ORIGINAL ARTICLE

Prevalence of Bacterial Pathogens and their Anti-microbial Resistance in Tilapia and their Pond Water in Trinidad

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Impacts

- Effect of bacterial pathogens on Tilapia health and production.
- Public health significance of resistance of bacterial pathogens isolated from Tilapia to antimicrobial agents.
- Environmental contamination by Zoonotic agents from Tilapia ponds.

Keywords:
Bacterial pathogens; anti-microbial resistance; Tilapia; pond water; Trinidad

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Summary

In Trinidad, Tilapia (*Oreonchromis* spp.) is one of the most important fresh water food fish and the number of farms has been increasing annually. A study was conducted in the local tilapia industry to determine the microbial quality of pond water, prevalence of bacterial pathogens and their anti-microbial resistance using the disk diffusion method. Seventy-five apparently healthy fish and 15 pond water samples from three of the four commercial tilapia fish farms in the country were processed. The 202 bacterial isolates recovered from fish slurry and 88 from water, belonged to 13 and 16 genera respectively. The predominant bacteria from fish slurry were *Pseudomonas* spp. (60.0%), *Aeromonas* spp. (44.0%), *Plesiomonas* (41.3%) and *Chromobacterium* (36.0%) ($P < 0.05$; χ^2) compared with isolates from pond water where *Bacillus* spp. (80.0%), *Staphylococcus* spp., *Alcaligenes* spp. and *Aeromonas* spp. (60.0%) were most prevalent ($P < 0.05$; χ^2). Using eight anti-microbial agents, to test bacteria from five genera (*Aeromonas*, *Chromobacterium*, *Enterobacter*, *Plesiomonas* and *Pseudomonas*), 168 (97.1%) of 173 bacterial isolates from fish slurry exhibited resistance to one or more anti-microbial agents compared with 47 (90.4%) of 52 from water ($P > 0.05$; χ^2). Resistance was high to ampicillin, 90.2% (158 of 173), erythromycin, 66.5% (115 of 173) and oxytetracycline, 52.6%, (91 of 173) but relatively low to chloramphenicol, 9.8% (17 of 173) and sulphamethoxazole/trimethoprim, 6.4% (11 of 173) ($P < 0.05$; χ^2). For pond water isolates, the frequency of resistance across bacterial genera ranged from 75% (nine of 12) for *Chromobacter* spp. to 100% found amongst *Enterobacter* spp. (six of six), P*lesiomonas* spp. (nine of nine) and *Pseudomonas* spp. (eight of eight) ($P < 0.05$; χ^2). Resistance was generally high to ampicillin, 78.8% (41 of 52), erythromycin, 51.9% (27 of 52) and oxytetracycline, 34.5% (18 of 52) but low to sulphamethoxazole/trimethoprim, 7.7% (four of 52) and norfloxacin, 3.8% (two of 52) ($P < 0.05$; χ^2). It was concluded that the rather high prevalence of bacterial pathogens in tilapia along with their high prevalence of resistance to anti-microbial agents might pose therapeutic problems as well as health risk to consumers. The microbial presence and their anti-microbial resistance in the tilapia industry are being reported for the first time in the country.

Introduction

Aquaculture is increasingly becoming one of the fastest growing aspects of agricultural industry worldwide. With the continuing decline in fishery reserves, and an increasing demand for food fish and other aquaculture products, aquaculture has the potential of becoming an important alternative supply of these products (Lucas, 2003). More

specifically, tilapia production worldwide was <200 000 metric tons (mt) in 1984, and the global production of tilapia is projected to increase to 2.5 million tons by 2010, with a sales value of more than US $5 billion according to the Fisheries Global Information System, Nile Tilapia Cultured Aquatic Species Fact Sheet (FAO, 2006). One of the factors affecting fish culture in Trinidad, and possibly in other parts of the world, is the quality of water, which in turn, determines the incidence of microbial pathogens particularly those of bacterial origin (Austin and Austin, 1999; Owens, 2003). Poor water quality including elevated nitrite and ammonia levels have been reported to be responsible for both morbidity and mortality in fish ponds worldwide (Branson and Southgate, 1992; Bunch and Bejerano, 1997).

