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Incidence of Putative Virulence Factors and Antimicrobial Resistance in *Aeromonas caviae* Isolated from Livestock in Northeast India

K.L Prashant[1], S. Ghatak[2]*, A. Kumar[1], K.N. Bhilegaonkar[1], S. Dandapat[1], A. Sen[2], I. Shakuntala[2], K. Puro[2], S. Das[2], R. Pegu[2], A. Karam[2], A. Ahuja[2], U. Bhattacharjee[2], L. Minakshi Singha[2], T. K. Dey[2], A. Chakraborty[2], A. Dutta[2].

ABSTRACT

Processing of 172 rectal swab samples comprising swabs from cattle (n = 26), pigs (n = 127) and dogs (n = 19) for isolation of *Aeromonas* spp. revealed incidence of 0%, 10.5% and 3.9%, respectively. Overall incidence was 4.06%. Identification of isolates through automated ID/AST system (Phoenix 100) revealed all isolates belonging to *A. caviae*. Determination of virulence potential by haemolysin assay and lecithinase production indicated that all canine isolates were haemolytic and 50% of them produced lecithinase, while porcine isolates were neither haemolytic nor lecithinase producing. Detection of three virulence genes (AHCYTOEN, cytotonic enterotoxin, and aerolysin) through polymerase chain reaction revealed all isolates of canine origin and 80% isolates of porcine origin harboured the AHCYTOEN gene, 50% of the canine isolates and 40% of swine isolates possessed cytotonic enterotoxin gene, 50% of the canine isolates and 20% of the porcine isolates 40% carried the aerolysin gene. Determination of antimicrobial resistance among the isolates indicated widespread resistance against a number of antimicrobials including penicillin, ampicillin, cephalexin, cefazolin, cefoxitin and nalidixic acid. Multiple antibiotic resistance was also common. Results of the present study highlighted the incidence of virulent, drug resistant *A. caviae* isolates in canines and pigs from northeast India with possible risk of human infection or contamination of food and water from these sources.

Keywords: *Aeromonas*, Virulence, Incidence, Drug resistance, PCR, Canine, Porcine.

INTRODUCTION

Aeromonas species are gram-negative, cocccobacillary, non-spore forming, motile, facultatively anaerobic organisms that are also catalase and oxidase positive (Abott et al. 2003). Though these organismd are widely distributed in nature, they are also known to cause a number of infections in humans and animals (Janda and Abott 2010; Evangelista-Barreto et al. 2010).

As with other bacterial pathogens, the ailments caused by aeromonads are linked to a number of virulence properties including aerolysin, hemolysin, enterotoxins, cytotoxins, adhesins, extracellular lipase, etc. (Cahill 1990; Janda et al. 2002).

Though many previous researchers documented incidence and virulence properties of *Aeromonas* spp. in humans (Janda and Abott 2010; Evangelista-Barreto et al. 2010; Messi et al. 2003) and too some extent from animals (Gray 1984; Gray and Stickler 1989; Figura and Marri 1985), there is paucity of similar literature corresponding to animals especially from India.

In recent years development of resistant or multidrug resistant pathogens has become a major problem in India and many countries (WHO 2013). Bacterial pathogens, that are capable of infecting both humans and animals are considered major offender for the origin of antibiotic resistance in the environment (Allen et al. 2010). Since

[1] IVRI, Izatnagar, India.
[2] ICAR RC for NEH Region, Umiam, Meghalaya, India
* Corresponding author's E-mail: ghataksnd@rediffmail.com

Aeromonas spp. has been implicated in human and animal diseases, and many members of the organisms are inherently resistant to beta-lactam antibiotics, they may play important role in emergence of antimicrobial resistance. In India, antimicrobial resistance of *Aeromonas* spp. have been studied by previously (Thayumanavan et al. 2003) though these studies involved food isolates and reported qualitative data only.

Considering the gaps in the scientific data on virulence potential and drug resistance profiles of aeromonads from animal sources, present study was undertaken to assess the incidence, virulence properties and antimicrobial resistance patterns of *Aeromonas* spp. isolated from domestic animals from north-eastern part of India.

