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Research Article

Prevalence and Antimicrobial Susceptibility of Bacterial Organisms in Raw Fish of Pond and Retail Market

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Abstract | Current study aimed to explore the prevalence and antimicrobial susceptibility profile of bacterial organisms isolated from fish samples. A total of 120 fish samples were collected from fish retail market and ponds (60 from each source) comprising of three fish species i.e., *Labeo rohita* (LR; local name Rohu), *Catla catla* (CC; local name Thaila) and *Cirrhinus mrigala* (CM; local name Morakhi). The samples were processed for isolation of bacterial organisms using conventional culture method and isolates were tested for antimicrobial sensitivity according to CLSI method. According to results, the prevalence of bacterial organisms was high ($p < 0.05$) in CC than CM and LR. *E.coli* were the most common organism in market and pond fish samples with occurrence of 86.6% and 66.6% respectively. Next most prevalent pathogens were *Salmonella* spp. (78.3% & 58.3%), *Shigella flexneri* (61.6% & 53.3%), *Pseudomonas flavescens* (55% & 53.3%), *Pseudomonas aeruginosa* (40% & 35%), *Staphylococcus aureus* (33.3% & 25%), *Streptococcus agalactiae* (21.6% & 18.3%), *Klebsiella pneumoniae* (15% & 13.3%), *Staphylococcus epidermidis* (13.3% & 10%), *Klebsiella oxytoca* (10% & 8.3%), and *Streptococcus pyogenes* (6.6% & 1.6%) in market and pond fish samples respectively. According to antibiotic susceptibility tests, among 11 tested antimicrobials, *S. agalactiae* and *S. pyogenes* were found resistant to 9 antibiotics; followed by *P. aeruginosa* and *P. flavescens* 8; *S. flexneri* 7; *K. oxytoca* and *S. epidermidis* 6; and *E.coli*, *Salmonella* spp., *K. pneumoniae* and *S. aureus* 4 antibiotics. In conclusion, these results demonstrated that market fishes are more contaminated than pond fishes. The occurrence of bacterial organisms was highest in CC than CM and LR. Moreover, all bacterial isolates were recorded as multi drug resistant, they were found resistant to at least four antimicrobial agents.

Keywords | Antimicrobial resistant, pond, *Catla catla, Cirrhinus mrigala, Labeo rohita*

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INTRODUCTION

Fish and other aquatic animals are the principal origin of animal protein (FAO 2009, Hastein et al., 2006). These are economically cheap origin of protein that comes after poultry (Wafaa et al., 2011). Globally it consumes about 17% as animal protein (FAO, 2014). In 2017, globally fish and sea food products consumes about 17.1% as animal protein and their consumption comes after the cerals and milk and greater than bovine, poutry, pig meat and poultry eggs (FAO, 2022). In 2016, global fish production was 170.9 million tons from which 90.9 million tons from capturing and 80 million tons from aquculture (FAO, 2018).

During past few decades, fish and other aquaculture export of Pakistan have increased significantly, playing a considerable role to boost up the economy by creating job oppurtunities and earning for ex reserves (Memon, 2015). In Pakistan, *Catla catla* (Thaila), *Labeo rohita* (Rohu) and *Cirrihinus mirrigala* (Mrigal or Morakhi) are well-known fish species and used as major source of protein. These species preferred by the consumers due to their high quality (Sheikh et al., 2017). Unluckily, similar to other food items, fish and fish products may transmit food borne pathogens. Disease outbreaks caused by food borne pathogens causing serious health problems to customers (Rohde et al., 2015). Food borne pathogenic infections in humans are marked by a symptoms limiting from milder complications such as muscle aches, abdominal pain, fever, diarrhea, vomiting and headaches, to rigorous health troubles like autoimmune complications, miscarriage in pregnant, bloody meningitis, hemorrhagic colitis, septicemia, hemolytic uremic syndrome (HUS), enterotoxin poisoning and diarrhea (Doyle 2007; D'Aoust and Maurer, 2007). Food borne pathogens don't affect the specific population. Nevertheless, the populations at risk that are mainly affected by these pathogens are infants, immune compromised adults and pregnant women (Forsythe, 2010).

