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Prevalence of *Edwardsiella tarda* in commercially important finfish and shellfish of Bihar and West Bengal, India

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ABSTRACT

Objective: To study the prevalence of *Edwardsiella tarda* (*E. tarda*) in finfish and shellfish of West Bengal and Bihar, India and their antibiogram.

Methods: Fish samples were enriched overnight in *Edwardsiella ictaluri* broth and plated onto *Edwardsiella ictaluri* agar. Typical colonies were identified conventionally and by VITEK 2 compact system. Antibiogram was done by agar disc diffusion assay.

Results: Of the 118 fish samples screened, only 14.41% had *E. tarda*. The incidence was marginally high in wild fish (15.30%) than in cultured fish (13.26%). Maximum incidence was in intestine (12.98%) followed by gills (6.60%) and skin (2.38%). All *E. tarda* strains were sensitive to ciprofloxacin and exhibited varying degrees of resistance to other antibiotics. Multiple antibiotic resistance was seen in 84.00%–87.50% of the *E. tarda* strains. Majority of them had high minimal inhibitory concentration values (200 µg/mL) for oxytetracycline and gentamycin.

Conclusions: The results suggested that considerable proportions of commercially important finfish are carriers of multiple antibiotic resistance *E. tarda*. This calls for proper sanitary measures to eliminate this pathogen in fish and fishery products.

1. Introduction

Edwardsiella tarda (*E. tarda*) is a Gram-negative, motile, short, rod-shaped bacterium of 2–3 µm long and 1 µm in diameter. It is one of the members of Enterobacteriaceae family and biochemically similar to *Escherichia coli* with the exception that it produces hydrogen sulfide (H₂S). *E. tarda* infects reptiles, amphibians, mammals and fish[1,2]. The incidence, identification and pathology of *E. tarda* infection (Edwardsiellosis) in various fish have been reviewed[3-5]. In humans, gastrointestinal disease is the most frequently reported infection caused by *E. tarda*. It has been isolated from stool samples obtained from patients with diarrheal illness[6]. In some cases, it was also isolated from stool samples of patients without any symptoms of gastrointestinal illness[7]. Extraintestinal infection with *E. tarda* has been rarely reported and this includes manifestations like biliary tract infection, bacteraemia, skin and soft tissue infection, liver abscess, peritonitis, intra-abdominal abscess, tubo-ovarian abscess, endocarditis, empyema, hepatobiliary infection, osteomyelitis, urosepsis and meningitis[8]. Gastroenteritis

and extraintestinal infections by *E. tarda* were more common in children and adults, respectively[9]. Although these infections are uncommon, they can still be severe and fatal. A mortality rate of 40%–50% in patients with bacteremia, due to *E. tarda* infections has been reported[10]. Since *E. tarda* is pathogenic to fish and not to mention its zoonotic nature[1], the prevalence of *E. tarda* in commercially important fish species of cultured and wild environments is investigated in the present study.

2. Materials and methods

2.1. Fish samples

Thirty-five commercially important fish species from two states, namely, Bihar and West Bengal, India were examined for the prevalence of *E. tarda* between 2009 and 2013. A total of 280 samples comprising gills (106), skin (84), intestine (77), muscle (11) and kidney (2) from 118 individual finfish and shellfish such as *Anabas testudineus* (*A. testudineus*) (8), *Anguilla* spp. (2), *Catla catla* (10), *Channa marulius* (2), *Channa striatus* (*C. striatus*) (3), *Chitala chitala* (*C. chitala*) (1), *Cirrhinus mrigala* (2), *Cirrhinus reba* (1), *Clarias batrachus* (*C. batrachus*) (6), *Ctenopharyngodon idella* (1), *Gudusia chapra* (1), *Harpodon nehereus* (5), *Heteropneustes fossilis* (*H. fossilis*) (10), *Johnius* sp. (1), *Labeo bata* (2), *Labeo calbasu* (1), *Labeo rohita* (14), *Lates calcarifer* (3), *Macrobrachium rosenbergii* (5), *Mystus vittatus* (1), *Notopterus notopterus* (*N. notopterus*) (6), *Ompak pabda* (1),