MATERIALS AND METHODS

Sampling

A total of 172 rectal swab samples were collected aseptically from various regions of Meghalaya and Assam over a period of October to February months (29/10/12 to 3/2/13). Samples comprised of faecal swab from cattle (n = 26), pigs (n = 127), dogs (n = 19). Of the 26 total animal sampled 20 (76.92%) cattle were healthy and 6 (23.07) % out of which were diarrhoeic; of the 127 isolates from rectal swab of pigs 13(10.23%) were healthy and 114(89.76%) were diarrhoeic. For dogs, the diarrhoeic cases comprised 18(94.73%) while 1(5.26%) were healthy canine subjects. Samples were shipped to laboratory immediately after collection under refrigeration temperature and were processed immediately. In no case processing of samples were delayed more than 24 h and till then they were stored in the laboratory at 2-8º C.

Isolation and identification of *Aeromonas* species

Collected samples were processed for isolation of *Aeromonas* spp employing Alkaline Peptone Water, pH 8.6 (APW, HiMedia, India) Ampicillin Dextrin Agar (ADA) containing ampicillin @ 10 mg / ml as described previously (Ghatak et al. 2009). Briefly, the samples were enriched for 16 – 18 h at 37 ºC followed by selective plating on ADA. Plates were incubated at 37ºC for 18 - 24 h. Smooth honey drop yellow colonies that were negative for oxidase reaction along with gram negative short coccobacillary morphology were presumptively identified as *Aeromonas* spp. Suspected isolates were saved on fresh nutrient agar (Hi-media, India) slants and were further processed for confirmation and speciation. Confirmation and speciation of presumptive isolates were undertaken by a combination of conventional (Carnahan et al. 1991) and automated identification systems (Phoenix ™ 100, Becton Dickinson, Singapore).

Haemolysin production

Aeromonas isolates were examined for their ability to produce haemolysin on 5% Sheep Blood Agar as described previously (Gerhardt et al. 1981; Ghatak, 2005) with suitable modifications. Ten microlitres of overnight grown broth cultures of *Aeromonas* isolates with OD A_{600} between 0.5 – 0.6 were spotted aseptically onto blood agar plates containing 5% defibrinated sheep blood. Plates were incubated for 24 – 48 h at 37 ºC. Production of haemolysin by an isolate was indicated by appearance of clear or opalescent zone around the colony.

Production of lecithinase

Lipase production by the isolates was estimated on Egg Yolk Agar containing 5% sterile egg yolk emulsion as described previously (Anguita et al. 1993). In short the method involved spotting of 10 µl of broth cultures of isolates (OD A_{600} 0.5 – 0.6) onto 5% egg yolk agar followed by incubation at 37ºC for 24 – 48 h. Expression of lecithinase was observed as a zone of opalescence around the spotting site at the end of incubation period.

Detection virulence genes by polymerase chain reaction (PCR)

Aeromonas isolates were characterized for three virulence genes namely, AHCYTOEN (a multivirulence gene that included mice lethality, haemolysin production, enterotoxigenicity and cytotoxicity), cytotonic enterotoxin, and aerolysin (Kingombe et al. 1999; Nam et al. 2007; Granum et al. 1998) (Table 1).

PCR for AHCYTOEN gene

Templates for PCR was prepared by boiling of overnight grown broth cultures of isolates for 15 minutes followed by snap chilling on crushed ice for 20 minutes. The lysate was centrifuged briefly

Table1: PCR primers for detection of virulence genes of *Aeromonas* isolates

Target gene	Primer sequence	Product size (bp)	Annealing temperature	Reference
AHCYTOEN	Forward: 5'-GAGAAGGTGACCACCAAGAACA- 3' Reverse: 5'-AACTGACATCGGCCTTGAACTC-3'	232	60°C	Kingombe et al. (1999)
Cytotonic enterotoxin	Forward: 5'-GCAAGTGTTCTAGTCTTTCCG-3' Reverse: 5'-ACCTGCCAAAGTTTGCTGTGA-3'	482	64°C	Granum et al. (1998)
Aerolysin	Forward: 5'-GAGCGAGAAGGTGACCACCAAGAAC-3' Reverse: 5'-TTCCAGTCCCACCACTTCACTTCAC-3'	417	60°C	Nam et al. (2007)

at 10000 g for 4 minutes and the supernatant was used as template for subsequent PCR reaction. For each PCR run templates were prepared afresh.