A number of pathogenic microorganisms including *Aeromonas, Pseudomonas, Edwardsiella* and *Streptococcus* have been implicated in bacterial epidemics in tilapia (*Oreochromis* sp.) cultures (Al-Harbi, 1994, 2003; Al-Harbi and Uddin, 2003). The number and types of these pathogens have been documented in other cultured fish species including mullet, salmon and turbot as well as ornamental species such as kois and goldfish (Noga, 1996; Austin and Austin, 1999).

Information on the prevalence of bacterial pathogens that may be present in the tilapia industry in Trinidad is unavailable. Additionally, there is paucity of data on the status of sensitivity of these bacteria to anti-microbial agents used in the livestock industry in the country. This study was carried out to identify the most prevalent pathogens present in the tilapia industry in Trinidad and to determine their anti-microbial resistance.

Materials and Methods

Farms studied

In Trinidad and Tobago, there are a total of four commercial food fish farms but only three (farms I, II and III) agreed to participate in the study. These farms are located in the East and Central Trinidad.

Collection of water samples and determination of water quality

The procedure described by Newaj-Fyzul et al. (2006b) was used to determine the chemical parameters of pond water. For the three farms, one farm was sampled monthly and on each farm, five ponds were randomly sampled and selected for investigation. A total of 15 pond water samples was therefore collected. The study was designed to determine the following water quality parameters: temperature, pH, nitrites and total ammonia contents. The pH and temperature were measured on the farm using a portable 704 Metrohm temperature-compensated pH meter (Metrohm Ltd, Oberdorfstr, Switzerland). The time of visit to each farm was standardized between 8.00 AM and 9.00 AM, during the months of May, June and July. Water samples (200 ml) were aseptically collected in sterile glass bottles and transported to the laboratory in a cooler within 1 h of collection to be used for chemical and bacteriological analysis. All samples were processed within 2 h of collection. Determination of total ammonia and nitrites was performed using the LaMotte freshwater aquaculture Test Kit AQ-2 (LaMotte Company, Chestertown, MD, USA) following manufacturers' instructions. The un-ionized and ionized ammonia were calculated from the total ammonia following the procedures outlined by Emmerson et al. (1975). A total of 15 water samples were processed.

Determination of coliform counts in pond water

For the enumeration of coliforms, the membrane filtration technique was used (American Public Health Association (APHA) (1998). Ten millilitre of water was filtered using a 0.45-μm Millipore (Millipore Millex, Edinburgh, UK) filtration membranes which were placed on endo agar (BD Diagnostic Systems, Franklin Lakes, NJ, USA) and incubated at 30°C for 24 h. All colonies with metallic sheen characteristic of coliform were counted using a Darkfield Colony Counter (Reichert Jung, Quebec, QC, Canada). When colonies were too numerous to count, samples were diluted by 10-fold and Millipore-filtered. All counts were expressed as total coliform per 100 ml of water.

Isolation of bacteria from water

One hundred millilitre of each water sample was centrifuged at 2500 rpm (Beckman Model TJ-6, Lab Recyclers Inc., Gaithersburg, MD, USA) for 20 min. The supernatant was decanted. The pellet was then re-suspended in 2 ml of the supernatant and mixed using a vortex mixer to ensure a good suspension. Sterile wire loops were dipped into the re-suspension and used to streak a series of media selected to ensure the recovery of a wide variety of organisms. MacConkey (MAC) agar (Oxoid, Basingstoke, UK) was used to isolate gram-negative lactose-fermenting and lactose non-fermenting bacteria. Eosin methylene blue (EMB) (Oxoid Ltd, Detroit, MI, USA) was used to isolate *Escherichia coli*, and thiosulfate citrate bile salts sucrose (TCBS) agar (Oxoid) was used as a selective medium for *Vibrio* spp. Trypticase soy agar (TSA) (Oxoid) was used as a general purpose agar, and blood agar was used to demonstrate characteristics which included haemolysis. *Aeromonas* medium with ampicillin selective supplement (SR 136, 2.5 mg/500 ml agar), (Oxoid Ltd) was

