Quantitative analysis of a fish pond for coliform bacterial content

Shagufta Andleeb*, Maria Maryam and Aqsa Aslam

Division of Science and Technology, University of Education, College Road Township Lahore (SA, AA); Department of Zoology, University of the Punjab, Lahore (MM), Pakistan.

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Abstract
A total of seventy five water samples were collected from three different levels: level 1 (surface), level 2 (sub-surface), and level 3 (bottom) of a fish pond and subjected to enumeration and isolation of colony forming units of coliform bacteria by growing on EMB agar. Highest number of CFUs/ml was obtained in the samples collected from level 3. Following pure culturing, each isolate was processed for colonial characteristics, biochemical characterization and staining reactions. These isolates exhibited gamma and beta hemolysis but none of them was found resistant against polymyxin B contrary to erythromycin. These isolates were identified as Klebsiella and Citrobacter and the latter genus dominant.

Key words: Enterobacteriacea, Klebsiella, Citrobacter, coliforms, polymyxin, erythromycin, fish pond

INTRODUCTION

Water pollution is one of the main causes which inhibit the proper growth of fish and ultimately decreases fish production (Benchalgo et al., 2014). The water sources are mostly contaminated with fecal wastes (Ponce-Terashima et al., 2014), which may cause various gastrointestinal diseases like diarrhea (Gruber et al., 2014) and even death (Atlas and Bertha, 1997). Evaluation of water quality has been conducted for a long time by measuring fecal coliforms such as Escherichia coli and Salmonella spp., etc. (Abdelzaher et al., 2010) that may cause water-borne diseases (Schets and de Roda Husman, 2014).

Bacterial as well as coliform count has been reported for pond water (Gogoi and Sharma, 2013). Fecal coliforms like Escherichia coli are markers of water quality (Leclerc et al., 2001). They have long been considered as an indicator of fecal contamination (McLellan and Eren, 2014). Enterobacteriacea is the most sensitive indicator of fecal pollution (Edberg et al., 2000) and is widely distributed in the intestines of humans and warm-blooded animals (Parveen et al., 1999). Klebsiellais another genus of family Enterobacteriacea. It is an important cause of nosocomial infections (Li et al., 2014). Another group of the family Enterobacteriacea is Citrobacter, causative agent of infections in the urinary tract, blood, superficial wounds, skin, peritoneum and several other normally sterile sites (Gupta et al., 2003a).

Rivers, beaches and ambient water may get contaminated with human waste (Yamahara et al., 2007; Gomi et al., 2014). Swimming at beaches polluted with human waste can cause diarrhea and eye, ear, skin and respiratory infections, and even hepatitis and meningitis. However, some studies were shown that fecal bacteria cannot be always correlated with pathogens in wastewater (Thompson et al., 2003) or environmental waters (McQuaig et al., 2006) which may reach groundwater and surface water via runoff (Ying et al., 2002).

Waterfowl are major excreters of fecal coliforms and Streptococci (Ashbolt et al., 2001). They harbour bacteria in their intestinal tract that are potential human pathogens like Salmonella and Campylobacter causing gastroenteritis (Timbury et al., 2002; Abulreesh et al., 2004). These organisms have also been found in the intestinal tract of ducks and geese (Refsum et al., 2002). It is known that fecal bacteria may contaminate fish (Cam et al., 2007) reflecting the bacteriological conditions of water (Bisht et al., 2014). Manure from livestock production is directly consumed by fish (Little...
and Edwards, 1999; Ahmed et al., 2011) as it contains almost all the essential nutrients (Jana et al., 2001), which stimulate the growth of planktons (Kadri and Emmanuel, 2003). Fish farming systems may introduce antimicrobials, their residues, and antimicrobial-resistant bacteria into fish ponds through animal manure (Petersen et al., 2002). Antimicrobial resistance has been emphasized in traditional fish farming systems (Schmidt et al., 2000); as residues of antimicrobials have been found in the sediments of marine fish farms too (Bjorklund et al., 1990).

This communication reports coliform content of University of the Punjab, Research Fish Farm.