PCR assay for detection of AHCYTOEN gene was standardized as described previously by Kingombe et al (1999) with necessary modifications. The reaction mixture (20 µl) was optimized with 10µl of 2 X PCR master mix (Fermentas MBI), 10 pmol each of forward and reverse primers, 6µl of nuclease free water and 2µl of DNA template.

Thermocycling conditions for AHCYTOEN gene comprised of following: an initial hold of 4 minutes at 94°C, denaturation at 94°C for 45 s, annealing at 60°C for 30 s, extension at 72°C for 30 s for a total of 35 cycles and a final extension of 72°C for 3 minutes. The polymerase chain reactions were carried out using a DNA thermal cycler with heated lid (iCycler, Bio-Rad, Germany). All PCR runs included appropriate negative control with DNA blank and were repeated at least thrice to ensure reproducibility of the assay.

Following electrophoresis products were electrophoresed through 1.5% agarose gel with 1X Tris-Acetate EDTA (TAE) buffer under electrical field strength of 6V/cm (Sambrook et al. 2001). Each run was accompanied with molecular weight marker of suitable range. Upon completion of the run, amplicons were visualized under UV illumination in a gel documentation system (DNR MiniLumi, Israel).

PCR for cytotonic enterotoxin gene

PCR for cytotonic enterotoxin gene was optimized as reported by Granum et al. (1998) with necessary adaptations. In short, the reaction mix was optimized with 10 pmol each of forward and reverse primers and 1 µl of template DNA prepared by boiling and snap chilling as described in preceding section. Thermocycing conditions involved denaturation at 94ºC for 45 s, annealing at 61ºC for 30 s, primer extension a 72ºC for 45 s for 30 cycles and a final extension step at 72ºC for 3 minutes. Appropriate PCR controls were included in each run. Finally products were electrophoresed in 1.5% agarose gel and visualized under UV illumination.

PCR for aerolysin gene

Similar to AHCYTOEN and cytotonic enterotoxin gene, PCR methodology described by Nam et al (2007) was adapted suitably for aerolysin gene. Reaction mix contained 10 pmol each of forward and reverse primers and 1.5µl of template DNA. PCR run conditions were optimized with denaturation at 94ºC for 1 minute, annealing at 62ºC for 40 s, primer extension a 72ºC for 1 minute for 30 cycles and a final extension step at 72ºC for 3 minutes. On completion of run, products were electrophoresed, visualized under UV and photographed.

Antimicrobial susceptibility testing and determination of Minimum Inhibitory Concentration (MIC)

Antimicrobial susceptibility tests along with determination of MIC of the isolates were performed using Phoenix ™ 100 automated ID/AST system employing NMIC/ID 55 panel. The panel contained following antimicrobials- amikacin, amoxicillin/clavulanate, ampicillin, aztreonam, cefazolin, cefepime, cefoperazone/sulbactam,

cefotaxime, cefoxitin, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, tetracycline, and trimethoprim/sulfamethoxazole. The results of MIC were interpreted as per CLSI and EUCAST guidelines along with BD Xpert™ rules of the Epicentre™ software.

RESULTS AND DISCUSSION

Incidence of aeromonads

Out of 172 rectal swab samples collected from canines, swine and bovines, 7 samples yielded *Aeromonas* spp. indicating overall incidence of 4.06%. All isolates were identified as *A. caviae* with no other species detected in the course of the present study. Incidences among canine and porcine samples were 10.5% and 3.9%, respectively. No *Aeromonas* could be isolated from cattle samples.

Of these seven isolates, two (28.6 %) originated from canines and rest five (71.4 %) were of swine origin (Table 2). Two of the canine subjects from which isolation of *Aeromonas* was possible had histories of elevated body temperature, anorexia, vomition and dysentery. On the other hand all swine subjects wherefrom aeromonads could be isolated, were normal healthy animals with no signs of illnesses.

Virulence factors

Of the seven isolates of *A. caviae*, two (28.6%) isolates caused haemolysis of SRBC on 5% sheep blood agar. While both these isolates originated from canines, none of the isolates from pigs were haemolytic of 5% sheep blood agar (Table 2).

