Multiple Drug Resistant Edwardsiella Tarda and Edwardsiella Hoshinae in an Integrated Fish-pig-duck Farm and Down Colony in Jharnapani, Nagaland

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Abstract

Integrated farming is popular in India but little is understood about circulation of multi-host pathogens under the emerging farming systems (IFS). Circulation of Edwardsiella a pathogen of public health importance has been studied in IFS. Gill swabs of fish (8), pond water (8), rectal swabs of pig (36) and cloacal swabs of duck (32) from an IFS and 24 samples each of rectal swabs of pigs and cloacal swabs of ducks from the down colony (300 m on down side from the farm) were analyzed for the presence of edwardsiellae and heterogeneity among the isolates. A total of 24 E. tarda and 6 E. hoshinae were isolated. No E. hoshinae was isolated from the IFS. We isolated both E. tarda and E. hoshinae from ducks but no E. tarda in down colony pigs. Though 55% E. tarda from the IFS were multi-drug-resistant (MDR) type, no MDR strain was detected in the down colony. Plasmid(s) were detected in E. tarda but not in E. hoshinae isolates. The study concluded that E. tarda may be detected in all components of the IFS but E. tarda from different sources may not be of similar type.

1. Introduction

Edwardsiella, a genus in Enterobacteriaceae family includes strains of three species (E. tarda, E. ictaluri and E. hoshinae). Of these, E. tarda is primarily a pathogens of fish associated with fish gangrene, emphysematous putrefactive disease of catfish, red disease of eels (3). Besides being a pathogen of aquatic animals, E. tarda is also an opportunistic pathogen of public health significance causing local wound, systemic, urinary tract and gastro-intestinal infections in human beings (1, 3, 7). It is present worldwide, spreading through feco-oral route or through contacts (1, 3). Though mostly isolated from fish, pathogenic strains of E. tarda have also been isolated from humans, pigs and ducks (1, 5, 8, 16). In India it is often isolated from fish, apparently healthy pigs and house geckos but rarely from human beings (10-15). Another zoonotic member of the genus is E. hoshinae, isolated first from lizards and birds (6), has also been isolated from house geckos in India (15).Although E. tarda isolated from fish of different species in natural environment are genetically different from each other (5), little is understood about the edwardsiellae circulating in different components of integrated farms having different animal units intricately connected with each other. In this study, we phenotypically characterised the edwardsiellae present in different components of an integrated fish-pig-duck farm and a nearby down colony to understand the circulation of edwardsiellae in IF components and environment.

2. Materials and Methods

Samples: Samples were collected from an integrated farm consisting of a fish (Labeo rohita) pond, a duckery (Khaki Campbel ducks) on the

pond bank and a piggery (Large Black Yorkshire) on the upper side of the pond. Water samples (50 ml) were collected from four different sides of the pond, 4 samples of top water and 4 samples with a sterilized siphon at depth of 50 cm in sterile glass container. Fish were caught (harvested) from the pond using net and gill swabs were collected from 8 of the fresh harvests. Gill swabs from 6-8 harvested fish were pooled to constitute one sample. Rectal swabs of all 36 sows and cloacal swabs of 32 ducks on the farm were collected in separate sterile tubes and brought to laboratory within an hour of collection. Similarly, rectal and cloacal swabs were collected from pigs and ducks, respectively in the down colony. However, from each household only one duck and one pigprovided by the owner were caught to sample.

3. Isolation and Characterization of Edwardsiella:

For isolation of Edwardsiella from water, 25 ml of water from the sample was transferred to 225 ml of sterilized MacConkey broth (Hi-Media, Mumbai) and incubated at 37C for 24 hrs. To each tube containing the swabs from different pigs, ducks and fish, 10 ml of sterilized MacConkey broth was aseptically added and all tubes were incubated at 37C for 24 hrs. Thereafter, from each broth, a loopful of culture was streaked on to Xylose-Lysine-Desoxycholate Agar (XLDA) plates (Hi-Media) and incubated at 37oC for 24 hrs. Red transparent colonies with or without black centre were picked up as suspected Edwardsiella and re-streaked on to blood agar plates to purify and observe haemolytic response. Well isolated 3 to 5 colonies from each sample were characterised using morphological (small Gram negative rods), biochemical and growth characteristics as described earlier (1, 10) using Hi25 Enterobacteriaceae identification kit (Hi-Media) and sugar fermentation tests (10). All suspected isolates of E. tarda and E. hoshinae were submitted for confirmation to bacteriology laboratory of Centre of Animal Disease Research and Diagnosis of the Institute for confirmation.