used as a selective medium for *Aeromonas* spp. Cytophaga agar (CA) was a selective medium used to detect *Flavobacterium* spp., and Baird-Parker agar (BPA) (Oxoid) was used as a selective medium for *Staphylococcus* spp.

To isolate *Salmonella* spp. from water and fish, a procedure earlier described was used (Newaj-Fyzul et al., 2006a). Briefly, 5 ml of double strength selenite cysteine (SC) broth (Oxoid) and 5 ml of double strength brilliant green bile broth were each inoculated with 5 ml of the re-resuspended water samples. Inoculated tubes were incubated at 42°C for 48 h. The enrichment broths were then used to inoculate both brilliant green agar (BGA) and xylose lysine desoxycholate (XLD) agar that were incubated aerobically at 37°C and then examined after 24 and 48 h for presumptive *Salmonella* colonies (MacFaddin, 2002). Presumptive *Salmonella* colonies on BGA appeared red and turned the media around red while colonies on XLD were red with black centres.

Collection of fish samples

Healthy fish were randomly selected by netting five fish from each of the 15 ponds studied. Water from the pond together with the live fish was placed in sterile plastic bags, which were oxygenated by pumping oxygen from oxygen tanks into the bags. These bags were transported to the laboratory, at ambient temperature, within 1 h. All fish samples and separate water samples were taken from each pond at the same time. A total of 75 fish samples were processed altogether.

Isolation of other bacteria from fish

The procedure described by Newaj-Fyzul et al. (2006a) was used to process fish for bacteria. Briefly, all samples of fish collected were humanely killed using five times the anaesthetic dosage of tricaine methane sulphonate (MS222; Sigma, Basingstoke, UK) as recommended by Richards (1997). The external surface of each fish was decontaminated by dipping them into 70% ethyl alcohol for 2 min. The fish was then washed three times with sterile distilled water; care was taken to wash the gills with alcohol and sterile water. Washed fish were then aseptically dissected and samples of kidney, liver, heart, spleen, swim bladder and gills were taken. These tissue samples from each fish were homogenized into a slurry using a sterile mortar and pestle in sterile phosphate buffered saline (PBS) of pH 7.5 to achieve 10% w/v suspension of fish. A total of 75 fish samples from 15 ponds were processed in this manner.

The slurry from each fish was used to inoculate the same set of media, earlier mentioned for water samples, as was the procedure for detecting *Salmonella*. The isolation of the various bacteria followed the procedure described for water (American Public Health Association – APHA, 1998; MacFaddin, 2002).

Identification of bacterial isolates from fish and water samples

All colonies on non-selective and selective media were examined for colonial morphology, pigmentation, shape and haemolysis. Colonies representative of each type of bacterium were stained by Gram's Method and examined microscopically. All bacterial isolates were identified using standard methods (MacFaddin, 2002). The biochemical tests used included: oxidase, catalase, triple sugar iron (TSI) agar, sulphur indole motility (SIM), urea slants (U), citrate slants (C), methyl red (MR), oxidation–fermentation (OF), nitrate reduction (NR), motility (M) and gelatin liquefaction (GL). After inoculation, all tubes, with the exception of GL tubes, were incubated at 30°C for 48 h and read, in some cases after adding appropriate chemical reagents. For GL tubes, the GL tubes were placed in an ice bath each day for 2 h, and all tubes that remained liquid were recorded as positive. All negative tubes were returned to the incubator. This process was repeated for a period of 10 days. For presumptive *Salmonella* spp, the *O*-nitrophenyl-β-D-galactopyranoside (ONPG) test was performed to detect β-D-galactosidase activity and isolates biochemically identified as *Salmonella* were tested by the slide agglutination test using Difco *Salmonella* O Poly A-I and Vi Antisera (BD Diagnostic Systems). To differentiate *Vibrio* spp. from *Aeromonas* spp. and *Plesiomonas* spp., the O/129 sensitivity test was performed (MacFaddin, 2002).