Foodborne pathogens present in fish are interrelated with the microbiological and environmental conditions of the water at the place of fishing, as the water pollution from agricultural, animal and human sources may affect microbiological quality of fish (Hosseini et al., 2004; Davies et al., 2001). Fish skin, gills and intestine are considered major source of microbial contamination because it have an average microbial load of 1.72 ± 0.68 × 10^8 to 7.00 ± 3.39 × 10^8 per gram (Mhango et al., 2010). The living environment (habitat) of fish is predominantly liable to contamination from household, agriculture and industrial liberations that includes chemicals, antibiotics, horomnes, pesticides etc, (Raufu et al., 2014). Skin and surface microflora of fish is affected by the water ecosystem including type and amount of other aquatic animals, nonliving material, as well as type and load of microorganisms present in water (Ribeiro et al., 2010).

Because of the stressful and unhygienic situations present in aquaculture facilities, the incidence of bacterial contamination in aquaculture fish is high, hence significant amounts of antibacterial agents are used in fish feed for curative and preventive purposes in aquaculture services all over the world (Sapkota et al., 2008). The extensive utilization of antibacterial componds in aquaculture leads to enhance the tolerance of bacterial strains to these chemicals. Such resistant bacterial organisms may have potential effects on the environment of the fish farms or on the fish and human diseases therapy (Smith et al., 1994). Keeping in view the above background, the current research was designed to isolate pathogenic bacterial organisms from common fish species including *Cirrhinus mrigala* (Morakhi), *Catla catla* (Thaila) and *Labeo rohita* (Rohu) from Dadu city of Sindh, Pakistan. Furthermore, study sorted out the antibacterial susceptibility profile of bacterial isolates of fish origin.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN AND COLLECTION OF SAMPLES

Fishes (n= 120) were collected from retail market and ponds of Dadu city, Sindh. Three fish species viz., *Labeo rohita* (Rohu), Thaila (*Catla catla*) and Morakhi (*Cirrhinus mrigala*) were used in the study. Each forty samples (20 from market fish and 20 from pond fish) were collected corresponding to each fish species and brought to the laboratory in cold storage at 4 °C. The fishes were slaughtered with sterilized knife, and to avoid the cross-contamination each fish was slaughtered distinctly. A 25g of fish meat was collected for bacteriological analysis and stored at -8°C until analyzed. All experimental procedure were carried out in line with animal ethics protocols and were approved by the Directorate of Advanced Studies, SAU, Tandojam (Approval No. DAS/2674 of 2019).

ISOLATION AND IDENTIFICATION OF BACTERIAL ORGANISMS

Fish carcass was used for the isolation and identification of bacteria using conventional culture technique (Baher and El-Said et al., 2022). Each fish sample was used to culture on bacteriological media under aseptic conditions. Before culturing 25g meat sample was mixed in the 225ml of peptone water and homogenized and incubated overnight at 37 °C. The samples were streaked on freshly prepared media (Oxoid, UK) including *Salmonella-Shigella* agar, MacConkey's agar, blood agar, etc, using sterilized wireloop. After streaking plates were incubated at 37°C for 24 hours in incubator to obtain bacterial growth. Suspected bacterial colonies were subcultured separately onto diverse bacteriological media under aseptic conditions and incubated for 24h at 37°C. Pure cultures were achieved accordingly. Morphological, cultural, biochemical and staining behaviors were noted to identify the bacterial species (Ansari et al., 2022).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Disc diffusion test was performed to check susceptibility and resistance of bacterial species to different antibiotics (Oxoid, UK) including oxytertracycline (30µg), cefoxitin (30µg), streptomycine (10µg), gentamycin (30µg), erythromycin (15µg), doxycycline (30µg), sulphamethxazole (25µg), bacitracin (10 units), ciprofloxacin (5µg), lincomycin (10µg), and norfloxacin (10µg). The test was performed following the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2011). 1000ml Muller Hinton agar was prepared and autoclaved at 121°C for 15 minutes. After cooling up to 45°C the medium was poured in Petri plates and allowed to solidify. Isolated colines from pure culture plates were inoculated into peptone water and kept at incubator for 24 hours. A sterile cotton swab was dipped in the peptone water and then spread over the surface of Muller Hinton Agar plate and covered completely. The antibiotic disc was placed over the surface of agar plate with the help of disc dispenser and slightly pressed with sterile forceps to make it fixed on the surface of the medium. The plates were then incubated for 24 hour at 37°C. After overnight incubation the diameter of zone of inhibition was measured and described as sensitive (S), intermediate (I) and resistance (R) (Sorour et al., 2022).