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Oreochromis mossambicus (*O. mossambicus*) (4), *Oreochromis niloticus* (1), *Pampus argenteus* (*P. argenteus*) (2), *Pampus chinensis* (1), *Pangasius pangasius* (2), *Penaeus monodon* (6), *Polynemus paradiseus* (1), *Sardinella longiceps* (2), *Sperata aor* (1), *Sperata seenghala* (*S. seenghala*) (5), *Speratas vittatus* (1), *Tenualosa ilisha* (*T. ilisha*) (5) and *Xenontodon cancila* (1) were screened for the prevalence of *E. tarda*. The fish samples of Bihar were collected from retail fish markets of Pusa, Samastipur district and Motijheel, Muzaffarpur district. In West Bengal, the fish samples were collected from the whole sale fish market, Howrah, Howrah district and retail fish markets such as Sealdah, Kolkata district, Jaguli, Nadia district and Garia, Santoshpur and Diamond harbour, 24 Parganas (South) district. Prior to the collection, the source, condition, length and weight, whether cultured or wild, diseased or healthy, iced or fresh etc were recorded on the sampling sheet.

2.2. Isolation and identification of *E. tarda*

Edwardsiella ictaluri broth (EIB) was used as enrichment medium for the isolation of *E. tarda*[11]. Briefly, samples of intestine, muscle, gill and kidney, and skin swabs of 5 cm² or 10 cm² were aseptically collected from the healthy/iced/fresh fish from different sampling areas and transferred into the tubes containing 10 mL sterile EIB. Samples of whole fish were also collected, packed individually in polythene bag and brought to the laboratory in iced condition in an insulated container within 2–3 h or 16 h of collection from Bihar and processed as above. All the inoculated tubes containing EIB were incubated in an incubator overnight at (30 ± 2) °C. Loopful of inocula from each tube were then streaked onto *Edwardsiella ictaluri* agar (EIA) plates with the help of sterile inoculation loop and incubated upright at (30 ± 2) °C for 24–48 h. On EIA, both *Edwardsiella ictaluri* and *E. tarda* produced 0.5–1.0 mm green translucent colonies after 48 h[11]. Typical and distinct green translucent colonies of 0.5–1.0 mm size were picked randomly from EIA plates, purified by repeated streaking on trypticase soy agar (TSA) plates and maintained on TSA slants. A series of biochemical reactions as described by Collins *et al.* were performed[12]. Taxonomic keys proposed by University of Idaho, USA[13] and Fisheries and Oceans Canada[14] were followed for the presumptive identification of *E. tarda*. Few isolates were subjected to confirmative identification by automated bacterial identification system, VITEK 2 compact (BioMerieux, France).

2.3. Antibiotic sensitivity testing by agar disc diffusion assay

A total of 33 strains of *E. tarda* were screened for their sensitivity to six antibiotics by agar disc diffusion method[12]. The antibiotic impregnated discs (HiMedia, Mumbai) and their concentration used in the present study included chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg) and co-trimoxazole (25 µg). Young cultures of *E. tarda* (20 h old) from TSA slants were inoculated into trypticase soy broth and

incubated for 10–12 h at (30 ± 2) °C. Inocula from these 10–12 h grown cultures were taken separately using sterile cotton swabs and spread onto antibiotic sensitive assay medium No. 37 supplemented with 1.5% agar. Antibiotic impregnated discs were placed aseptically onto the inoculated agar plates at least 15 mm away from the edge, at equal distance and sufficiently separated from each other to avoid overlapping of the zone of inhibition. The plates were then incubated for 24 h at (30 ± 2) °C and the diameter of zone of inhibition in mm was measured. Interpretation of sensitivity was based on the zone size interpretation chart provided by the manufacturer of the antibiotic impregnated discs.