Based on the series of biochemical tests used in the identification of the various groups of bacteria, representatives of each group were sent for confirmation to the Caribbean Epidemiology Centre (CAREC), Port of Spain, and the regional reference laboratory.

Determination of anti-biograms of bacterial isolates

Anti-microbial sensitivities of isolates were determined by the disc diffusion method using the National Committee for Clinical Laboratory Standards – NCCLS (2001) guidelines. For this investigation, on the basis of the reported implication of bacteria in fish and human diseases and their high prevalence compared with other bacteria isolated, only five genera of bacteria were tested for anti-microbial sensitivity. These genera were: *Aeromonas*, *Chromobacter*, *Enterobacter*, *Plesiomonas* and *Pseudomonas*. Eight anti-microbial agents were selected as representatives of the different classes of antibiotics used in aquaculture practice (Schmidt et al., 2000; Sorum and L'Abee-Lund,

2002). The anti-microbial agents (Oxoid Ltd) used and their concentrations were: ampicillin (10 μg), chloramphenicol (30 μg), norfloxacin (10 μg), oxytetracycline (30 μg), erythromycin (10 μg), nalidixic acid (30 μg), gentamicin (10 μg) and sulphamethoxazole/trimethoprim (25 μg). Briefly, to perform the test, isolated colonies were transferred to 10 ml of brain heart infusion (BHI; BD Diagnostic Systems) broth and standardized according to 0.5 McFarland turbidity standard. The isolate was plated onto Mueller Hinton agar (BD Diagnostic Systems) and the disc placed onto the inoculated agar after drying for 10 min at room temperature. All inoculated plates were incubated at 30°C for 24 h. The isolates were classified as sensitive or resistant using the criteria described by the disc manufacturer and according to NCCLS. Reference strains of *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, recommended by NCCLS, were used as controls each day testing was performed.

Statistical analysis

Statistical Package for Social Science (SPSS, Chicago, IL, USA) version 10 was used to process the data on the frequency and detection of the various bacteria from the various sources. The prevalence of resistance to the anti-microbial agents was compared using the chi-squared (χ^2) test and students *t*-test to determine statistically significant differences. All tests were two-tailed and tested at a level of 0.05 for significance.

Results

The pH of pond water, which ranged from 4.1 to 10.3, was significantly different ($P < 0.001$) across farms. Of the 15 ponds tested, 10 (66.7%), 9 (60.0%) and 8 (53.0%) exceeded the recommended limits of <0.01, <1.0, <0.03 and <0.2 ppm of un-ionized ammonia (NH_3), total ammonia (NH_4NH) un-ionized ammonium nitrogen (NH_3N) and nitrite (NO_2^-) respectively. The toxic, un-ionized ammonia (NH_3) content that ranged from 0.001 to 2.934 ppm was significantly ($P = 0.004$) different across ponds. The levels of nitrite (NO_2^-) in ponds were from 0.165 to 2.64 ppm, which were significantly different ($P < 0.001$; χ^2).

All water samples from the 15 ponds sampled were positive for total coliforms and the mean of the total coliform count (per 100 ml) of all ponds sampled was $9.3 \pm 1.6 \times 10^5$. The mean ± SD coliform count per 100 ml of water for farms I, II and III was $1.6 \pm 2.2 \times 10^5$, $3.3 \pm 3.9 \times 10^4$ and $8.6 \pm 9.5 \times 10^4$ respectively.