Statistical analysis

The data was entered into a computer database for calculation via Microsoft Excel (Microsoft Inc., USA). Prevalence of bacterial species in various types of fishes was compared by one way ANOVA. Level of antimicrobial resistance in bacterial isolates was compared by using JMP statistical Package Software (version 5.0.1.a SAS Institute Inc., Cary, N).

RESULTS

Number and percentage incidence of bacterial species in market fish samples

As shown in Table-1, among market fish samples load of bacterial contamination was recorded higher ($p < 0.05$) in *Catla catla* followed by *Cirrhinus mrigala* and *Labeo rohita.* In *Catla catla, E.coli* were found as most prevalent bacterial species (19/20; 95%) followed by *Salmonella* spp. (18/20; 90%), *Shigella flexneri* (16/20; 80%), *Pseudomonas flavescens* (10/20; 50%), *Pseudomonas aeruginosa/Staphylococcus aureus* (9/20; 45%), *Klebsiella pneumoniae* (5/20; 25%), *Streptococcus agalactiae/Klebsiella oxytoca* (4/20; 20%), *Staphylococcus epidermidis* (2/20; 10%) and *Streptococcus pyogenes* (1/20; 5%). Likewise, in *Cirrhinus mrigala, E.coli* were found as most contaminant organism (16/20; 80%), followed by *Salmonella* spp. (15/20; 75%), *Shigella flexneri/Pseudomonas flavescens* (12/20; 60%), *Pseudomonas aeruginosa/Staphylococcus aureus* (7/20; 35%), *Streptococcus agalactiae* (6/20; 30%), *Staphylococcus epidermidis* (5/20; 25%), *Streptococcus pyogenes* (3/20; 15%) and *Klebsiella pneumoniae/Klebsiella oxytoca* (2/20; 10%). Similar trend of bacterial contamination was observed in *Labeo rohita* i.e., *E.coli* (17/20; 85%) followed by *Salmonella* spp. (14/20; 70%), *Pseudomonas flavescens* (11/20; 55%), *Shigella flexneri* (9/20; 45%), *Pseudomonas aeruginosa* (8/20; 40%), *Staphylococcus aureus* (4/20; 20%), *Streptococcus agalactiae* (3/20; 15%), *Klebsiella pneumoniae* (2/20; 10%) and *Staphylococcus epidermidis* (1/20; 5%). However, there were no any *Streptococcus pyogenes* and *Klebsiella oxytoca* in *Labeo rohita.*

On overall basis *E.coli* were found as most prevalent bacterial organism in market fish samples i.e., 52/60 (86.6%) followed by *Salmonella* spp. (47/60; 78.33%), *Shigella flexneri* (37/60; 61.66%), *Pseudomonas flavescens* (33/60; 55%), *Pseudomonas aeruginosa* (24/60; 40%), *Staphylococcus aureus* (20/60; 33.3%), *Streptococcus agalactiae* (13/60; 21.6%), *Klebsiella pneumoniae* (9/60; 15%), *Staphylococcus epidermidis* (8/60; 13.3%), *Klebsiella oxytoca* (6/60; 10%) and *Streptococcus pyogenes* (4/60; 6.6%) (Table 1).

Number and percentage incidence of bacterial species in pond fish samples

Among pond fish samples, *Catla catla* exhibited higher ($p < 0.05$) microbial contamination followed by *Cirrhinus mrigala* and *Labeo rohita* (Table 2). In *Catla catla* fish, *E.coli* were observed as most prevalent bacterial organism with 80% (16/20) prevalence. It was followed by *Salmonella* spp. (15/20; 75%), *Shigella flexneri* (14/20; 70%), *Pseudomonas flavescens* (10/20; 50%), *Staphylococcus aureus* (8/20; 40%), *Pseudomonas aeruginosa* (7/20; 35%), *Streptococcus agalactiae* (4/20; 20%), *Klebsiella pneumoniae/Klebsiella oxytoca* (3/20; 15%) and *Staphylococcus epidermidis* (2/20; 10%). However, there was no any fish sample contaminated by *Streptococcus pyogenes* in *Catla catla*. Comparatively, in *Cirrhinus mrigala* fish, *E.coli* and *Pseudomonas flavescens* contamination was highest (13/20; 65%) than other bacterial species. It was followed by *Shigella flexneri* (10/20; 50%), *Salmonella* spp. (9/20; 45%), *Pseudomonas aeruginosa/Staphylococcus aureus/Streptococcus agalactiae* (5/20; 25%), *Staphylococcus epidermidis/Klebsiella pneumoniae* (3/20; 15%), *Klebsiella oxytoca* (2/20; 10%) and *Streptococcus pyogenes* (1/20; 5%). On the other hand, *Labeo rohita* fish highly laden with *E.coli* and *Salmonella* spp. (11/20; 55%), followed by *Pseudomonas flavescens/Pseudomonas aeruginosa* (9/20; 45%), *Shigella flexneri* (8/20; 40%), *Staphylococcus aureus/Streptococcus agalactiae/Klebsiella pneumoniae* (2/20; 10%) and *Staphylococcus epidermidis* (1/20; 5%). However, *Labeo rohita* was free from the *Klebsiella oxytoca* and *Streptococcus pyogenes* contamination (Table 2).

