INCIDENCE OF ANTIBIOTIC RESISTANT BACTERIA IN FISH SOLD IN LOCAL MARKET IN DHAKA CITY


Department of Microbiology, Stamford University Bangladesh, Dhaka-1217, Bangladesh

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Present study was designed to evaluate the microbial contamination in fish (Olive Barb, Bele, Pale-Carplet, Pabo Catfish, Olive, Tangra, Gizzard, Nola, Tatkini, Corica Soborna) samples were collected from the several local market of Dhaka city, Bangladesh. Most of the fish samples were found to be contaminated with huge number of micro-biota within the range of $10^3$ to $10^6$ CFU/g. The maximum total viable bacterial (TVB) load was estimated observed in Pale Carplet (Amblyparyngodon mola) ($4.8 \times 10^6$ CFU/g) and the lowest count was found ($1.5 \times 10^3$ CFU/g) in tangra (Mystus spp.)

Escherichia coli and Klebsiella spp. were present in olive barb, bele, tangra and gizzard while Salmonella spp. and Vibrio spp. were present in olive barb, bele, tangra and gizzard fish samples. Moreover, most of the isolated bacteria exhibited resistance against maximum antibiotics like trimethoprime/sulfamethoxazole (25 μg), erythromycin (15 μg), amoxicillin (30 μg), Ceftriaxon (30 μg), ciprofloxacin (5 μg), streptomycin (10 μg), Ampicillin (10 μg). Microbial contamination in the fish samples especially those were resistant to drugs may pose a serious threat to public health.

Keywords: Food safety, Fish pathogen, Drug resistant, Consumer’s risk.

INTRODUCTION

Animals and humans have traditionally relied heavily on fish and fish products as a source of nutrition. Being the second-highest source of foreign exchange earnings and employing 10% of Bangladesh’s workforce directly or indirectly, the fisheries industry is extremely vital for the economy of Bangladesh (1-3). In addition, consumption of fish offers many health benefits alike expand life span of human being by preventing many diseases like breast cancer and heart diseases (1-4). However, the incidence of foodborne outbreaks brought on by microbial infection or intoxication associated with fish pathogens can pose a serious threat to public health (5-7).

Fish quality generally depends on the microbiological and chemical quality of environments where they grow up (6). Both opportunistic and pathogenic bacteria including Staphylococcus aureus, Salmonella spp., E. coli, fecal coliform and streptococci are directly responsible for different food borne outbreaks (8). As described in several studies that the overall environment of the local markets in Bangladesh is unhealthy. Due to the unhygienic processing and dirty storage condition the microbial contamination in fish is expanding (9, 10). However, in Bangladesh, limited research has been conducted on the microbiological quality of local fish, with a focus on identification of specific pathogens with their antibiotic resistance profiling. Therefore, present study was conducted to find out the overall microbial contamination in different fish samples as well as the drug susceptibility pattern of the isolated bacteria.

MATERIALS AND METHOD

Collection and preparation of samples: For the isolation of fish pathogens (10) fish samples (olive, barb, bele, pale-carplet, pabo catfish, olive, tangra, gizzard, nola, tatkini, corica soborna) were collected randomly from different local markets of Dhaka city within the time frame from August, 2022 to November, 2022. Samples were collected aseptically early in the morning and taken in sterile sample collection box with ice and transported immediately to the laboratory for further analysis (11). An amount of 25 g of each fish sample was cut out using a sterile knife then sterile mortar was used to grind the fish into small pieces, which then mixed with approximately 250 ml of normal saline. Each sample were diluted up to $10^6$ following the standard methods by adding a 1 ml aliquot of the crushed sample to 9 ml of normal saline. (11, 12, 13).

Microbiological analysis of each sample: A volume of 0.1 ml from $10^0$ dilutions of each sample suspension was spread onto nutrient agar (NA) and Sabouraud dextrose agar (SDA) with appropriate incubation period (NA at 37°C for 24 hours and SDA at 25°C for 48 hours) for the enumeration of total viable bacteria (TVB) and fungal count (TF), respectively.