Production of lecithinase was assessed on egg yolk agar, which indicated that only one isolate (14.3%) of canine origin produced lecithinase observed as zone of lecithin precipitation around the colonies of the isolate (Table 2).

Detection of virulence genes

The PCR assays optimized for detection of three virulence genes (AHCYTOEN, cytotonic enterotoxin, and aerolysin) proved to be efficacious, as all three assays yielded PCR amplicons of expected molecular weight (Fig. 1, 2 & 3).

Majority (85.7%) of the *A. caviae* isolates possessed AHCYTOEN gene. All isolates of canine origin and 80% isolates of porcine origin harboured the AHCYTOEN gene. On the other hand, cytotonic enterotoxin gene was present in 42.9% of isolates

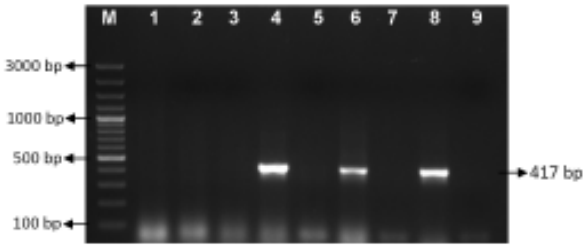

Fig. 1: PCR amplification of Aerolysin gene (417) from *Aeromonas caviae* isolates, Lane 1: Molecular weight marker (100 bp); Lanes 4, 6, 8: *A. caviae* isolates with positive amplifications (417 bp); Lane 8: Positive control (*A. hydrophila* ATCC 35654); Lane 9: Negative control

Table 2: Sources and virulence properties of *A. caviae* isolates

Isolate code	Source	Virulence factors		Virulence genes		
		Haemolysis of sheep RBC	Lecithinase production	AHCYTOEN	Cytotonic enterotoxin	Aerolysin
ARSDG6GH	Canine	+ve	-ve	+ve	+ve	+ve
ARSDG10GH	Canine	+ve	+ve	+ve	-ve	-ve
ARSPG4a	Swine	-ve	-ve	-ve	-ve	-ve
ARSPG5a	Swine	-ve	-ve	+ve	+ve	-ve
ARSPG38a	Swine	-ve	-ve	+ve	+ve	-ve
ARSPG39a	Swine	-ve	-ve	+ve	-ve	+ve
ARSPG50a	Swine	-ve	-ve	+ve	-ve	-ve
	Canine: 28.6% Swine: 71.4%	Haemolytic: 28.6%	Lecithinase +ve: 14.3%	AHCYTOEN +ve: 85.7%	Cytotonic enterotoxin +ve: 42.9%	Aerolysin +ve: 28.6%

+ve = presence, -ve = absence

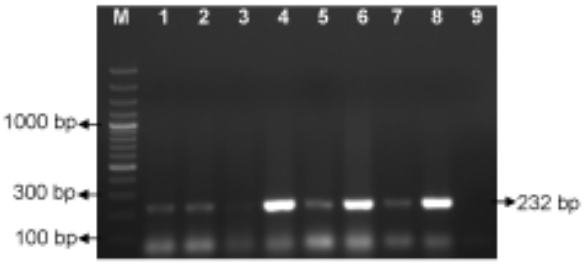

Fig. 2: PCR amplification of *AHCYTOEN* gene (232) in *Aeromonas caviae* isolates, Lane M: Molecular weight marker (100 bp); Lanes 1, 2, 3, 4, 5, 6, 7: *A. caviae* isolates with positive amplifications (232 bp); Lane 8: Positive control (*A. hydrophila* ATCC 35654); Lane 9: Negative control

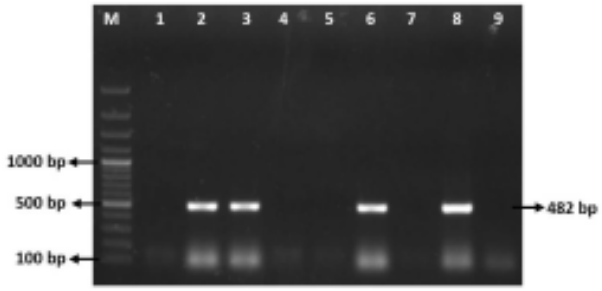

Fig. 3: PCR amplification of Cytotonic Enterotoxin gene (482bp) in *Aeromonas caviae* isolates, Lane M: Molecular weight marker (100 bp); Lanes: 2, 3, 4, 6: *A. caviae* isolates with positive amplifications (482 bp); Lane 8: Positive control (*A. hydrophila* ATCC 35654); Lane 9: Negative control.