The frequency of isolation of aerobic bacteria from fish tissues and pond water are shown in Table 1. Overall, a total of 17 genera of bacteria were isolated from both fish and water samples but 13 and 16 genera were recovered from fish tissues and pond water respectively. In the 75 fish slurries processed, the predominant genera of bacteria isolated were *Pseudomonas* spp., 45 (60.0%), *Aeromonas* spp., 33 (44.0%) and *Chromobacter* spp., 27 (36.0%). The isolates recovered from 15 pond water samples were mainly *Bacillus* spp., 12 (80.0%), *Staphylococcus* spp., 9 (60.0%), *Aeromonas* spp., 9 (60.0%), *Alcaligenes* spp., 9 (60.0%). All samples were negative for S*almonella*.

Table 2 shows the frequency of resistance to anti-microbial agents amongst bacterial isolates from pond water. Of a total of 52 bacterial isolates belonging to five

Table 1. Frequency and mean counts of total coliform in pond water of Tilapia farms studied

Farm identification	No. pond water sampled	No. (%) pond water positive for total coliforms	Mean (±SD)[a] total coliform count per 100 ml
I	5	5 (100.0)	$1.6 \times 10^5 \pm 2.2 \times 10^5$
II	5	5 (100.0)	$3.3 \times 10^4 \pm 3.9 \times 10^4$
III	5	5 (100.0)	$8.6 \times 10^4 \pm 9.5 \times 10^4$

[a]Based on a total of 15 pond water samples positive for total coliforms; World Health Organization and US regulations recommend that water used in aquaculture contain $<5 \times 10^3$ coliforms per 100 ml of water.

Table 2. Frequency of aerobic bacteria in fish tissues slurry and pond water of Tilapia

	Source of isolates	
	Fish tissue	Pond water
Type of bacteria	No. (%)[a] positive	No. (%)[b] positive
Aeromonas spp.	33 (44.0)	9 (60.0)
Alcaligenes spp.	1 (1.3)	9 (60.0)
Acinobacter spp.	3 (4.0)	5 (33.3)
Bacillus spp.	11 (14.7)	12 (80.0)
Chromobacter spp.	27 (36.0)	6 (40.0)
Escherichia coli	7 (9.3)	1 (6.1)
Enterobacter spp.	16 (21.3)	6 (40.0)
Hafnia spp.	0 (0.0)	2 (13.3)
Micrococcus spp.	16 (21.3)	1 (6.1)
Necromonas spp.	3 (4.0)	0 (0.0)
Plesiomonas spp.	31 (41.3)	3 (20.0)
Pseudomonas spp.	45 (60.0)	8 (53.3)
Proteus spp.	0 (0.0)	4 (26.7)
Serratia spp.	4 (5.3)	4 (26.7)
Staphylococcus spp.	5 (6.7)	9 (60.0)
Citrobacter spp.	0 (0.0)	1 (6.1)
Flavobacterium spp.	0 (0.0)	8 (53.3)

[a]Based on a total of 75 tissue slurries of Tilapia processed.
[b]Based on a total of 25 pond water samples processed.

genera tested, 47 (90.4%) were resistant to one or more anti-microbials. The prevalence of resistance ranged from 75% (nine of 12) for *Chromobacter* isolates to 100% for *Enterobacter* (six of six), P*lesiomonas* (nine of nine) and *Pseudomonas* (eight of eight) isolates. The difference in prevalence of resistance to anti-microbials between the genera of bacteria was statistically significant ($P < 0.05$; χ^2).

Amongst all genera of bacteria, resistance was higher to ampicillin, 78.8% (41 of 52), erythromycin, 51.9% (27 of 52) and oxytetracycline, 34.5% (18 of 52) than to sulphamethoxazole/trimethoprim, 7.7% (four of 52) and norfloxacin, 3.8% (two of 52). The differences were statistically significant ($P < 0.05$; χ^2). The frequency of resistance to ampicillin, oxytetracycline, erythromycin and sulphamethoxazole/trimethoprim was significantly ($P < 0.05$; χ^2) different for the five genera of bacteria.