Isolation pathogenic microorganisms: For the isolation of coliform bacteria (Escherichia coli, Klebsiella spp.), Pseudomonas spp. and Staphylococcus aureus, 0.1 ml of each sample from $10^0$ was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar, Pseudomonas agar (Oxoid Ltd., Basingstoke, Hampshire, England) and Mannitol Salt Agar (MSA) respectively. Further plates were incubated at 37°C for 24 hours to observe the results (12-15).

Isolation of Salmonella spp., Shigella spp. and Vibrio spp.: The in-vitro cultivation of the species of Salmonella, Shigella and Vibrio often appears difficult or with faulty results (false-negative) due to their viable but non-culturable (VBNC) attributes (16, 17). Therefore, enrichment technique was used prior to isolating these bacteria (12, 16, 17). Enrichment was performed for Salmonella spp. and Shigella spp. in the saline cystene broth (SCB). 1 ml of homogenized sample was transferred to SCB followed by incubation at 37°C for 4 hours and serial dilutions were made up to $10^6$, and from $10^4$ dilution 0.1 ml sample was spread onto Salmonella Shigella (SS) agar (Himedia, India) followed by the incubation at 37°C for 24 hours. For the enrichment of Vibrio spp., 0.1 ml of the homogenized sample was transferred to alkaline peptone water (APW) and incubated at 37°C for 4 hours and serially diluted to $10^6$ and from $10^4$ dilution, 0.1 ml was spread onto TCBS (Oxoid Ltd., Basingstoke, Hampshire, England) agar followed by the incubation at 37°C for 24 hours (16, 17).

*Corresponding Author: Kamal Kanta Das. Lecturer, Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh. E-mail: kkanta_36@yahoo.com or kkanta_36@stamforduniversity.edu.bd.
Determination of antibiotic susceptibility pattern of the isolates: Isolates were tested for antibiotic susceptibility on Mueller-Hinton agar (Difco, Detroit, MI) against trimethoprim/sulfamethoxazole (25 μg), erythromycin (15 μg), amoxicillin (30 μg), Ceftriaxone (30 μg), ciprofloxacin (5 μg), streptomycin (10 μg), Ampicillin (10 μg), by modified Kirby-Bauer method (12, 14, 19). In 2 ml of Mueller-Hinton broth, a single colony was injected (compared with 106 cells of McFarland standard) and it was then incubated at 37°C for 4 hours. The growing cultures turbidity was then adjusted to a 0.5 McFarland standard. After 15 minutes of incubation, a sterile cotton swab was inserted into the adjusted solution, the surplus broth was removed by forcefully pushing and spinning the swab against the tube's interior above the fluid level. To obtain homogenous inoculums, the swab was then uniformly dispersed across the whole surface of the MHA plate. The plates were left to dry for 10 to 15 minutes. Using sterile forceps, antibiotic-impregnated discs were subsequently placed onto the inoculated plate surface. All the plates were incubated at 37°C and examined the zone diameters to measure the susceptible, intermediate and resistant pattern of the isolates according to the CLSI guidelines, 2013 (12, 14, 19).

RESULTS AND DISCUSSION

Presence of microorganism in the of fish samples: In the present study, 10 fish samples were studied to isolate fish pathogens, where all samples were found to exhibit huge load of total viable bacteria within a range of 106 to 108 CFU/g (Table 1). Total fungi count was always found to be lower and out of 10 samples only 4 samples were contaminated with fungi (range of 103 CFU/g which exceeded the standard microbial limit according to the International Commission on Microbiological Specifications for food (ICMSF) (26, 27). The biochemical identification of the isolates from fish samples were presented in Table 2. In case of specific pathogens E. coli and Klebsiella spp. were found in 4 samples out of 10 and their count were up to 106 CFU/g. Presence of these groups of organisms indicated the faecal contamination though polluted water. Based on the results of the research, most of the local fish samples exceeded the IAMS (International Association of Microbiological Societies) limits for total coliform (100/g) and fecal coliform (11/g), which indicates that most of our fish markets sell low-quality fish (28).

The most alarming issue is Salmonella spp. was found in all fish samples collected from local market.

Pseudomonas spp. was found to be present in 6 samples within a range of 102 to 106 and Aeromonas spp. were found only in two samples. On the other hand, Vibrio spp. was predominant in most of the samples with in a range of 102 to 104 CFU/g (Table 1). Some the studies showed that they found the similar amount of microbial contamination in fish caused by the polluted external environment (6, 9).

