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Prevalence and Antimicrobial Resistance of bacterial genera *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp. from fish samples

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Abstract:

The prevalence of food-borne infections has grown worldwide and is still a significant public health issue. In the current investigation, the incidence of Salmonella spp., Staphylococcus aureus, and Escherichia coli were assessed from fish species Mystus seenghala as well as the antimicrobial susceptibility pattern against eight selected antibiotics. From 100 samples, a total of 53 E. coli bacteria, 37 S. aureus strains, and 10 Salmonella spp. strain were isolated and extensively described using the usual culture method and biochemical testing. The Kirby-Bauer disc diffusion technique was used to screen the isolates for antimicrobial resistance against 8 different antibiotics. According to an antibiogram research, S. aureus isolates had a high resistance to amoxicillin (89.19%), whereas E. coli isolates had a high resistance to cephazolin (68.67%), erythromycin (66.26%), and chloramphenicol (65.06%). Only one positive Salmonella species (2%), resistant to Amoxycillin, Penicillin and Ciprofloxacin, was found in the test. In order to ensure healthy fish, adequate staff hygiene is crucial while processing and handling fish. To prevent the creation of numerous antibiotic resistant types of bacteria, excessive usage of antibiotics should be avoided.

Keywords: Antibiotics, *Escherichia coli*, *Staphylococcus aureus, Salmonella* spp., *Mystus seenghala*, antimicrobial resistance.

Introduction:

Freshwater fish called *Mystus seenghala*, sometimes known as "sykes" because of its barbels, can be found in rivers in Pakistan, India, and Myanmar. It is very nutritious and has a great food value. This fish is referred to as *Mystus seenghala* because it possesses four pairs of barbels.

The bacteria associated with fish can be divided into three groups: pathogenic bacteria, opportunistic microorganisms, and members of the normal flora (Roy et al., 2017). The management of the fish, presence of pathogens, health state are some of the variables that determine the kind of microorganism present in the organism. Therefore, harmful microorganisms including *Staphylococcus aureus, Salmonella spp.,* and *Escherichia coli* that can cause diseases to humans by fish consumption might impair the development of fish. The symptoms of *E. coli* infections in humans include diarrhoea, nausea, vomiting and abdominal pain (Wyatt et al., 1979).

A vast range of disorders, including *salmonellosis*, which may affect both humans and fish species can be caused by *Salmonella spp*. This bacteria is found in gills and internal organs: liver, kidney, spleen, heart, head kidney and kidney of fish. The eyes of infected fishes became opaque and bloody fluid filled in the body cavity, catarrhal lesions and congestion is observed in the mucous membranes of the stomach and intestines.

Since antibiotics are crucial to treating the infection resulted from bacteria like *Escherichia coli, Staphylococcus aureus,* and *Salmonella* spp. the isolation of these bacterial species from infected fish and their antimicrobial susceptibility pattern against eight selected antibiotics were examined in this work.

Material & Methods:

The microbiological quality of freshwater fish species *Mystus seenghala* obtained from local retail fish shops in Indore city was recorded. A total of 30 healthy indigenous fish, including ranging in size from 90 to 250 grams. Cotton swaps from fish surfaces were transferred and cultured on different agar plates for microbiological detection for 24 hours at 37^{o} C under sterile circumstances (aerobic culture). Gram staining was performed on selected colonies, and were subjected to a biochemical test. Standard antibiotic discs of different antibiotics (BIOANALYS) were placed on top of the plates and incubated for 4 hours at 37°C using sterile swap (NCCLS procedures) A ruler was used to measure the diameter of the inhibitory zone.

Isolation of *Escherichia coli*

A loopful of inoculum was streaked onto Eosin Methylene Blue (EMB) agar medium (Hi-media, India) after enrichment in brilliant green broth and incubated for 24 hours at 37 ^{o}C. To create a pure culture with uniform colonies, the green metallic colony morphology with E. coli traits was

subcultured onto nutrient agar. For further examination, the pure isolates were stored in slant agar at 4 ^{o}C.

Isolation of *Staphylococcus aureus*

From enriched samples, a loopful of inoculum was scattered onto Vogel and Johnson (VJ) agar, where it was then incubated for 24 hours at 37 ^{o}C. Then, to promote the development of S. aureus, bacterium colonies on VJ agar were injected onto Mannitol Salt (MS) agar as a selective medium and incubated for 24 hours at 37 ^{o}C (Shareef et al., 2009; Hamad et al., 2012; Palanisamy and Bamaiyi 2015). For further examination, the pure isolates were stored in slant agar at 4 ^{o}C.

Isolation of *Salmonella* spp.