2.4. Determination of minimal inhibitory concentration (MIC) of antibiotics

The MIC of five antibiotics was determined against 33 *E. tarda* strains following the agar dilution method[12]. The details of commercial antibiotics and the solvents used were presented in Table 1. Each antibiotic was initially dissolved in appropriate solvent at concentrations 100 times higher than the most concentrated level to be tested. Further dilutions were made in physiological saline (0.85% NaCl). Various concentrations (µg/mL) tried for the MIC determinations were 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.2, 0.1 and 0.05. Antibiotic-agar plates were prepared by incorporating appropriate concentration of respective antibiotic into the antibiotic sensitive assay medium No. 37 supplemented with 1.5% agar, mixed and poured into sterile Petri plates. The agar plates with or without antibiotics at varying concentrations were spot inoculated with 1 µL (~10⁴–10⁵ cells) from 10–12 h old cultures, grown on trypticase soy broth at (30 ± 2) °C. The plates were incubated upright at (30 ± 2) °C for 24 h and observed for growth or no growth. The MIC was determined as the minimal concentration (µg/mL) showing no growth at 24 h. The *Chi-square* (χ^2) test was followed to determine the significance of differences in the incidence of *E. tarda* in fish samples and antibiotic resistance pattern[15].

3. Results

The incidence rate of *E. tarda* in fish samples of West Bengal and Bihar, cultured and wild fish, finfish and shellfish, different fish families, different organs of fish and different districts of West Bengal and Bihar were depicted in Figures 1–6, respectively. Only 14.41% of the 118 different freshwater, marine and brackishwater fish had *E. tarda*. Of the 35 different fish species screened, only 10 fish species, viz., *A. testudineus*, *C. striatus*, *C. chitala*, *C. batrachus*, *H. fossilis*, *N. notopterus*, *O. mossambicus*, *P. argenteus*, *S. seenghala* and *T. ilisha* were positive for *E. tarda*. The results of *E. tarda* identification by VITEK 2 compact (BioMerieux, France) system were presented in Table 2. Representative isolates were confirmed as *E. tarda* with 94%–98% probability. Nevertheless, minor discrepancies were noticed among the tested *E. tarda* strains in citrate utilization, urease reaction, H₂S production, tyrosine arylamidase, L-lactate alkalisation and succinate alkalisation.

Table 1

Details of commercial antibiotics and solvents used in the MIC study.

Antibiotics	Product name	Concentration	Company	Solvent
Chloramphenicol	Paraxin	500 mg/capsule	Nicholas Piramal India Ltd., Himachal Pradesh, India	Ethanol 1:10 dilution
Ciprofloxacin	Cipla	500 mg/tablet	Cipla Ltd., Mumbai, India	0.01 mol/L hydro-chloric acid
Co-trimoxazole	Sepmax	800 mg/tablet	Glaxo SmithKline, Mumbai, India	0.125 mol/L sodium hydroxide
Gentamycin	Genticin	40 mg/mL	Nicholas Piramal India Ltd., Madhya Pradesh, India	Physiological saline
Oxytetracycline	Terramycin	250 mg/capsule	Pfizer, Bangalore, India	Physiological saline

Table 2

Biochemical characteristics of representative *E. tarda* strains ($n = 8$) as assessed by VITEK 2 compact system (Biomerieux, France).

Characteristics	Reaction
D-Glucose	+
Saccharose/Sucrose	-
D-Cellobiose	-
Adonitol	-
L-Arabitol	-
D-Mannitol	-
D-Sorbitol	-
D-Maltose	+
D-Mannose	+
D-Tagatose	-
D-Trehalose	-
H ₂ S production	(+) ^c
Lysine decarboxylase	+
Ornithine decarboxylase	+
L-Malate assimilation	-
Citrate (sodium)	(-) ^d
Urease	(-) ^d
Fermentation/Glucose	+
Phosphatase	+
Beta-glucuronidase	-
Lipase	-
Alpha-glucosidase	-
Beta-xylosidase	-
Beta-galactosidase	-
Ala-Phe-Pro-arylamidase	-
L-Pyrrolidonyl arylamidase	-
Beta-N-acetylglucosaminidase	+
Glutamyl arylamidase pNA	-
Gamma-glutamyl transferase	-
Beta-alanine arylamidase pNA	-
L-Proline arylamidase	-
Tyrosine arylamidase	(-) ^c
Beta-N-acetyl-galactosaminidase	-
Glycine arylamidase	-
Glu-Gly-Arg-arylamidase	-
Ellman	+
L-Lactate assimilation	-
L-Histidine assimilation	-
5-Keto D-gluconate	-
Palatinose	-
Malonate	-
L-Lactate alkalisation	(-) ^a
Succinate alkalisation	(+) ^b
Alpha-galactosidase	-
Beta-glucosidase	-
Coumarate	+
O/129 Resistance	(+) ^b