with 50% of canine isolates 40% of the swine isolates harbouring the gene. PCR for aerolysin gene indicated that 28.6% of the isolates yielded positive amplification. Aerolysin gene was detected in 50% of the canine isolates and 20% of the porcine isolates (Table 2).

Antimicrobial resistance

The strains of *A. caviae* isolated from both canine and porcine were examined for their resitance pattern against 23 antimicrobials including determination of MIC values employing BD Phoenix™ 100 automated ID/AST system.

Results revealed that all isolates were resistant to 6 antimicrobials (penicillin, ampicillin, cephalexin, cefazolin, cefoxitin and nalidixic acid). High degree of resistance (71.5%) was also noted against tetracycline with most observed MIC being >8 µg / ml.

Most effective antimicrobials as revealed in the study were amikacin, imipenem, meropenem, levofloxacin and ciprofloxacin with 100% sensitivity for all isolates. Among penicillin group piperacillin-tazobactam (most prevalent MIC - <=4/ 4 µg/ml) and amoxicillin-clavalunate (most prevalent MIC - 8/4 µg/ml) combinations were most effective, with 14.3% isolates being resistant to these antimicrobials. Similar sensitivities were also observed for aztreonam (monobactam), third generation cephalosporins (cefotaxime, ceftazidime) and gentamicin (aminoglycoside).

Compared to isolates of porcine origin, isolates of canine origin were clearly more resistant with 41-67% antimicrobials rendered ineffective. Isolates of porcine origin, on the other hand, revealed uniform sensitivities to a number of antimicrobials including amoxicillin-clavulanate, piperacillin-tazobactam, azetreonam, cefotaxime, ceftazidime, amikacin, imipenem, meropenem, levofloxacin, ciprofloxacin and gentamicin with MIC values of 8/4 µg /ml, <=4/4 µg /ml, <=2 µg / ml, <=4 µg /ml, <=0.5 – 2 µg /ml, <=8 µg /ml, <=1 µg /ml, <=1 µg /ml, <=1 µg /ml, <=0.5 µg /ml and <=2 µg /ml, respectively (Table 3).

All isolates were multidrug resistant with resistance recorded for >= 3 antimicrobials. Overall resistance rate varied from 27 – 67%.

The present study reports incidence, virulence properties and antimicrobial resistance patterns of a collection of *Aeromonas* (*A. caviae*) isolates that were obtained from canines and porcines of northeast India (Assam and Meghalaya).

The *Aeromonas* species isolated from rectal swabs were identified at phenotypic level by integrating an automated system BD Phoenix™ 100 (Singapore). This approach proved to be useful in terms of rapidity and convenience with an average identification / confirmation time of 12 h.

Overall incidence of *Aeromonas* species was 4.06%, which is slightly in variation with the previous report by Ghatak et al. (2009) who reported an incidence of 4.35% in canine species. This minor difference may be attributed to difference in geographical location and difference in host species studied. All isolates obtained from canines and pigs were identified as *A. caviae*. This was rather unusual because previous works documented variety of species of aeromonads from animal sources (Gray and Stickler 1989; Ghatak et al. 2009; Ghengesh et al. 1999). According to

Table 3: MIC values and drug resistance patterns of *A. caviae* isolates of canine and porcine origin