Swabs in Selenite Cystine Broth (Hi-media) were placed in bottles, which were then incubated aerobically for 24 hours at 37 ^{o}C. The selective medium, Xylose Lysine Deoxycholate agar (XLD) (Himedia), was then streaked with a loopful of each broth culture, and the plates were incubated at 37 ^{o}C for 24 hours. *Salmonella spp.* isolates with the usual XLD agar colony colour of pink to red with a black core were selected, subcultured on XLD agar to create pure cultures, and incubated at 37 ^{o}C for 24 hours. For further examination, the pure isolate was stored in slant agar at 4 ^{o}C. For further examination, the pure isolate was stored in slant agar at 4 ^{o}C. stain

Gram staining

Gram staining was used to determine the bacteria's morphology. The representative *E. coli*, *S. aureus* and *Salmonella* spp. colonies were characterized microscopically using Gram's stain according to the method described by James and Natalie (2005). Briefly, the cells were heated, fixed and the stain with the following procedures: Crystal Violet (1 min), Iodine-Lugol (1 min), Decolourization (1 min), and Safranin (1 min). Microscopic examination was done under microscope with high power objectives 100X using immersion oil. A microscopic inspection was performed using immersion oil and high power 100X lenses under a microscope.

Biochemical test

Escherichia coli and *Salmonella spp.* were identified using biochemical tests such as the catalase, oxidase, and triple sugar iron (TSI) tests, whereas *Staphylococcus aureus* was identified using the coagulase, oxidase, and catalase tests.

Test for antimicrobial sensitivity

All *E. coli, S. aureus,* and *Salmonella spp.* isolates' antibiotic sensitivity patterns were assessed using the standard disc diffusion method (Saifullah et al., 2016), and the results were classified

as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI, 2014). In Muller-Hinton agar, this antibiotic disc diffusion (CLSI 2014) test was conducted (Hi-Media, India). The isolates are tested for resistance to commonly used antibiotics against these, including erythromycin (15 mg), cephazolin (30 mg), gentamycin (10 mg), tetracycline (30 mg), doxycycline (30 mg), chloramphenicol (10 mg), ciprofloxacin (30 mg), sulfamethoxazole (30 mg), sulfisoxazole (25 mg), streptomycin (25 mg), and amikacin (30 mg) pathogenic bacteria. The plates were inoculated using a sterile swab. Swab was then gently dispersed from side to side over Muller Hinton (MH) agar (Hi-Media, India) plates to create homogenous inoculums. On the inoculated plates, antibiotic discs were aseptically positioned. Incubation took place at 37 °C with the plates inverted. The zone of inhibition diameters were measured 24 hours after incubation (mm).

Statistic evaluation

All information was entered into a Microsoft Excel 2016 file. *E. coli*, *S. aureus*, and *Salmonella spp.* infections were used as dependent variables in the study using the Chi square method for independent statistical analysis. P-values under 0.05 were taken into account for statistical significance.

Results:

Fig: Infected *Mystus seenghala*

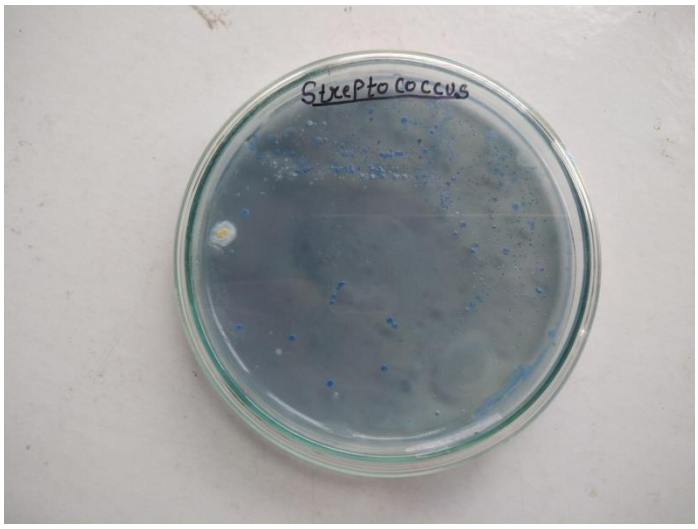

Fig: *Streptococcus* culture Plate

Prevalence of *Salmonella spp., E. coli, S. aureus*

The overall incidence of *E. coli, Salmonella spp., S. aureus* was 53%, 37% and 10% respectively.

Cultural, staining and motility characteristics

On EMB agar, typical E. coli colonies have a metallic sheen and are greenish black. Due to their capacity to convert tellurite to metallic tellurium, Salmonella spp. colonies on XLD agar appear as pink to red colonies with black centers, whereas *S. aureus* colonies appear as black, convex glossy colonies bordered by a yellow zone (Chong 2014).

Biochemical test

While all *S. aureus* isolates were catalase and coagulase positive but oxidase negative, all E. coli and Salmonella spp. isolates were catalase, TSI positive but oxidase negative.

Antibiogram profiles

Salmonella spp., E. coli, and S. aureus isolates from infected fish were tested for antibiotic sensitivity, and the results are displayed in Tables. For instance, Chloramphanicol (30 µg), the diameter of zone of inhibition is scaled as resistance (≤12), intermediate (13 to 17) and susceptible (≥18) in the antimicrobial disc used.