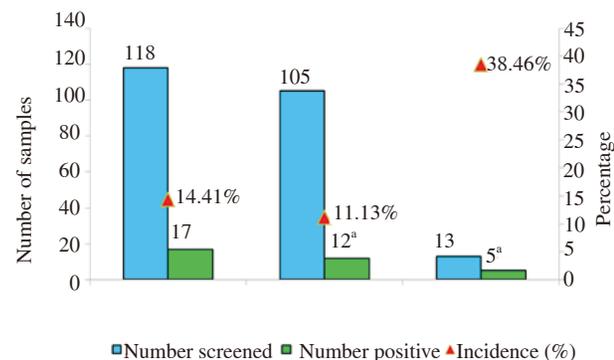
^a: Two isolates were positive; ^b: Two isolates were negative; ^c: Three isolates were positive; ^d: One isolate was positive; ^e: One isolate was negative.

Table 3

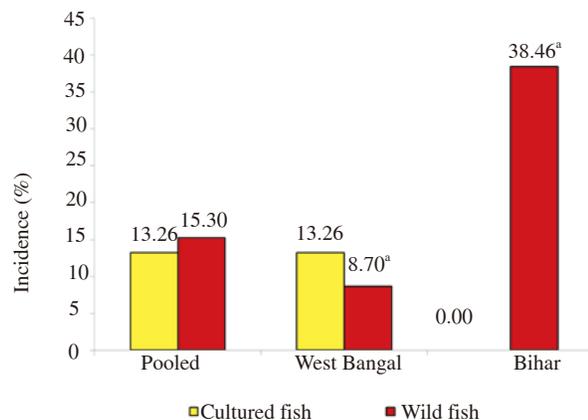
The ranges of MIC of antibiotics against *E. tarda* strains.

Source region and fish species ($N = 33$)	MIC of antibiotics ($\mu\text{g/mL}$)				
	Chloramphenicol	Oxytetracycline	Gentamycin	Ciprofloxacin	Co-trimoxazole
West Bengal (25)	3.13–25.00	12.50–>200.00	6.25–200.00	0.20–0.39	3.13–50.00
Bihar (8)	3.13–12.50	12.50–>200.00	6.25–100.00	0.20–0.39	3.13–6.25
<i>H. fossilis</i> (14)	3.13–25.00	50.00–>200.00	6.25–200.00	0.20–0.39	3.13–50.00
<i>T. ilisha</i> (7)	3.13	12.50–>200.00	6.25–200.00	0.20–0.39	3.13–12.50
<i>C. striatus</i> (2)	6.25–12.50	50.00–>200.00	12.50–100.00	0.20–0.39	6.25
<i>C. batrachus</i> (1)	3.13	> 200.00	50.00	0.39	3.13
<i>C. chitala</i> (1)	6.25	> 200.00	50.00	0.20	6.25
<i>A. testudineus</i> (2)	3.13	> 200.00	6.25–100.00	0.20–0.39	3.13–6.25
<i>O. mossambicus</i> (2)	12.50–25.00	50.00–200.00	6.25–100.00	0.20	12.50
<i>S. seenghala</i> (2)	3.13–6.25	12.50–200.00	50.00	0.39	6.25–50.00
<i>P. argenteus</i> (1)	6.25	200.00	25.00	0.20	6.25
<i>N. notopterus</i> (1)	3.13	> 200.00	50.00	0.39	3.13