Of a total of 173 isolates from fish slurries, 168 (97.1%) were resistant to one or more anti-microbial (Table 3). For the five genera tested, the frequency of resistance ranged from 89.5% (17 of 19) for *Enterobacter* spp. to 100.0% (33 of 33) for *Pseudomonas* spp. The differences were statistically significant ($P < 0.05$; χ^2). Resistance was higher to ampicillin, 90.2% (158 of 173), erythromycin, 66.5% (115 of 173) and oxytetracycline, 52.6%, (91 of 173) than compared with chloramphenicol, 9.8% (17 of 173) and sulphamethoxazole/trimethoprim, 6.4% (11 of 173). The differences were statistically significant ($P < 0.05$; χ^2). The frequency of resistance to oxytetracycline, nalidixic acid and gentamycin was statistically significantly different ($P < 0.05$; χ^2) for the five genera of bacteria.

Overall, resistance to anti-microbial agents (ampicillin, oxytetracycline, erythromycin, nalidixic acid and gentamycin) was significantly higher ($P < 0.05$; χ^2) amongst fish slurry isolates compared with pond water isolates.

Discussion

The finding that all 15 ponds tested were contaminated by coliforms with counts that exceeded the limit of 5×10^3 per 100 ml for water used in aquaculture recommended by WHO (Mara and Cairncross, 1989; WHO, 1989) was not unexpected since these farmers fertilize their ponds with manure to enhance algal bloom. Furthermore, many of these ponds were further integrated with duck rearing.

In this study >50% of the ponds were found to be unfit for fish rearing based on their chemical parameters. Poor water quality is known to induce weakness in fish resulting in greater susceptibility to bacterial infection (Branson and Southgate, 1992; Bunch and Bejerano, 1997). The bacterial activity in pond water may also result in changes in chemical content of the water. Newaj-Fyzul et al. (2006b) had earlier reported that for the water samples from some tilapia ponds studied in 2003, 65.9% and 46.8% exceeded the recommended values of un-ionized ammonia and nitrite levels respectively. The possible effect of the toxic levels of these compounds on the health, growth rate, appearance, and reproductive rates of the tilapia reared in these ponds, is unknown. In many countries (Khalil and Hussein, 1997; Escher et al., 1999), the use of raw domestic sewage or effluent from treatment plants in fish farming has been advocated with the main objective being to promote primary and secondary productivity following fertilization of the ponds. The number of bacteria in the pond water has been documented to determine the presence and concentration of bacteria in fish. It has also been reported that for bacteria in pond water to appear in fish muscles, the 'threshold concentration' must be exceeded (Plumb, 1994; Escher et al., 1999). The exposure potential of fish reared in such ponds to bacterial pathogens cannot be over-emphasized (Tables 1 and 2).

Table 3. Frequency of resistance amongst bacterial isolates from pond water

Type of microorganism	No. isolates tested[a]	No. isolates resistant[b]	No. (%) isolates resistant to							
			AMP	OX	E	NA	C	SXT	CN	NOR
Aeromonas spp.	17	15 (88.2)	14 (82.3)	4 (23.5)	5 (29.4)	2 (11.8)	3 (17.6)	0 (0.0)	2 (11.8)	1 (5.9)
Chromobacter spp.	12	9 (75.0)	8 (66.7)	4 (33.3)	6 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter spp.	6	6 (100.0)	3 (50.0)	2 (33.3)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)
Plesiomonas spp.	9	9 (100.0)	8 (88.9)	2 (22.2)	5 (55.6)	1 (11.1)	2 (22.2)	1 (11.1)	2 (22.2)	0 (0.0)
Pseudomonas spp.	8	8 (100.0)	8 (100.0)	6 (75.0)	8 (100.0)	5 (62.5)	1 (12.5)	3 (37.5)	1 (12.5)	1 (12.5)
Total	52	47 (90.4)	41 (78.8)	18 (34.5)	27 (51.9)	8 (15.4)	6 (11.5)	4 (7.7)	6 (11.5)	2 (3.8)

AMP, ampicillin; OX, oxytetracyline; E, erythromycin; NA, nalidixic acid; C, chloramphenicol; SXT, sulphamethazole/trimethoprim; CN, gentamycin; NOR, norfloxacin.