As shown in Table 2, on overall basis pond fish samples exhibited the highest microbial contamination of *E.coli* (40/60; 66.6%) followed by *Salmonella* spp. (35/60; 58.33%), *Shigella flexneri/Pseudomonas flavescens* (32/60; 53.3%), *Pseudomonas aeruginosa* (21/60; 35%), *Staphylococcus aureus* (15/60; 25%), *Streptococcus agalactiae* (11/60; 18.3%), *Klebsiella pneumoniae* (8/60; 13.3%), *Staphylococcus epidermidis* (6/60; 10%), *Klebsiella oxytoca* (5/60; 8.3%) and *Streptococcus pyogenes* (1/60; 1.6%).

Table 1: Number and percentage incidence of bacteria found in market fish samples.

Isolated bacteria	Fish type*			Overall positive number (%age)**
	Labeo rohita (Kurala)	*Catla catla* (Thaila)	*Cirrhinus mrigala* (Morakhi)	
	Positive number (%age)	Positive number (%age)	Positive number (%age)	
E.coli	17 (85)^{b}	19 (95)^{a}	16 (80)^{b}	52 (86.66)
Salmonella spp.	14(70)^{b}	18(90)^{a}	15(75)^{b}	47(78.33)
Shigella flexneri	9(45)^{b}	16(80)^{a}	12(60)^{b}	37(61.66)
Pseudomonas aeruginosa	8(40)^{a}	9(45)^{a}	7(35)^{ab}	24(40.00)
Pseudomonas flavescens	11(55)^{a}	10(50)^{ab}	12(60)^{a}	33(55.00)
Klebsiella pneumoniae	2(10)^{b}	5(25)^{a}	2(10)^{b}	9(15.00)
Klebsiella oxytoca	0(0)^{c}	4(20)^{a}	2(10)^{b}	6(10.00)
Staphylococcus aureus	4(20)^{c}	9(45)^{a}	7(35)^{b}	20(33.33)
Staphylococcus epidermidis	1(5)^{b}	2(10)^{b}	5(25)^{a}	8(13.33)
Streptococcus agalactiae	3(15)^{bc}	4(20)^{b}	6(30)^{a}	13(21.66)
Streptococcus pyogenes	0(0)^{b}	1(5)^{b}	3(15)^{a}	4(6.66)

* n= 20 for each fish type; ** total n= 60

a-c: different superscript in a row are significantly different at $p < 0.05$

Table 2: Number and percentage incidence of bacteria found in pond fish samples.

Isolated bacteria	Fish type*			Overall positive number (%age)**
	Labeo rohita (Kurala)	*Catla catla* (Thaila)	*Cirrhinus mrigala* (Morakhi)	
	Positive number (%age)	Positive number (%age)	Positive number (%age)	
E.coli	11 (55)^{c}	16 (80)^{a}	13(65)^{b}	40(66.66)
Salmonella spp.	11(55)^{b}	15(75)^{a}	9(45)^{c}	35(58.33)
Shigella flexneri	8(40)^{c}	14(70)^{a}	10(50)^{b}	32(53.33)
Pseudomonas aeruginosa	9(45)^{a}	7(35)^{b}	5(25)^{c}	21(35.00)
Pseudomonas flavescens	9(45)^{b}	10(50)^{b}	13(65)^{a}	32(53.33)
Klebsiella pneumoniae	2(10)	3(15)	3(15)	8(13.33)
Klebsiella oxytoca	0(0)^{b}	3(15)^{a}	2(10)^{a}	5(8.33)
Staphylococcus aureus	2(10)^{c}	8(40)^{a}	5(25)^{b}	15(25.00)
Staphylococcus epidermidis	1(5)^{ab}	2(10)^{a}	3(15)^{a}	6(10.00)
Streptococcus agalactiae	2(10)^{b}	4(20)^{a}	5(25)^{a}	11(18.33)
Streptococcus pyogenes	0(0)	0(0)	1(5)	1(1.66)