The study by Nur et al., 2020, found that rawest fish samples were contaminated with TVB with the range of 103 to 106 CFU/g, Pseudomonas spp. was predominant (33). Similar results were reported by Hassan et al. 2013, Antony et al. 2002, and Novotny et al. 2004. A number of studies have also reported the presence of S. aureus (26, 29-30).

In the present study, fish samples exceed this limit for bacterial count, thereby, demonstrating a substantial risk on the public health. A period of time between harvesting and processing determines the quality of fish and fish products if these are not preserved properly. During this period, the quality of fish continues to deteriorate (30).

Moreover, aseptic handling and applying gutting as soon possible can prevent the growth of pathogenic bacteria and spoilage of fish (31, 32).

Antibiotic sensitivity pattern of bacteria:
Most of the isolates cultivated during the current investigation were found to be resistant against commonly used antibiotics (Table 3). The development of drug-resistance might be due to amazing genetic abilities of microbes, misuse of antibiotic and several epidemiologic influences (21-23). For fish borne disease outbreaks to be effectively managed, such resistance to drugs must be resolved (24, 25). Furthermore, with increased antibiotic resistance and their side effects, using herbal products to eliminate fish pathogens could be a safer, more cost-effective and more effective solution than antibiotics (24, 25).

Table 1: Microbial analysis of fish samples (CFU/g).

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</thead>
<tbody>
<tr>
<td>Olive Barb</td>
<td>1.2×10⁶</td>
<td>2.6×10⁴</td>
<td>3.0×10²</td>
<td>1.1×10³</td>
<td>4.5×10³</td>
<td>4.6×10³</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Bele</td>
<td>3.6×10⁶</td>
<td>4.2×10⁴</td>
<td>2.2×10²</td>
<td>0</td>
<td>9.3×10³</td>
<td>2.6×10³</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pale Carpet</td>
<td>4.8×10⁷</td>
<td>3.0×10⁴</td>
<td>0</td>
<td>3.6×10³</td>
<td>6.5×10⁴</td>
<td>9.8×10⁵</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pabo Catfish</td>
<td>1.2×10⁶</td>
<td>3.5×10³</td>
<td>0</td>
<td>1.4×10³</td>
<td>1.2×10³</td>
<td>2.5×10³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Olive</td>
<td>1.5×10⁵</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.5×10³</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tangra</td>
<td>1.5×10⁶</td>
<td>3.0×10³</td>
<td>0</td>
<td>1.0×10³</td>
<td>3.0×10³</td>
<td>3.0×10⁴</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gizzard</td>
<td>3.0×10⁶</td>
<td>3.0×10³</td>
<td>0</td>
<td>6.0×10³</td>
<td>0</td>
<td>6.0×10³</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nola</td>
<td>1.7×10⁷</td>
<td>0</td>
<td>0</td>
<td>TNTC</td>
<td>1.7×10³</td>
<td>TNTC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tatkin</td>
<td>2.0×10⁶</td>
<td>0</td>
<td>0</td>
<td>6.0×10³</td>
<td>8.6×10³</td>
<td>2.2×10³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corica Soborna</td>
<td>6.0×10⁶</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.5×10⁴</td>
<td>TNTC</td>
<td>-</td>
<td>-</td>
</tr>
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Note: IAMS limits (100/g) for total coliform and (11/g) for fecal coliform.


8979-8984.


CONCLUSIONS

The current study indicates that the experimental fish sold from the local market did not meet the requirements as most microbial loads were found to exceed the limit values. The presence of multidrug resistance traits among bacterial isolates has also accelerated the threat to public health. Given these results, this study recommended adherence to appropriate guidelines in order to preserve the microbiological quality of fish. To overcome this situation, it is necessary to improve practices for handling, freezing, post-harvest procedures, and storage, including cleaning and hygiene measures, must be applied to the catch. Additionally, appropriate training programs for fish farmers on fish management should be organized to reduce the risk of cross-contamination.

REFERENCE


Incidence of antibiotic resistant bacteria in fish

28. IAMS (International Association of Microbiological Societies), 1962. (www.microbial standard.com)