Antimicrobials	MIC values for *A. caviae* isolates							Resistance (%)
	Canine isolates		Swine isolates					
	ARSDG6GH	ARSDG10GH	ARSPG4a	ARSPG5a	ARSPG38a	ARSPG39a	ARSPG50a	
Penicillins								
Ampicillin	**>16** R	**>16** R	**>16** R	**>16**R	**>16** R	**>16** R	**>16** R	100%
Amoxicillin-Clavulanate	**>16/8** R	<=4/2 S	8/4 S	8/4 S	8/4 S	8/4 S	8/4 S	14.28%
Piperacillin-Tazobactam	>**64/2** R	<=4/4 S	<=4/4 S	<=4/4 S	<=4/4 S	<=4/4 S	<=4/4 S	14.28%
Ticarcillin-Clavulanate	**>8** R	<=8/2 S	**16/2** R	<=8/2 S	**16/2** R	<=8/2 S	<=8/2 S	42.85%
Monobactams								
Azetreonam (ATM)	**>16** R	<=2 S	<=2 S	<=2 S	<=2 S	<=2 S	<=2 S	14.28%
Cephahalosporins								
Cephalexin	**>16** R	**>16** R	<=4 R	8 R	**>16** R	**>16** R	**>16** R	100%
Cefazolin	**>16** R	**>16** R	<=4 R	8 R	**>16** R	**>16** R	**>16** R	100%
Cefotaxime	**>32** R	<=4 S	<=4 S	<=4 S	<=4 S	<=4 S	<=4 S	14.28%
Cefoxitin	**>16** R	<=4 R	<=4 R	<=4 R	<=4 R	<=4 R	<=4 R	100%
Ceftazidime	**>16** R	<=0.5 S	<=0.5 S	1 S	2 S	2 S	1 S	14.28%
Carbapenems								
Imipenem	<=1 S	<=1 S	<=1 S	<=1S	<=1 S	<=1 S	<=1 S	-
Meropenems	<=1S	<=1 S	<=1 S	<=1 S	<=1 S	<=1 S	<=1 S	-
Aminoglycosides								
Amikacin	<=8 S	<=8 S	<=8 S	<=8 S	<=8 S	<=8 S	<=8 S	-
Tobramycin	**>8** R	**>8** R	8 R	<=2 S	<=2 S	<=2 S	<=2 S	42.85%
Gentamicin	<=2 S	**>8** R	<=2 S	<=2 S	<=2 S	<=2 S	<=2 S	14.28%
Tetracyclines	**>8** R	**>8** R	<=2 S	**>8** R	**>8** R	**>8** R	<=2 S	71.48%
Fluoroquinolones								
Nalidixic acid	**>16** R	**>16** R	**>16** R	**>16** R	**>16** R	**>16** R	**>16** R	100%
Levofloxacin	<=1 S	<=1 S	<=1 S	<=1 S	<=1 S	<=1 S	<=1 S	-
Ciprofloxacin	<=0.5S	1 S	<=0.5 S	1S	2 I	2 I	<=0.5 S	-
Trimethoprim-Sulphamethoxazole	**>2/38** R	**>2/38** R	**>2/38** R	<0.5/9.5S	1/19 S	1/19 S	<0.5/9.5S	42.85%
Miscellaneous								
Colistin	<=1S	<=1S	<=1S	<=1S	<=1S	<=1S	<=1S	
Nitrofurantoin	32S	<=16S	<=16S	<=16S	<=16S	<=16 S	<=16S	-
Ineffective antimicrobials (%)	67%	41%	37%	27.27%	32%	27.27%	23%	

Highest MIC breakpoints- mentioned resistance in bold.

researchers, *A. hydrophila* and *A. caviae* were most prevalent in clinical samples (Figueras 2005; Janda and Abbott 1998, 2010). Similar observations were also revealed in the present study with all isolates from canine clinical cases were of *A. caviae*. Incidence of aeromonads in porcine samples (rectal swabs) were considerably lower (3.93%) than 9.6% (11 of 115) as reported previously (Gray 1984). However, in contrast to the report of Gray and Stickler (1989), no aeromonads could be isolated from bovine samples.

It has been reported that *Aeromonas* species which were isolated from the species of dog and cat (Boynukara et al. 2002; Ceylan et al. 2003; Ghenghesh et al. 1999) may cause fatal septicaemia in dogs and puppies (Andre fontaine et al. 1995; Zdovc et al. 2004). In the present study the canine subjects from which *A. caviae* were isolated, suffered with clinical symptoms of anorexia, vomition, gastroenteritis. Moreover, for these cases no conclusive diagnosis could be established. Therefore, though it could not be ascertained that whether the symptoms were due to *A. caviae* infection or not, present findings indicate possible role of aeromonads in gastrointestinal illnesses of canines under study.