4. Antimicrobial Sensitivity Assay:

Antibiotic sensitivity of Edwardsiella isolates was determined through disc diffusion methods (4) on Muller Hinton agar (BBL BD), using amikacin 10 g, ampicillin 10 g, cefotaxime 30 g, ceftazidime 30 g, chloramphenicol 30 g,ciprofloxacin 10 g, cotrimoxazole 25g, gentamicin 30 g, kanamycin 10 g, nalidixic acid 30 g, netilmicin 30 g, nitrofurantoin 300 g, norfloxacin 10 g, ofloxacin 5 g, streptomycin 10 g and tetracycline 30 g discs(Hi-Media). Diameter of growth inhibition zone around antimicrobial disc measured in mm was used to classify an isolate either as sensitive or resistant according to CLSI (4) standards. Multiple drug resistance (MDR) of a bacterial strain was defined as resistant to three or more of following drugs: ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamicin, nitrofurantoin and tetracycline. Reference E. coli K12 strain (E-382) was used as sensitive control. From any one animal all the strains having similar morphological, cultural and biochemical characteristics and similar antimicrobial sensitivity pattern were counted as one isolate. For determining minimum inhibitory concentration of tetracycline, streptomycin and chloramphenicol E-test was used using Ezy-MIC strips (Hi-Media) as per directions of the manufacturer.

5. Plasmid Profiling:

To isolate plasmid from Edwardsiella isolates, bacteria was grown overnight at 37C in LB broth. For plasmid isolation QIAGEN plasmid mini kit (Qiagen India Pvt. Ltd. New Delhi) was used as per directions of the manufacturer and plasmid(s) was eluted in 25 l nuclease free water. Ten l of plasmid elute was digested with 2 units of EcoRI (Fisher Scientific, Pittsburgh, USA) for 30 min at 37C. Thereafter, horizontal electrophoresis for digested and undigested plasmid preparations in 1% agarose gel was performed at 200V for 2 hrs in Tris borate EDTA (TBE) buffer. Thereafter, gels were visualized and photographed under UV-gel documentation system (Alpha Innotech Co., USA).

6. Statistical Analysis:

All data related to isolation of Edwardsiella and antimicrobial sensitivity were analyzed and compared using Chi-squares test to determine any relationship between source of the isolate and its characteristics including antimicrobial susceptibility and plasmid profiles.

7. Results:

Of the 132 samples analyzed (Table 1) in the study, E. tarda and E. hoshinae could be detected in 24 (18.2%) and 6 (4.5%) samples, respectively. Though there was no significant difference in Edwardsiella detection in samples from IF and down colony pigs (P, 0.29) and ducks (P, 0.37), but the species of Edwardsiella detected in two varied significantly. Edwardsiella hoshinae was isolated only from 5 (20.8%) of down colony pigs and 1 (4.2%) of the down colony duck samples. However, E. tarda was detected in all types of IF sam-

ples (water, fish, pig and ducks) and down colony ducks (Table 1). Of the 16 antimicrobials tested for their activity on E. tarda and E. hoshinae (Table 1), ampicillin was least effective and inhibited only 12.5% of E. tarda and 50% of E. hoshinae isolates. On the other hand nalidixic acid was the most effective antimicrobial drug inhibiting 95.8% E. tarda and 100% E. hoshinae. In general, E. tarda isolates from the IF samples were more resistant to several antimicrobials than E. hoshinae. All MDR strains belonged to E. tarda species (P, 0.025) and were isolated from the IF samples (P, 0.007) only (Table. 1). Statistical analysis revealed that significantly (p, 0.05) more number of E. tarda were resistant to ampicillin and nitrofurantoin and had more MDR than E. hoshinae isolates. Duck and pig isolates signified that more isolates of pig origin were resistant to nitrofurantoin (P, 0.02) and chloramphenicol (P, 0.047) than isolates of duck origin while more E. tarda of duck origin were resistant to ceftazidime (P, 0.04) than strains of pig origin.

Thirty edwardsiellae tested for antimicrobial sensitivity could be classified (Table 2) into 22 antibiogram types (AT). Strain with similar antibiogram were few, two AT-2 strains (resistant to ampicillin) were detected both in down colony and IF ducks. Four AT-3 (resistant to ampicillin and ceftazidime) strains belonged to both species and were isolated from the IF as well as the down colony ducks. Three AT-5 (resistant to ampicillin and nitrofurantoin) strains also belonged to both of the species and were isolated from the IF pond water, the down colony pig and the IF pig. Two AT-8 (resistant to ampicillin, cotrimoxazole and nitrofurantoin) E. tarda were detected both in the IF pond water and fish. Both of the AT-13 (resis-

tant to ampicillin, ceftazidime, streptomycin, cotrimoxazole and nitrofurantoin) isolates were isolated from the IF ducks. There was no similarity in antibiotic sensitivity pattern of Edwardsiella isolated from pigs, ducks and fish.