Antibiotic resistance in *E. tarda* strains of West Bengal and Bihar were presented in Figure 7. The *E. tarda* strains of the present study exhibited varying degrees of resistance antibiotics. Most of the *E. tarda* strains were resistant to oxytetracycline (69.70%) followed by nitrofurantoin (66.70%), gentamycin (54.50%) and co-trimoxazole (18.20%). The ranges of MIC of antibiotics ($\mu\text{g/mL}$) against *E. tarda* strains from different regions and fish species were presented in Table 3. Majority of the strains screened had high MICs (200 $\mu\text{g/mL}$) to oxytetracycline and gentamycin and low MICs to ciprofloxacin (0.20–0.39 $\mu\text{g/mL}$). The MICs of chloramphenicol and co-trimoxazole ranged from 3.13 to 25.00 $\mu\text{g/mL}$ and 3.13 to 50.00 $\mu\text{g/mL}$, respectively.

**Figure 1.** Incidence of *E. tarda* in West Bengal and Bihar fish samples.

^a: Bars sharing common superscript are significantly different ($P < 0.008$).

**Figure 2.** Incidence of *E. tarda* in cultured and wild fish of West Bengal and Bihar.

^a: Bars sharing common superscript are significantly different ($P < 0.008$).

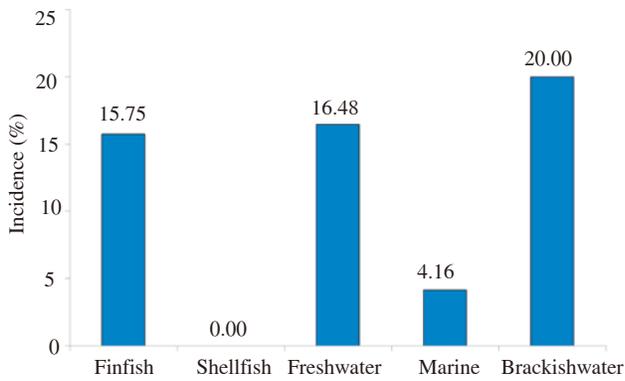


Figure 3. Incidence of *E. tarda* in finfish and shellfish and from different habitats.

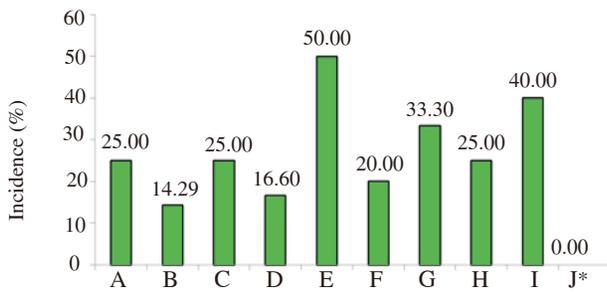


Figure 4. Incidence of *E. tarda* in different fish families.
*: Others include Cyprinidae, Pangasiidae, Polynemidae, Anguillidae, Belonidae, Latidae, Siluridae and Harpodontidae. A: Notopteridae; B: Clupeidae; C: Bagridae; D: Clariidae; E: Heteropneustidae; F: Channidae; G: Stromateidae; H: Anabantidae; I: Cichlidae; J: Others.

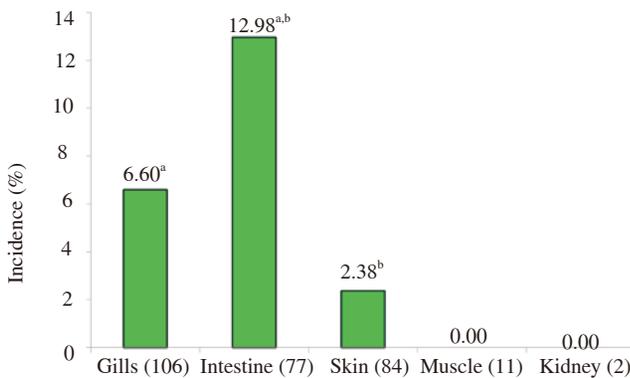


Figure 5. Incidence of *E. tarda* in different organs of fish.
^{a, b}: Bars sharing common superscripts are significantly different ($P < 0.05$).