All isolates of canine origin were haemolytic while 20% of porcine isolates were so. This finding is in line with previous report by Ghengesh et al (1999).

Lipase play important role in bacterial virulence since insertion mutants for the lipase gene *plc* reduces the lethal dose (LD_{50}) in mice and fish (Anguita et al. 1993; Merino et al. 1999). In the present study, 50% of canine isolates and none of porcine isolated exhibited lipase activity indicating virulent nature of the canine isolates. However, the overall lipase activity was lower than previous report by Merino et al (1999).

Molecular characterization of isolates for virulence genes indicated that most (85.7%) of the isolates harboured AHCYTOEN gene. This is somewhat in excess of the previous report by Kingombe et al. (1999) who documented 58% incidence of the same gene. The higher incidence in the present study might be due to the clinical nature of the isolates while Kingombe et al. (1999) reported the same for environmental isolates.

Incidence of aerolysin gene on the other hand, was somewhat lower (28.6% overall). Previous workers recorded incidence of this gene in the range between 20 – 84% (Ghatak 2005; Gonzalez-Rodriguez et al. 2002; Ottaviani et al. 2011). Therefore, results of the present study rest well within reported range.

Possession of cytotonic enterotoxin gene by aeromonads is indicative of enterotoxigenic potential (Janda and Abott 1998). In the present study 42.9% of isolates harboured cytotonic enterotoxin gene which was contributed by 50% of canine isolates and 40% of swine isolates. However, the incidence of cytotonic enterotoxin gene recorded in the present study was lower than previous reports with incidence range of 65 – 75%. (Granum et al. 1998; Abdullah et al. 2003; Ottaviani et al. 2011). This apparent anomaly may be attributable to the difference in the set isolates studied by various authors.

Emergence of drug resistant microbe is a global concern. Wide spread use of antibiotics for treating bacterial diseases sub-therapeutic use of antibiotics in animal husbandry and aquaculture are held responsible for emergence of antibiotic resistance (WHO 2013). In developing countries including India, the situation is more precarious due to less stringent regulatory control of antibiotics with extensive use of antibiotics in animal husbandry and aquaculture (Vivekanandhan et al. 2002; WHO 2000). In the present study, therefore, attempts were made to document antibiotic susceptibility/ resistance pattern of Aeromonas isolates that were obtained from animal sources. All isolates were tested against a panel of 22 antimicrobial agents.

In a previous study (Overman and Janda 1999), reported antimicrobial resistance in clinical isolates with resistance to ampicillin (94.9%), cephalexin (76.3%), Trimethoprim (37.3%), tetracycline (11.9%). However, the results of the present study revealed higher resistance to these antimicrobials which may be due to difference in previous exposure to these antimicrobials. Similarly, nalidixic acid resistance was higher in the present study (100%) than that reported by Sinha et al. (2004) who recorded nalidixic acid resistance in 54 – 62% isolates. Similar variation in incidence of antimicrobial resistance among *Aeromonas* isolates have been reported by previous researchers also (Diker et al. 1984; Megraud 1986; Das and Paranjape 1990; Jindal et al. 1993; Kienzle et al. 2000).

A number of researchers have previously reported the MIC values for aeromonads (Ko et al. 1998; Kim et al. 2011; Bakken et al. 1988). However, there have been no studies reporting the same from animal isolates. Therefore the MIC data of the present study could not be compared with previous reports.

Though gastrointestinal infection of *Aeromonas* is a self-limiting disease and antimicrobials are indicated for only severe and unresponsive cases of *Aeromonas* gastroenteritis (Phavichitr and Catto-Smith 2003), resistant strains of aeromonads as identified in the present study do pose a clinical challenge.

Multiple antibiotic resistance further complicates the situation. Previous researchers also documented, multiple resistance in aeromonads (Vivekanandhan et al. 2002). In the present study, all isolates were multi-resistant with canine isolates harbouring more resistance than swine isolates. This perhaps indicated greater antimicrobial usage in canine clinical cases compared to porcine cases.

CONCLUSIONS

The results of the present study reported incidence of virulent, drug resistant *A. caviae* isolates in canines and pigs from northeast India highlighting possible risk of human infection or contamination of food and water from these sources.

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