[a]Recovered from 25 ponds containing Tilapia.

[b]Resistant to one or more of the eight anti-microbial agents tested.

Table 4. Frequency of resistance amongst bacterial isolates from tissues of Tilapia

Type of microorganism	No. isolates tested[a]	No. isolates resistant[b]	No. (%) isolates resistant to							
			AMP	OX	E	NA	C	SXT	CN	NOR
Aeromonas spp.	52	51 (98.1)	46 (88.5)	29 (55.8)	30 (57.7)	10 (19.2)	5 (9.6)	4 (7.7)	11 (21.2)	3 (5.8)
Chromobacter spp.	34	33 (97.1)	30 (88.2)	15 (44.1)	22 (64.7)	7 (20.6)	2 (8.8)	1 (2.9)	9 (26.5)	1 (2.9)
Enterobacter spp.	19	17 (89.5)	15 (78.9)	5 (26.3)	12 (63.2)	3 (15.8)	1 (5.3)	0 (0.0)	1 (5.3)	1 (5.3)
Plesiomonas spp.	35	34 (97.1 0	32 (91.4)	15 (42.9)	22 (62.9)	3 (8.6)	3 (8.6)	1 (2.9)	7 (20.0)	2 (5.7)
Pseudomonas spp.	33	33 (100.0)	33 (100)	27 (81.8)	29 (87.9)	23 (69.7)	6 (18.2)	5 (15.2)	24 (72.7)	11 (33.3)
Total	173	168 (97.1)	156 (90.2)	91 (52.6)	115 (66.5)	46 (26.6)	17 (9.8)	11 (6.4)	52 (30.10	18 (10.4)

AMP, ampicillin; OX, oxytetracyline; E, erythromycin; NA, nalidixic acid; C, chloramphenicol; SXT, sulphamethazole/trimethoprim; CN, gentamycin; NOR, norfloxacin.

[a]Recovered from 75 tissue slurries of Tilapia.

[b]Resistant to one or more of the eight anti-microbial agents tested.

Aeromonas spp., a known fish and human pathogen (Mateos et al., 1993; Thune et al., 1993; Austin and Adams, 1996; Huys et al., 1997; Camus et al., 1998), was recovered from 44.0% and 60.0% of fish slurry and pond water respectively. This indicates that should the fish carrying the pathogen or exposed to the organism in contaminated pond water be subjected to stress, that clinical disease may occur. The potential losses to the farmer, as well as the risk of fish-borne *Aeromonas* gastroenteritis in consumers of improperly cooked tilapia, exist. Gastroenteritis due to consumption of *Aeromonas*-contaminated fish and fish products have been reported elsewhere (Altwegg and Geiss, 1989; Altwegg et al., 1991).

Al-Harbi and Uddin (2003) reported 17 genera of bacteria isolated from tilapia during different seasons in Saudi Arabia. This number of genera is in agreement with the present study; however, the type and prevalence of organisms differ. Our study found 60% for *Pseudomonas* spp. while Al-Harbi and Uddin (2003), found 21.5% during summer months. In agreement with the present study is the finding of *Aeromonas* spp. as the most prevalent pathogen. Interestingly, our study found no *Vibrio* spp. and a prevalence of 14.7% and 80% of *Bacillus* sp. in the fish tissue slurry and pond water respectively. Al-Harbi and Uddin (2003) found 6.5% *Vibrio* spp., 2.4% and 1.5 8% *Bacillus* spp. in tilapia intestines during summer and winter respectively. Our findings of *Aeromonas* spp. and *Pseudomonas* spp. being the predominant bacterial genera in the tilapia fish are in agreement with the report of Sugita et al. (1982). However, our results differ from the findings of Chowdhury et al. (1989) who found the predominant bacteria recovered from tilapia intestine to be *Micrococcus*. Although there is a seasonal effect on bacterial presence as demonstrated by other researchers (Sugita et al., 1982; Sakata et al., 1984; Eves et al., 1995), in Trinidad there are no winter and summer months. Thus, there is less likely to be an effect on the microflora due to seasonality changes.