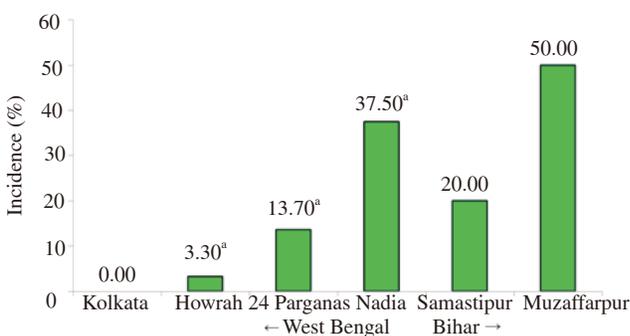


Figure 6. Incidence of *E. tarda* in fish from different districts of West Bengal and Bihar.
^a: Bars sharing common superscripts are significantly different ($P < 0.05$).

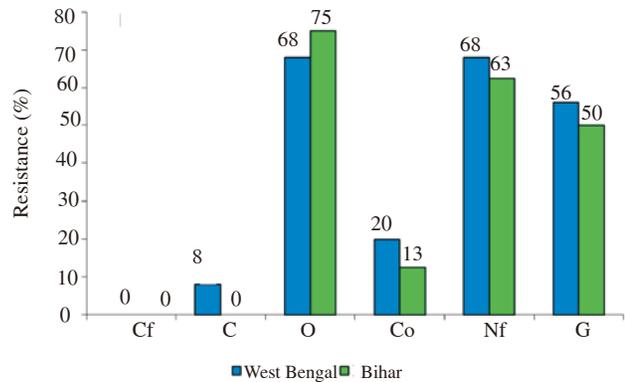


Figure 7. Antibiotic resistance in *E. tarda* strains of West Bengal and Bihar.

Cf: Ciprofloxacin (5 µg); C: Chloramphenicol (30 µg); O: Oxytetracycline (30 µg); Co: Co-trimoxazole (25 µg); Nf: Nitrofurantoin (300 µg); G: Gentamycin (10 µg).

4. Discussion

Based on the medium formulated for the isolation of *E. tarda*[11], which was effective in recovering even two *E. tarda* cells/mL in 24 h, 280 samples from 118 fish were enriched in EIB overnight and subsequently plated onto EIA. The typical green translucent bacterial colonies with 0.5–1.0 mm size on EIA were picked, purified and identified presumptively as *E. tarda*. It was found that 17 (14.41%) of the 118 different freshwater, marine and brackishwater fish were positive of *E. tarda*. The incidence rate was marginally high in wild fish (15.30%) than in cultured fish (13.26%) and the differences were insignificant ($P > 0.05$). In West Bengal, its prevalence was 11.13% (Figure 1). The fish were mostly from aquaculture environment, except the marine fish and the prevalence of *E. tarda* was high in cultured fish (13.26%) than the wild fish (8.70%). In Bihar, the *E. tarda* incidence rate was 38.46% and all fish screened were of feral stocks (Figure 2). There existed significant difference ($P < 0.008$) in the incidence rate of *E. tarda* between Bihar and West Bengal fish. *E. tarda* has been reported from a variety of cultured fish in Asia, USA and other parts of world[1,3,5,16]. As shown in Figure 3, the incidence of *E. tarda* in finfish was 15.75%. None of the shellfish screened recorded *E. tarda*. Occurrence of *E. tarda* in aquatic invertebrates was documented earlier[5]. When pooled according to the habitat, maximum incidence was found in brackishwater fish (20.00%), followed by freshwater (16.48%) and marine (4.16%) fish. The results corroborate earlier studies[16,17], who reported that *E. tarda* is capable of adapting to survive within a broad host and different environments. The differences in the incidence rate were, however, insignificant ($P > 0.05$). Maximum incidence of *E. tarda* was found in fish of the family Heteropneustidae (50.00%) followed by Cichlidae (40.00%), Stromateidae (33.30%), Notopteridae, Bagridae and Anabantidae (25.00%), Channidae (20.00%), Clariidae (16.60%), Clupeidae (14.29%) and others (Figure 4). The high incidence of *E. tarda* in Heteropneustidae might be because of their habitat as they prefer to live in pond mud, which is suitable for *E.*

tarda growth. High temperature, overcrowding and high organic load may also favour the growth of *E. tarda*[3].