Fig. 1: Table 1.

Minimum inhibitory concentration (MIC) of all the three antibiotics (chloramphenicol, streptomycin and tetracycline) was lower for E. hoshinae strains than for E. tarda isolates (Table 3). Edwardsiella tarda isolates of IF origin had the highest MIC. Strains isolated from pigs of IF were more resistant than of fish, water and duck origin.

Fig. 2: Table 2.

All E. tarda isolates had one or more plasmid (Fig 1, 2) except one AT-15 (resistant to ofloxacin, norfloxacin, netilmicin, streptomycin, kanamycin, cotrimoxazole and nitrofurantoin) from pig. None of the six E. hoshinea isolates had detectable plasmid. On the basis of undigested plasmid profile (Fig. 1) plasmid bearing strains were divided into 8 types, while after digestion (Fig. 2) they fell into six PF types. All plasmid bearing strains had one or more heavy plasmid except the two strains of E. tarda isolated from the IF fish and pond water. These two strains had no similar PF and AT, i.e., strains from different as well as from similar sources could be differentiated using PF and AT patterns.

Table 3. Range of minimum inhibitory concentration (MIC) of antibiotics (µg/ ml) against *Edwardsiella tarda* and *E. hoshinae* isolates from integrated farm and down colony animals

Bacteria	Source	Tetracycline	Chloramphenicol	Streptomycin
Edwardsiella hoshinae	Down Colony pigs	0.1-10.0	0.1-2.0	0.1-1.0
	Down Colony Ducks	1.0	2.0	1.0
Edwardsiella tarda	Farm pigs	0.25-120	0.5-120	1.0-30.0
	Farm ducks	1.0-30.0	0.5-2.0	1.0-30.0
	Farm pond	1.0-2.0	0.5-1.0	1.0-2.0
	Farm fish	1.0-2.0	0.5-1.0	1.0-2.0

Fig. 3: Table 3.

Fig. 4: Fig. 1. Uncut plasmids of Edwardsiella tarda isolates from integrated farm (IF) and down colony (DC) animals. Lane 1 to 18 are from Edwardsiella tarda isolates from: 1, integrated IF fish (IFF); 2, IF pig (IFP); 3, IFP; 4, IF duck (IFD); 5, DC duck (DCD); 6, IFP; 7, IFP; 8, IFP; 9, IFP; 10, IFD; 11, IFD; 12, IFD; 13, IFD; 14, IFD; 15, IFD; 16, IF pond water (IFPW); 17, IFP; 18, IFP.

8. Discussion:

Edwardsiella causes systemic as well as local infections in fish and often causes extra-intestinal infections in fishermen after puncture wounds or after consumption of contaminated fish or fish products (7). Isolation of Edwardsiella tarda from fish, pigs, fish ponds and ducks in the study is not a novel finding and it has frequently been isolated from similar sources all over the world (1, 13, 14, 16). Edwardsiella infections are common in aquatic environment but the bacteria has been sporadically isolated from animals, birds, lizards and humans with or without clinical illness (1, 8, 13, 14, 16). Isolation rate of E. tarda was significantly higher than E. hoshinae strains in our study and the observations are in concurrence to past observations on occurrence of edwardsiellae in different environments (1, 8, 13, 14, 16). Although E. hoshinae has been isolated from birds and lizards earlier (5, 13, 14), isolation from pigs is very rare. The isolation of E. hoshinae from pig might be an indication of the widening of its host range which may be very important from public health point of view.
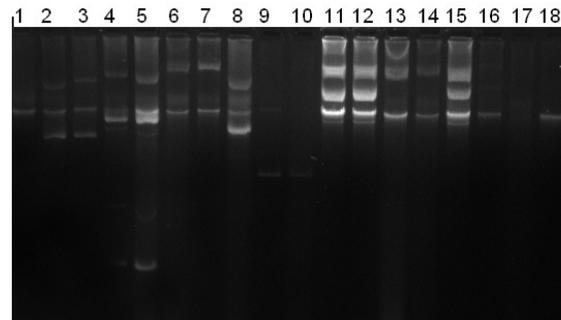
Edwardsiella are often reported to be sensitive to gentamicin, amoxicillin, trimethoprim-sulfamethoxazole, cephalosporins and oxyquinolones (7). Isolation of strains resistant to one or more of the drugs from the group of commonly considered effective drugs indicated the need of antimicrobial sensitivity assays before instituting the antimicrobial therapy in clinical Edwardsiella infections. It also signifies the need of regular monitoring of drug resistance in Edwardsiella. In earlier studies in Nagaland (14), E. tarda strains from pigs of an organized farm were reported resistant to several drugs on contrary, at least one similar type of drug resistance pattern (AT-10) observed in the present study. Similar antimicrobial susceptibility patterns as of E. hoshinae and E. tarda isolated from down colony animals were reported earlier in Edwardsiella isolates of lizards origin in Dimapur area (14, 15). The results indicated that isolates from farm had more resistance towards common antimicrobials than strains from down colony probably due to regular use of antibiotics either for therapeutic or preventive purposes on integrated farm.