Table: Susceptibility pattern of all the isolate *Salmonella* to different antibiotics

Types of antibiotics	No. of positive *Salmonella* spp.		
	Resistance	**Intermediate**	**Susceptible**
Amoxycillin	(90%)	(10%)	(0%)
Penicillin	(90%)	(10%)	(0%)
Chloramphenicol	(8%)	(0%)	(92%)
Ciprofloxacin	(50%)	(50%)	(0%)

Salmonella

Presumably, the pathogen *Salmonella* had been derived from the aquarium water or from fish (carriers) (Kodama *et al.,* 1987). Lesions and congestions were recorded in the mucus membranes of the stomach and intestine of fish (Kodama *et al,* 1987). *Salmonellosis* infections are one of the most concerning in terms of human and animal health. These are brought on by a number of climatic factors that contribute to the organism's environmental expansion. Salmonella spp. were susceptible to ciprofloxacin and imipenem but resistant to tetracycline and erythromycin by Zihadi et al., 2018, but in the current study, Salmonella spp. were also found resistant to tetracycline. *Salmonella* was shown to be resistant to the antibiotics tetracycline, neomycin, ampicillin, and novobiocin in a research by Uddin et al., (2018) on chickens. According to Palanisamy and Bamaiyi's (2015) investigation, *Salmonella spp*. only exhibited antimicrobial resistance to ampicillin. However, only a small percentage of the Salmonella isolates included in this investigation were resistant to doxycycline, sulphamethoxazole, and chloramphenicol.

Table: Susceptibility pattern of all the isolate *E. coli* to different antibiotics

Types of antibiotics	**No. of positive *Escherichia coli***		
	Resistant	Intermediate	Susceptible
Amikacin	(0%)	(0%)	(100%)
Amoxicillin	(33.33%)	(7.69%)	(58.97%)
Cephazolin	(68.67%)	(28.20%)	(5.12%)

Chloramphenicol	(66.66%)	(0%)	(33.33%)

Escherichia coli

Escherichia coli is a significant bacteria responsible for food poisoning. It infects fish and show Clinical signs included haemorrhagic lesions on the skin, pale gills, digestive tract full of bloody exudate, haemorrhaging in the gonads, and yellow liver with hyperaemic areas (Aydin *et al* 1997). According to the results of the antibiotic susceptibility test, E. coli were extremely susceptible to amikacin (30 g), gentamycin (10 g), and doxycycline (30 g). Similar findings were seen with Malaysian farms, where *E. coli* isolates exhibit a range of 11–95% tetracycline and gentamicin resistance. According to other investigations, fish E. coli was extremely resistant to gentamicin, erythromycin, tetracycline, and chloramphenicol (Rahman et al., 2008). *Escherichia coli*. The antimicrobial resistance profile among *E. coli* isolates from fish and pond water in demonstrated varying levels of resistance against the thirteen antibiotics tested. The highest level of resistance was observed for erythromycin (fish: 98.7%) (Rita et al., 2022). From the antibiotic susceptibility test, it was observed that *E. coli* were highly susceptible to amikacin (30 µg), and Amoxicillin (30 µg). Highest resistant was shown for antiobiotic Cephazolin and Chloramphenicol in present study.

Table : Percentage of antibiotic sensitivity profile of *S. aureus* isolated from fish.

Antimicrobial class	**Antimicrobial Agent**	**No. of isolates (%)**		
		Resistance	Intermediate	Susceptible
β-lactams	Amoxicillin	(90%)	(2.70%)	(8.10%)
Cephalosporin	Cephazolin	(32.43%)	(18.91%)	(48)
Penicols	Chloramphanicol	(24.32%	(37.84%)	(64)
Quinolones	Ciproflaxacin	(2.70%	(5.41%	(91.89%)

Staphylococcus

The prevalence of *S. aureus* isolated in our investigation was comparable to that reported by Ganaie & Sharma (2022). Their results of the Antibiotic Sensitivity test were mixed. Most *Staphylococcus* species were resistant to Ampicillin (AM), but responsive to Ciprofloxacin 5g

(CIP) and Ofloxacin (OF), with varying susceptibility to other antibiotics such as chloramphenicol (30 g) (CL), polymaxin-300 (Poly), and CO-Trimaxazol-25g (CO- T).

In the current study, the pathogen *S. aureus* showed the variation in antibiotic sensitivity. There was a resistance of about 90% for antibiotic Amoxicillin. The pathogen was found most Susceptible to antibiotic Ciproflaxacin with a percentage of about 91.89%. For antibiotic Cephazolin, the Susceptibility was about 48% and for antibiotic Chloramphanicol the Susceptibility was about 64%. In order to manage and treat S. aureus infections, it is crucial to continuously monitor clinical isolates for antibiotic resistance since the organism has a predisposition to develop antimicrobial resistance.

Conclusion:

In conclusion, the bacterial pathogens *E. coli*, *S. aureus* and *Salmonella* spp. isolated from fish species *Mystus seenghala* show antimicrobial resistance towards a number of antibiotics tested in this study. Thus, careful and systematic usage of antibiotics in fish farms is important to reduce the emergence of resistanc strains bacteria.

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