* n= 20 for each fish type; ** total n= 60

a-c: different superscript in a row are significantly different at $p < 0.05$

Antimicrobial susceptibility profile of bacteria isolated from fish samples

Antimicrobial susceptibility profile of bacterial organisms isolated from fish samples have been presented in Table 3 and 4. The results demonstrated that *E.coli* isolates of fish samples were found susceptible to ciprofloxacin, oxytetracycline, sulfamethoxazole-trimethoprim, norfloxacin and doxycycline (zone of inhibition 24.2, 24, 21.6, 20.8 and 20.1 mm respectively). While, *E.coli* were intermediate to gentamicin and streptomycin (zone of inhibition 19 and 14.6 mm respectively). However, it was observed that the *E.coli* were resistant to bacitracin, lincomycin, erythromycin and cefoxitin. Among these antimicrobials, only the cefoxitin exhibited a little zone of inhibition (8.4mm). *Salmonella* spp. were found susceptible to sulfamethoxazole-trimethoprim, norfloxacin, oxytetracycline and doxycycline (zone of inhibition 25.4, 22.9, 22.7 and 21.0 mm respectively); while it was intermediate to ciprofloxacin, gentamicin and

Table 3: Antibiotic susceptibility profile of bacteria isolated from fish samples*.

Bacterial isolate	Antibiotics (µg)										
	NOR (10)	SXT (25)	CIP (5)	CN (30)	DO (30)	S (10)	FOX (30)	B (10)	MY (10)	OT (30)	E (15)
E.coli	20.8 (S)	21.6(S)	24.2 (S)	19.0(I)	20.1 (S)	14.6(I)	8.4 (R)	0.0(R)	0.0(R)	24.0(S)	0.0(R)
Salmonella spp.	22.9(S)	25.4(S)	22.4(I)	18.8 (I)	21.0(S)	12.3 (I)	0.0(R)	0.0(R)	0.0(R)	22.7 (S)	0.0 (R)
Shigella flexneri	15.1(I)	0.0(R)	12.5 (R)	21.3 (S)	13.1 (I)	13.0(I)	0.0(R)	0.0(R)	0.0(R)	0.0(R)	0.0 (R)
Pseudomonas aeruginosa	0.0(R)	0.0 (R)	26.0 (S)	25.8 (S)	0.0 (R)	7.8(R)	0.0(R)	0.0(R)	5.8 (R)	23.0 (S)	5.2 (R)
Pseudomonas flavescens	0.0(R)	0.0 (R)	25.6 (S)	25.1 (S)	0.0(R)	7.3 (R)	0.0(R)	0.0(R)	6.2(R)	23.2 (S)	0.0 (R)
Klebsiella pneumoniae	0.0(R)	23.8 (S)	26.6 (S)	26.1 (S)	21.8 (S)	15.0(S)	0.0(R)	0.0(R)	21.6(S)	20.1(S)	4.0 (R)
Klebsiella oxytoca	0.0(R)	22.4 (S)	25.2 (S)	25.0(S)	23.6(S)	0.0(R)	0.0(R)	0.0(R)	22.5(S)	0.0(R)	8.0 (R)
Staphylococcus aureus	13.1 (I)	17.4 (S)	19.2(I)	25.4 (S)	22.0(S)	7.5 (R)	26.0(S)	0.0(R)	0.0 (R)	0.0(R)	21.6 (I)
Staphylococcus epidermidis	13.0(I)	0.0(R)	20.5(S)	22.3 (S)	20.8 (S)	7.6(R)	24.2 (R)	0.0(R)	6.2 (R)	0.0(R)	20.5 (I)
Streptococcus agalactiae	14.1 (I)	6.0(R)	12.0 (R)	23.3 (S)	12.0(R)	8.2 (R)	6.2 (R)	0.0(R)	0.0 (R)	0.0 (R)	0.0(R)
Streptococcus pyogenes	14.6(I)	0.0(R)	12.0 (R)	19.4(S)	12.0 (R)	9.6 (R)	6.0(R)	0.0(R)	0.0 (R)	0.0(R)	0.0(R)