MATERIALS AND METHODS

Seventy five samples were collected from the fish pond at The University of Punjab, near Zoology Department. Twenty five samples were collected randomly from level 1, level 2 and level 3 i.e from surface, sub surface and bottom zonations of the pond, respectively. The samples were processed within 1 hr of collection and 0.1ml of the original sample was spread over prepared EMB agar plates. Similarly a 100-fold dilution of each sample was also spread in the same way with subsequent incubation at 37°C for 48 hrs. The count was expressed as colony forming units (CFUs/ml). Well separated colonies were purified by alternative streaking on EMB agar and nutrient agar for five times. Mature colonies were observed for various characteristics like elevation, shape, color, size, consistency, elevation, motility and optical nature. Furthermore, each isolate was processed for Gram's and endospore staining in addition to various biochemical tests viz: catalase, indole, citrate utilization, oxidase, methyl red and VogesProskauer tests I & II and pathogenicity. The isolates were also assessed for drug resistance (Pelczar et al., 1986; Benson, 2001).

Statistical analysis

The data were analyzed by one way ANOVA using Microsoft Excel 2010.

RESULTS

Significant (P<0.05) higher coliforms' density was found in sample collected from level 3 than those of levels 1 and 2 with the mean values of 370,700 and 1050×10^2 CFUs/ml of the pond water, respectively (Fig 1). Colonies obtained of the isolates for samples collected from different levels were round, butterious and opaque. Isolates were identified as Citrobacter and Klebsiella. All species of Citrobacter were found oxidase and VogesProskauer -ve, whereas +ve for methyl red, catalase, indole and citrate. All the isolates of Klebsiella were +ve for citrate, catalase and VogesProskauer, giving –ve response for oxidase, methyl red and indole tests.

Table I: Colonial and biochemical characteristics of coliform bacteria isolated from egg shell surface

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genus</th>
<th>Size (mm)/Color</th>
<th>Elevation/ Motility</th>
<th>MR/ Citrate</th>
<th>Indole/ Oxidase</th>
<th>Catalase</th>
<th>VP-I/ II</th>
<th>Hemolysis</th>
<th>Antibiotic sensitivity test (D= mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PB</td>
</tr>
<tr>
<td>1</td>
<td>Citrobacter</td>
<td>3/ Purple</td>
<td>Raised/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-/-</td>
<td>γ</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>Citrobacter</td>
<td>2-3/ Blue</td>
<td>Raised/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-/-</td>
<td>β</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>Citrobacter</td>
<td>1.5/ Purple</td>
<td>Droplike/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-/-</td>
<td>γ</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella</td>
<td>3.5/ Orangish pink</td>
<td>Droplike/-</td>
<td>-/+</td>
<td>-/-</td>
<td>+</td>
<td>+/+</td>
<td>γ</td>
<td>I</td>
</tr>
<tr>
<td>5</td>
<td>Klebsiella</td>
<td>4/ Light purple</td>
<td>Droplike/-</td>
<td>-/+</td>
<td>-/-</td>
<td>+</td>
<td>+/+</td>
<td>B</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>Klebsiella</td>
<td>6/ Pink</td>
<td>Droplike/-</td>
<td>-/+</td>
<td>-/-</td>
<td>+</td>
<td>+/+</td>
<td>B</td>
<td>I</td>
</tr>
</tbody>
</table>

PB: Polymyxin B with disk potency of 300µg and E: Erythromycin with disk potency of 15µg (Kirby-Bauer method).
Gamma hemolysis was shown by the isolates 1, 2 and 3, whereas all other isolates gave beta hemolysis when grown on blood agar. None of the isolates showed resistance to polymyxin B, and only isolate 4 and 6 showed intermediate behavior. In case of erythromycin, the isolates 4 and 6 were found resistant, 1 and 3 gave intermediate response, whereas 2 and 5 were found sensitive (Table I). Contents of the culturable coliforms increased progressively from surface towards bottom of the fish pond (Fig. 1). *Citrobacter* was found dominant over *Klebsiella* at each level (Fig. 2).

**DISCUSSION**

In the present study, lowest number of CFUs/ml of original solution was found at level 1 as compared to levels 2 and 3 of the pond. The level 3 contained highest number of coliform (Fig. 1). As coliforms are facultative anaerobes, so it may be assumed that their number should be less at bottom of water body than its surface or sub surface part. But the reason for the highest number of coliforms at bottom of the pond in our experiment may be shallowness of pond. Kroger and Noll (1969) found *E. coli* and Gram’s negative rod shaped bacteria in tube well water due to the low depth of water source.