Isolation of *E. tarda* has been reported from a wide range of fish hosts[4,5,18]. Recently, *E. tarda* infections in *Pangasius hypophthalmus*[19], *Pangasianodon hypothalamus*[20] and *Paralichthys lethostigma*[21] have been reported. In the present study, *E. tarda* was isolated from 10 fish species (Table 3). Earlier studies from India reported isolation of *E. tarda* in diseased *C. batrachus* and *A. testudineus*[3], *Pangasius hypophthalmus*[19], *Pangasianodon hypothalamus*[20], *Labeo rohita*[22] and *Clarias gariepinus*[23]. This is the first study to report the incidence of *E. tarda* in *T. ilisha*, *C. striatus*, *P. argenteus*, *C. chitala*, *O. mossambicus*, *S. seenghala* and *N. notopterus* from India. *C. batrachus* and *A. testudineus* prefer to live in pond water and mud and, thus, *E. tarda* is likely to be present at higher percent in those fish[3]. Further, *E. tarda* could not be isolated from fish families such as Cyprinidae, Pangasiidae, Polynemidae, Anguillidae, Belonidae, Latidae, Siluridae and Harpodontidae. As shown in Figure 5, the intestine had high incidence of *E. tarda* (12.98%) followed by gills (6.60%) and skin (2.38%). No *E. tarda* could be recorded from muscles and kidney samples. Significant difference existed in the prevalence of *E. tarda* among different organs of fish ($P < 0.05$). Likewise, Wyatt *et al.* isolated *E. tarda* from skin and viscera, but with higher proportions[24]. They recorded higher prevalence of *E. tarda* in viscera (88.00%) followed by dressed fish (79.00%) and skin (47.00%). While, Muratori found *E. tarda* from external surface (17.20%), muscle (14.30%) and intestine (11.20%) of tilapia from an integrated fish farm that used pig excrements as food[25]. Contrarily, Galal *et al.* reported the presence of *E. tarda* in *Oreochromis niloticus* with higher proportion in kidney, spleen and liver followed by intestine; while the samples from gills and dorsal musculature were negative for *E. tarda*[26]. Among the sampling areas, the prevalence of *E. tarda* was high (37.50%) in Nadia followed by South 24 Parganas (13.70%) and Howrah (3.30%) districts of West Bengal (Figure 6). In Bihar, maximum incidence was in Muzaffarpur district (50.00%) followed by Samastipur district (20.00%). The differences in the incidence of *E. tarda* among the districts of West Bengal were significant ($P < 0.05$), while those of Bihar were insignificant ($P > 0.05$). The possible explanation for the high incidence of *E. tarda* in Muzaffarpur district might be that the fish samples screened were collected from a domestic sewage polluted water body. On the other hand, the samples of Samastipur district were from the riverine source which was comparatively less polluted.

Stock and Wiedemann created a database concerning the natural susceptibility to a wide range of antibiotics to all known *Edwardsiella* species originating from materials such as water, human faeces, fish, monitor lizard and female puffin originated from countries such as Sweden, UK, USA, Germany, Japan, France and Austria[27]. According to them, all *Edwardsiella* were naturally sensitive to tetracycline, aminoglycosides, most β -lactams, quinolones, antifolates, chloramphenicol, nitrofurantoin and fosfomycin, and naturally resistant to macrolides, lincosamides, streptogramins,