* Zone of inhibition (mm) categorized as Susceptible (S), Intermediate (I) and Resistant (R) according to CLSI, 2012.
NOR: Norfloxacin; SXT: Sulfamethoxazole-trimethoprim; CIP: Ciprofloxacin; CN: Gentamicin; DO: Doxycycline; S: Streptomycin; FOX: Cefoxitin; B: Bacitracin; MY: Lincomycin; OT: Oxytetracycline; E: Erythromycin.

Table 4: Antimicrobials susceptible, intermediate and resistant to various bacterial isolates of fish samples.

Bacterial isolate	Susceptible	Intermediate	Resistant
E.coli	NOR, SXT, CIP, OT, DO	CN, S	FOX,B,MY,E
Salmonella spp.	NOR, SXT, OT, DO	CIP, CN, S	FOX,B,MY,E
Shigella flexneri	CN	NOR, DO, S	SXT, CIP, OT, FOX,B,MY,E
Pseudomonas aeruginosa	CIP, CN, OT	-	SXT, NOR, DO, S, FOX,B,MY,E
Pseudomonas flavescens	CIP, CN, OT	-	SXT, NOR, DO, S, FOX,B,MY,E
Klebsiella pneumoniae	SXT, CIP, CN, OT, DO,S,MY	-	FOX, NOR, B, E
Klebsiella oxytoca	SXT, CIP, CN, DO, MY	-	OT, S, FOX, NOR, B, E
Staphylococcus aureus	SXT, CN, DO, FOX,	NOR, CIP, E	OT, S, B, MY
Staphylococcus epidermidiss	CIP, CN, DO	NOR,E	SXT, FOX, OT, S, B, MY
Streptococcus agalactiae	CN	NOR	SXT, FOX, OT, S, B, MY, CIP, DO, E
Streptococcus pyogenes	CN	NOR	SXT, FOX, OT, S, B, MY, CIP, DO, E

NOR: Norfloxacin; SXT: Sulfamethoxazole-trimethoprim; CIP: Ciprofloxacin; CN: Gentamicin; DO: Doxycycline; S: Streptomycin; FOX: Cefoxitin; B: Bacitracin; MY: Lincomycin; OT: Oxytetracycline; E: Erythromycin.