In the study, Edwardsiella strains isolated from ducks were quite different from those of fish or pigs. The differentiation was possible even without exhaustive and costly molecular studies. Recently Griffin et al. (5) revealed the same fact of strain difference among E. tarda isolated from 4 different fish species in United States, this phenomena is referred as the intra-specific variability of E. tarda in the study. The understanding of the reasons for host specificity among Edwardsiella may be helpful in future to design disease control programmes. However, urgent need is to understand the mechanism underlying for the development of host specific clone in an integrated farm with interlinked components but carrying different Edwardsiella strains. In the study, of the 30 Edwardsiella tested, 12 (40%) were resistant to ceftazidime while 4 (13.3%) were resistant to cefotaxime (P, 0.02). The observations were in contrast to earlier observations (9) reporting ceftazidime more effective than cefotaxime

in controlling infections caused by Pseudomonas spp., Acinetobacter spp. and Enterobacteriaceae strains. This may be due to preferred use of ceftazidime over cefotaxime in last few decades which might have induced silent selection of ceftazidime resistant Edwardsiella strains over the years or due to difference in susceptibility of Edwardsiella from the bacteria studied earlier. Similarly, there was numerical difference in susceptibility of strains to quinolones (Table 1) but it was not significant (P, 0.16) and might be due to random appearance of a few variants in the population.
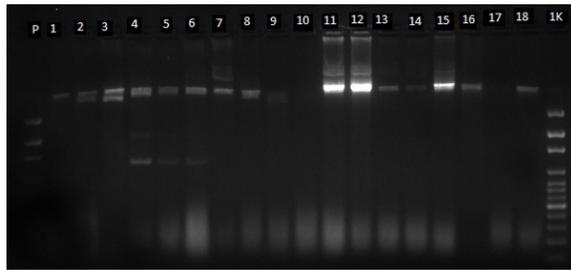

Fig. 5: Fig. 2. ECORI digested plasmids of Edwardsiella tarda isolates from integrated farm and down colony animals. Lane 1 to 18 are from Edwardsiella tarda isolates from: 1, integrated IF fish (IFF); 2, IF pig (IFP); 3, IFP; 4, IF duck (IFD); 5, DC duck (DCD); 6, IFP; 7, IFP; 8, IFP; 9, IFP; 10, IFD; 11, IFD; 12, IFD; 13, IFD; 14, IFD; 15, IFD; 16, IF pond water (IFPW); 17, IFP; 18, IFP; P, plasmids from an Escherichia coli (E-383 Strain from National Salmonella Centre Vet., IVRI, Iatnagar; 1K, 1Kb ladder.

Susceptibility of Edwardsiella strains to 5 different aminoglycosides (netilmicin, gentamicin, streptomycin, amikacin and kanamycin) indicated that there was some variation in number of susceptible strains to different aminoglycosides. However, the variation was significant (P, 0.02) between gentamicin (13.3% resistant) and streptomycin (40% resistant) susceptibility only; it was not significant when compared among other aminoglycosides. The most effective was gentamicin (86.7%), followed by amikacin (76.7%), netilmicin (73.3), kanamycin (66.7%) and streptomycin (60%). The results are in concurrence to earlier observations on better efficacy of gentamicin over streptomycin (2). However, amikacin has been advocated to treat the gentamicin resistant infections being safer and more effective (2) in our observations it was evident that none of the gentamicin resistant Edwardsiella was susceptible to amikacin. The observation indicated that if gentamicin failed to eliminate Edwardsiella no other aminoglycosides may be of clinical utility.

The study concludes that though Edwardsiella may be isolated from different sources in an integrated farm, they may not be similar. Edwardsiella in IF system might be comparatively more drug resistant type than those isolated from other animal husbandry settings.

9. References

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