Another reason for the highest count at bottom may be that fecal coliforms, after settling down might had encountered a favorable environment for reproduction (Davies et al., 1995). The sediment’s surface area and nutrient content promote the growth of bacteria (Sherer et al., 1988). Fecal coliforms can survive for up to 60 days in freshwater sediments (Davies et al., 1995) and can persist for a long time under hot and dry summer range conditions (Okafo et al., 2003). As samples were collected following a rainy day, so it may be another reason. For the higher counts of coliform Qureshi and Dutka (1979) had declared that coliform population increases during monsoon months due to rain water. Kistemann et al. (2002) observed that in case of rainfall, the microbial loads of runoff water may suddenly increase and reach the lakes very quickly. As the sampling was done in summer, so it provided favorable condition for multiplication and growth of coliforms. As Badge and Varma (1982) had reported that multiplication of coliform is retarded at low temperature.

Different isolates obtained in the present study belonged to genus *Citrobacter* and *Klebsiella*. Although these genera are generally thought as non-fecal coliforms but have been reported from a treated sewage effluent with multiple drug resistance too (Silva et al., 2006). *Citrobacter* was found as the most dominant species in the present study (Fig 2). Daboor (2008) found *E. coli* as the most dominant bacterial species in most samples of fish farm. In another study *Salmonella* has been isolated from tannery polluted fish pond with higher number of total coliforms as compared to fresh water (Begum et al., 2007).

Birds visiting the ponds may be a source of fecal contamination. In fact magnitude and diversity of microbial load of a water bodies from many factors (Jones, 2002). Generally, it is believed that fecal coliforms are present only in feces of warm-blooded animals but it may be
spected that the two genera i.e., Klebsiella and Citrobacter isolated in the present study may be present in feces of cold-blooded fish inhabiting the fish pond; as several coliforms including fecal Citrobacter, Enterobacter and Klebsiella have been isolated by Harwood et al. (1999) from the fecal matter of fresh water turtle which is also a cold blooded animal.

Another source of contamination of pond may be the water entering the pond through water pipeline. A study done by Kirmeyer et al. (1999) showed that even post-treatment contamination may be detected in surrounding distribution system of pipelines. Enterobacter and Klebsiella have been found to multiply in the water mains and storage tanks under favourable environment (Edberg et al., 2000).

Polymyxin B showed excellent activity against Citrobacter (Table I). Comparable results have been documented in previous studies (Parchuri et al., 2005; Gales et al., 2006). Polymyxin B is considered as final treatment of many infections caused by multidrug resistant (MDR) Gram-negative bacteria, such as Pseudomonas, Acinetobacter and Klebsiella (Zavascki et al., 2007).

Klebsiella has been declared as ESBLs (extended spectrum beta-lactamase producer) thus as multidrug resistant (Polishko et al., 2011), and had been found resistant against many drugs (Gundogan et al., 2011) owing to a group of enzymes known as KPC (Miriagou et al., 2003) for inducing resistance in several pathogens (Smith et al., 2003; Bratu et al., 2005). Another enzyme NDM-1 carbapenemase has been held responsible to induce resistance in Enterobacteriacea against several groups of antibiotics in Pakistan, India and UK (Kumarasamy et al., 2010). Perhaps this is the reason that Klebsiella is β-hemolytic (Li et al., 2014). Erythromycin has been found as most effective against many Gram’s negative bacilli like Klebsiella and E.coli (Lorian and Sabath, 1970) as was also found in present study. Less resistant and sensitive response of Citrobacter was observed against erythromycin as compared to findings of Fass (1993), where none of the three drugs tested including erythromycin were found active against members of the family Enterobacteriaceae or nonfermentative gram-negative bacilli. Citrobacter has been reported as infectious agent in immunosuppressed host being opportunistic pathogen (Gupta et al., 2003b). Such pathogens in water may cause infections in fish too (Sanaa, 2009; Tetlock et al., 2012). Introduction of antimicrobial-resistant bacteria from animal manure seems to favor prevalence of antimicrobial-resistant bacteria in the pond environment (Petersen et al., 2002).

The present study suggests that fish reared in ponds receiving waste water passes danger of contaminated fish flesh with microorganisms pathogenic to humans as observed by Mandal et al. (2009). Fish ponds should have a proper boundary to check the approach of tetrapods. A proper netting above the pond may be used to avoid fecal contamination of visiting birds. Sterilization of feed/manure may minimize the chances of contamination. Equipping of ponds with emergency shades will certainly reduce the risks of introduction of pathogens via rain. Disinfection of pond water at regular time intervals and sanitization of water supply as well as drain will assure the decrease in level of contamination. Such practices must be considered compulsory in order to increase fish productivity and avoid post-harvest infections to the consumers.

REFERENCES


BACTERIA IN FISH POND


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