streptomycin (zone of inhibition 22.4, 18.8 and 12.3 mm respectively). However, *Salmonella* spp. exhibited 100% resistant (no zone of inhibition) against bacitracin, lincomycin, erythromycin and cefoxitin. *Shigella flexneri* were found susceptible to gentamicin (zone of inhibition 21.3 mm) and intermediate to norfloxacin, doxycycline and streptomycin (zone of inhibition 15.1, 13.1 and 13.0 mm respectively). However, it was observed that the *Shigella flexneri* exhibited 100% resistant against oxytetracycline, sulfamethoxazole-trimethoprim, bacitracin, lincomycin, erythromycin and cefoxitin by showing 0 mm zone of inhibition, while ciprofloxacin formed a small zone (12.5 mm) that was within the limit of resistance. *Pseudomonas aeruginosa* and *Pseudomonas flavescens* both found susceptible to ciprofloxacin, gentamicin and oxytetracycline (zone of inhibition 26.0, 25.8, 23.0 and 25.6, 25.1, 23.2 mm respectively). While both were resistant to norfloxacin, doxycycline, streptomycin, sulfamethoxazole-trimethoprim, bacitracin, lincomycin, erythromycin and cefoxitin. They did not make the zone of inhibition except the streptomycin, lincomycin and erythromycin that exhibited smaller zones of inhibition which were within resistant limit. *Klebsiella pneumoniae* were found susceptible to ciprofloxacin, gentamicin, sulfamethoxazole-trimethoprim, doxycycline, lincomycin, oxytetracycline and streptomycin (zone of inhibition 26.6, 26.1, 23.8, 21.8, 21.6, 20.1 and 15.0 mm respectively); and were resistant to norfloxacin (0mm), erythromycin (4.0mm), cefoxitin (0mm) and bacitracin (0mm). *Klebsiella oxytoca* were found susceptible to ciprofloxacin, gentamicin, doxycycline, lincomycin and sulfamethoxazole-trimethoprim (zone of inhibition 25.2, 25.0, 23.6, 22.5 and 22.4 mm respectively). While it was resistant to oxytetracycline (0mm), streptomycin (0mm), norfloxacin (0mm), erythromycin (8.0mm), cefoxitin (0mm) and bacitracin (0mm). *Staphylococcus aureus* were found susceptible tocefoxitin, gentamicin, doxycycline and sulfamethoxazole-trimethoprim (zone of inhibition 26.0, 25.4, 22.0 and 17.4 mm respectively) and intermediate to erythromycin, ciprofloxacin and norfloxacin (zone of inhibition 21.6, 19.2 and 13.1 mm respectively), however, it was found resistant to lincomycin (0mm), oxytetracycline (0mm), streptomycin (7.5mm) and bacitracin (0mm). *Staphylococcus epidermidis* were found susceptible to gentamicin, doxycycline and ciprofloxacin (zone of inhibition 22.3, 20.8 and 20.5 mm respectively); intermediate to erythromycin (20.5mm) and norfloxacin (13.0mm), and resistant to lincomycin (0mm), oxytetracycline (0mm), streptomycin (7.6mm), bacitracin (0mm), sulfamethoxazole-trimethoprim (0mm) and cefoxitin (24.2mm). *Streptococcus agalactiae* were found susceptible to gentamicin (23.3 mm), while it was intermediate to norfloxacin (14.1mm). However, *Streptococcus agalactiae* were resistant to lincomycin, oxytetracycline, streptomycin, bacitracin, sulfamethoxazole-trimethoprim, cefoxitin, doxycycline, ciprofloxacin and erythromycin. There were no any zone of inhibition except ciprofloxacin, doxycycline, streptomycin, cefoxitin and sulfamethoxazole-trimethoprim that exhibited 12.0, 12.0, 8.2, 6.2 and 6.0 mm zones respectively. *Streptococcus pyogenes* were found susceptible to gentamicin (19.4 mm), intermediate to norfloxacin (14.6 mm) and resistant to lincomycin (0mm), oxytetracycline (0mm), streptomycin (9.6mm), bacitracin (0mm), sulfamethoxazole-trimethoprim (0mm), cefoxitin (6.0mm), doxycycline (12.0mm), ciprofloxacin (12.0mm) and erythromycin (0mm).

DISCUSSION

Many pathogenic bacteria including *Escherichia coli, Staphylococcus, Salmonella, Shigella* and *Pseudomonas* were commonly found in fish samples from the ponds and rivers linked with integrated farming systems. Their presence was accredited to the pollution of the fish ponds by livestock waste (Abdelhamid et al., 2006). The isolation of *E.coli, Shigella* and *Salmonella* from the fish samples shows faecal contamination of the ponds resulting from the farm animal manure that they add to the fish ponds as feed.

According to our results, the market and pond fish showed load of *E.coli* 86.6% and 66.6%, respectively. The present findings are varied with the observations of Dutta and Sengupta (2016) where the occurrence of *E.coli* was recoded 65% in fish and 85% in shrimps collected from retail market. The variation in *E.coli* occurance in above mentioned fish samples and our market samples might be occurred because of the environmental contamination or the unhygienic handling by the seller at the market level, as it is well known that poor handling of fishes at whole sell or retail level may increase their microbial contamination (Noor et al., 2021). High occurance of *E.coli* may be related to their resistance characters which help them to survive in harsh conditions (Saharan et al., 2020). However, (Saharan et al., 2020) observed the prevalence of *E.coli* 51% in feacal material of fish. (Udeze et al., 2012) stated that, *Klebsiella, Staphylococcus aureus* and *E.coli* are the microorganism from human origin and has been observed to live and grow in the gut and tissues of fish that cause the fish a major source of human disease for long periods of time.

On the basis of present findings, *Salmonella* spp. in market fish and pond fish were 78.3 and 58.3%, respectively. While the prevalence of *Shigella flexneri* was found 61.6%. These results are some what similar to the study of (Nilla et al., 2012) who reported the 75% prevalence of *Salmonella/Shigella* organisms in Mola fish. However, (Saharan et al., 2020) observed the 45% prevalence of *Salmonella* in feacal material of fish. *Salmonella* is not a part of normal microflora in fishes. Its' occurance in fish potentially reflects contaminated aquaculture environment and/or cross contamination during handling, storage or transportation (Fernandes et al., 2018), and could be present as feed contamint in feed of farm aniamls (Iqbal et al., 2023).