The potential for *Pseudomonas* spp. to be a pathogen of fish was demonstrated by Morinigo et al. (2003) who isolated the organism from 9.7% of diseased cultured sea bream (*Spatus aurata* L.) in Southwestern Spain. Other studies have documented *Pseudomonas* spp. as a fish pathogen (Garcia-Lopez et al., 1998; Garcia-Lopez et al., 2002) but in tilapia, as in other food fish, the microorganism could persist even after processing posing a health hazard in consumers. Lyhs et al. (1998) reported that pseudomonas was responsible for 15.3% of spoilage of preserved fish products, making the organism important in food spoilage with potential economic losses. Finally, just like *Aeromonas* spp., *Pseudomonas* spp., (Garcia-Lopez et al., 1998; Lyhs et al., 1998; Wong et al., 2000) may infect workers on fish farms especially if they are immuno-compromised or if their skin integument is compromised in any way. The high frequency of resistance by bacteria may result in therapeutic failure not only in sick fish, but also in people who may have become infected with pathogens from fish since plasmid transfer of resistance to more pathogenic bacteria may take place (Schmidt et al., 2000; Barat et al., 2002).

The significantly higher prevalence of resistance to anti-microbial agents in bacteria recovered from fish tissue may have public health consequences in consumers of food fish. There are numerous reports of people becoming infected by pathogenic organisms while handling fish and eating raw fish and shrimps (Altwegg and Geiss, 1989; Altwegg et al., 1991; Hassan et al., 1994; Garcia-Lopez et al., 2002).

The relatively high resistance of bacterial pathogens to anti-microbial agents in the current study agrees with the findings of Barat et al. (2002), who also found a high frequency of antibiotic resistance to gram-negative bacteria in

fish farms. The predominance of resistance to ampicillin (90.2%) observed for the five genera of bacteria isolated from fish slurry is also comparable to the findings by Barat et al. (2002) who found a prevalence of 93.4% resistance of gram-negative bacteria isolated from fish to ampicillin.

The present study found a resistance prevalence of 52% by pathogens to tetracycline which is higher than the 14% reported by Gofti-Urriza et al. (2000) but comparable with the 47% reported by Castro-Escarpulli et al. (2003) for isolates recovered from Tilapia (*Oreochromis niloticus niloticus*) intended for human consumption in Mexico. Our finding of 0–15% resistance of selected pathogens to trimethoprim/sulphamethaxazole (SXT) is much lower than the 25–67% resistance to SXT amongst *Aeromonas* spp. reported by Castro-Escarpulli et al. (2003). The fact that tetracycline and SXT are among the approved drugs for treating food fish is of concern when such a high level of resistance is encountered. These findings suggest either overuse of the anti-microbial agents or a new method of treatment is urgently needed in the food fish industry in Trinidad.

In conclusion, it is apparent that water in many of the ponds studied present unsuitable bacteriological and chemical environments for fish health, growth and reproduction. Furthermore, the data have demonstrated the presence of bacterial flora, including facultative pathogens, which under adverse environmental conditions could give rise to fish epidemics with potential economic consequences to the farmer. The public health significance of anti-microbial residues in food fish as well as the potential environmental health impact of pond water with exceedingly high coliform counts released to the rivers and other catchment areas cannot be ignored. Finally, the high prevalence of these zoonotic pathogens in food fish in the country has public health implications for consumers of food fish and workers in the fish industry.

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