glycopeptides, rifampicin, fusidic acid, benzylpenicillin and oxacillin. As seen in Figure 7, all the 33 strains of *E. tarda* were sensitive to ciprofloxacin. Few isolates of this species were resistant to chloramphenicol so also in DePaola *et al.*[28]. Contrary to the database[27], most of the *E. tarda* strains of the present study from fish were resistant to oxytetracycline, nitrofurantoin, gentamycin and co-trimoxazole. DePaola *et al.* reported that the tetracycline resistant *E. tarda* isolates from catfish ponds were susceptible to all other Uniscept KB drugs, which contained 18 antibiotics[28]. They have also reported that 4.00% and 1.90% of the *E. tarda* isolates from intestine and pond water respectively were resistant to tetracycline. Similar to the present study, Mallick reported that the *E. tarda* isolates from diseased catfish, *Clarias gariepinus* from West Bengal were resistant to oxytetracycline (36.37%) and trimethoprim (26.67%) and sensitive to chloramphenicol and gentamycin[23]. The present study further revealed no marked differences ($P > 0.05$) in the antibiotic sensitivity of *E. tarda* strains from West Bengal and Bihar. The strains of Bihar were sensitive to ciprofloxacin and chloramphenicol and resistant to oxytetracycline (75%) followed by nitrofurantoin (63%), gentamycin (50%); whereas the *E. tarda* strains of West Bengal were more resistant to the tested antibiotics than Bihar strains except oxytetracycline (Figure 7). The insignificant difference in antibiotic sensitivity among the *E. tarda* isolates of Bihar and West Bengal indicated that these strains from different geographical origin possibly share a common phenotypic and genotypic characteristics. Multiple antibiotic resistance (MAR), *i.e.*, resistant to at least two antibiotics, was found to be 87.50% for Bihar and 84.00% for West Bengal *E. tarda* strains. The difference in MAR of *E. tarda* isolates of West Bengal and Bihar was insignificant ($P > 0.05$). The results of the present study are in accordance with earlier studies from West Bengal[23,29].

A wide range of MIC values among the *E. tarda* strains was found in the present study. West Bengal *E. tarda* strains showed high MIC values than Bihar isolates. The ranges of MICs of antibiotics such as ciprofloxacin, chloramphenicol, oxytetracycline and gentamycin were found to be almost the same except co-trimoxazole. The *E. tarda* strains from *H. fossilis* and *O. mossambicus* recorded high MIC values for majority of the antibiotics tested. Likewise, Mallick reported that *E. tarda* strains from diseased catfish were resistant to oxytetracycline, gentamycin and trimethoprim with MIC values $> 200 \mu\text{g/mL}$ [23]. On the other hand, Stock and Wiedemann recorded low MIC for tetracycline (0.50–16.00 $\mu\text{g/mL}$ and only one strain with $> 128 \mu\text{g/mL}$), gentamycin (0.13–1.00 $\mu\text{g/mL}$), ciprofloxacin (0.03 $\mu\text{g/mL}$), co-trimoxazole (0.03–0.50 $\mu\text{g/mL}$), chloramphenicol (0.13–4.00 $\mu\text{g/mL}$) and nitrofurantoin (4.00–8.00 $\mu\text{g/mL}$) against 102 *E. tarda* strains from various sources including fish from different countries[27]. Wei and Musa recorded MIC values of tetracycline in the range of 0.02–0.39 $\mu\text{g/mL}$ against 18 *E. tarda* isolates pathogenic to fish[2]. The increased prevalence of MAR and high MIC values for antibiotics among *E. tarda* of the present study may limit the effectiveness of the treatment, since many of the *E. tarda* strains are potential fish pathogen[1-5,16] and human

pathogen[6-9]. Conceivably, the high MIC values for antibiotics may cause problems for chemotherapy in the future. These results, in general, suggested that considerable proportion of commercially important finfish of West Bengal and Bihar are carriers of multiple antibiotic resistant *E. tarda*. This is a cause for concern for health authorities as *E. tarda* is a known pathogen involved in gastroenteritis and extraintestinal infections in human beings. This calls for appropriate sanitary and control measures to eliminate this pathogen in fish and fishery products.

Conflict of interest statement

We declare that we have no conflict of interest.

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