Mann and Wozniak. (2012) reported that the *Pseudomonas flavescens*is known as the major species of spoilage bacteria pervaling in animal and aquaculture products because of its short generation time, high activites of extracellular enzymes and resistant to heat treatment. The prevalence of *Pseudomonas flavescens* and *Pseudomonas aeruginosa* was found 55.0 and 40.0% in market fish, respectively. These observation are related to the research of Abd El Tawab et al. (2016) who found the prevalence of *Pseudomonas flavescens* and *Pseudomonas aeruginosa* 50% and 37.5% in the kidney and different organs (liver, kidney, gills and skin), respectively. Ikeagwu et al. (2008) reported that *Staphylococcus aureus* and *Staphylococcus epidermidis* are present in the gastrointestinal tract of fish because of contamination from the skin of individuals handling the fish culture. In current investigation, the prevalence of *Staphylococcus aureus* was 33.3% and 25.0% in market and pond fish, respectively. These results are agree with the observation of Jatt et al. (2019) who found the prevalence of *Staphylococcus aureus* 23.07% in *Cirrhinus mrigala* (fish). Likewise, Saharan et al. (2020) isolated *Staphylococcus aureus* 45% from feacal material of fish. The prevalence of *Streptococcus pyogenes* was 6.6% and 1.6% in market and pond fish. These findings are similar to the study of Chonoko et al. (2014) who found 09 positive samples among 300 samples of pond fish for *Streptococcus pyogenes*. Al-Imarah, (2008) reported the prevalence of *Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus epidermidis* and *Streptococcus pyogenes* as 39.99, 20, 19.99 and 13.325%, respectively.

Our study results shown that all bacterial isolates were multi resistant to antimicrobial agents. These results are in agrrement to previous reports that exhibited the prevalence of multidrug resistant pathogens in ornamental (Saengsitthisak et al., 2020) and retail (Basha et al., 2019) fishes. It is well established that continuous use of antibiotics in aquaculture cause a selective pressure on the microbial communities that lead to development of antimicrobial resistant starins that may form environmental reservoir (Preena et al., 2020). Fish farms and aquaculture sytems were recognized as 'hotspots' for antimicrobial resistant genes (Watts et al., 2017). The transfer of resistant organisms from aquaculture environment to natural aquatic environment may leads to development of antibacterial resistance in wild fishes and other marine life (Cizek et al., 2010). Our study have provided strong evidence that non-judicial use of antibiotics in developing countries like Pakistan lead to development of multidrug resistant organisms that had thoroughly spread in the environment of wild as well as farm animals in both land and water. This alaraming propagation of drug resistant strains in Pakistan in backyard and commercial poultry (Kamboh et al., 2018) and meat (Koondhar et al., 2021) has already been reported by our research group.

CONCLUSION

In present study, eleven bacterial species were isolated from both market and pond fish samples. Among these, *Escherichia coli* was most and *Streptococcus pyogenes* was least prevalent organism in fishes regardless of their source. It was also found that the prevalence of bacterial organisms was highest in *Catla catla* than *Cirrhinus mrigala* and *Labeo rohita.* In present research, it was also observed that all bacterial isolates were multi drug resistant. They were found resistant to at least four antmicrobials. Among the antibiotics used in this study, the most sensitive antibiotic against the bacterial isolates was gentamicin, while the least sensitive (100% resistant) antibiotic was bacitracin.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This is first study that have isolated pathogenic bacterial organisms from common fish species of Sindh province of Pakistan including Cirrhinus mrigala (local name Morakhi), Catla catla (local name Thaila) and Labeo rohita (local name Rohu). Moreover, the study reported the antibacterial susceptibility profile of bacterial isolates of fish origin.

AUTHORS CONTRIBUTIONS

Asghar Ali Kamboh conceptualized the study and design; Jameel-ur-Rehman performs the research; Ahmad Ali Moryani, Riaz Ahmed Leghari, Abdullah Sethar contributed in data analysis and manuscript writing; Abdul Ahad Soomro, Ali Raza Nizamani, Kanwar Kumar Malhi helped in interpretation of data analysis and proofread the manuscript.

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