun, I. Dalsgaard, K. Pedersen and J.L. Larsen, 2000. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four danish rainbow trout farms. Applied Environ. Microbiol., 66: 4908-4915.

Singh, U., 1997. Isolation and identification of *Aeromonas* sp. from ground meats in Eastern Canada. J. Food Prot., 60: 125-130.

Wang, C. and J.L. Silva, 1999. Prevalence and characteristics of *Aeromonas* sp. isolated from processed channel catfish. J. Food Prot., 62: 30-34.