Full Length Article

CONVENTIONAL ISOLATION AND MOLECULAR-LEVEL IDENTIFICATION OF AEROMONAS FROM MEAT SAMPLES: A FOOD SAFETY PERSPECTIVE

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ABSTRACT

Foodborne diseases pose a significant threat to public health, with bacterial contamination of meat being a major concern. Among emerging foodborne pathogens, Aeromonas species have been linked to gastroenteritis, septicemia, and wound infections. This study aimed to isolate and identify Aeromonas species from retail meat samples using conventional cultural and biochemical methods, followed by molecular confirmation through polymerase chain reaction (PCR). A total of 200 meat samples (50 from each type: fish, chicken, mutton, and pork) were collected from various retail markets in Chennai, India. Presumptive Aeromonas isolates were identified using selective plating on Ampicillin Dextrin Agar (ADA), followed by biochemical tests such as oxidase, catalase, Triple Sugar Iron (TSI) test, and citrate utilization. Molecular characterization was performed using species-specific primers targeting 16S rRNA, gyrB, and rpoB genes. The highest prevalence of Aeromonas species was found in pork (62%), followed by mutton (48%), fish (44%) and chicken (36%). PCR analysis confirmed the presence of Aeromonas species and distinguished different strains, validating the accuracy of biochemical identification methods. The study underscores the necessity of integrating conventional and molecular techniques for reliable identification and surveillance of Aeromonas in food sources. Enhanced regulatory measures and routine monitoring of meat products are essential to mitigate the risks associated with Aeromonas contamination and to safeguard public health.

Keywords: *Aeromonas*, conventional isolation, molecular identification, food safety, PCR.

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INTRODUCTION

Meat remains one of the most valuable sources of high-quality protein, essential amino acids, and micronutrients in the human diet. However, its high moisture and nutrient content also make it highly susceptible to microbial contamination during slaughter, processing, transportation, and retail handling. Foodborne pathogens such as *Salmonella, Escherichia coli, Listeria monocytogenes*, and *Aeromonas* spp. have been widely implicated in meat-associated outbreaks and pose significant public health concerns (Janda and Abbott, 2010). Among these, *Aeromonas* spp. are increasingly recognized as emerging foodborne pathogens capable of causing gastroenteritis, septicemia, and extraintestinal infections in humans (Huys *et al*., 1997).

The frequent detection of *Aeromonas* in retail meat is attributed to cross-contamination from water, equipment, and poor hygiene during processing (Persson *et al*., 2006). These bacteria are psychrotolerant and resilient, enabling them to survive refrigeration, withstand processing stress, and persist throughout the meat supply chain (Varela *et al*., 2016). Their ability to colonize a wide range of environments, coupled with the rising prevalence of antimicrobial resistance within the genus, further intensifies their significance as potential meatborne hazards (Fosse *et al*., 2012).

Accurate identification of *Aeromonas* species is crucial for food safety surveillance. Conventional culture-based methods remain the first step for isolating presumptive colonies, yet species-level differentiation is challenging due to phenotypic similarities among closely related taxa (Janda and Abbott, 2010). Molecular assays, including PCR targeting conserved genes, provide more reliable and rapid confirmation, facilitating precise identification and supporting risk assessment in the food sector (Sen and Rodgers, 2004). In this context, the present study focuses and offering a food safety perspective on their occurrence in retail markets. Understanding their prevalence and accurate detection is essential for designing effective interventions, improving hygienic practices, and reducing public health risks associated with contaminated meat.

MATERIALS AND METHODS

Sample Collection

A total of 200 meat samples, including fish (n=50), chicken (n=50), mutton (n=50), and pork (n=50), were collected from retail shops in Chennai (Table 1). The samples were collected in sterile sample bags and transported to the laboratory under refrigerated conditions (4°C) within two hours to maintain microbial integrity. The samples were stored at -20°C until further processing.

Pre-enrichment and Selective Isolation

To increase the recovery of *Aeromonas* spp., 25 g of each meat sample was homogenized in 225 mL of Alkaline Peptone Water (APW) and incubated at 37°C for 18–24 hours. The enriched samples

were streaked onto Ampicillin Dextrin Agar (ADA), a selective medium for *Aeromonas* species, and incubated at 37°C for 24 hours. Presumptive *Aeromonas* colonies exhibiting yellow pigmentation with a characteristic opaque center were selected for further analysis.

Biochemical Identification

Presumptive *Aeromonas* isolates obtained from selective culture media were subjected to standard biochemical assays for confirmation. All isolates were first examined by Gram staining to determine cell morphology and staining characteristics. Consistent with *Aeromonas* spp., all presumptive colonies were identified as Gram-negative, rod-shaped bacteria.

The isolates were further characterized using the following biochemical tests:

- **Oxidase Test:** Performed using oxidase reagent; development of a purple color within 10 seconds was interpreted as a positive reaction, indicative of *Aeromonas* species.
- **Catalase Test:** A drop of 3% hydrogen peroxide was added to fresh colonies; immediate bubble formation confirmed catalase activity.
- **Triple Sugar Iron (TSI) Reaction:** Slants were inoculated to assess glucose, lactose, sucrose fermentation and gas production. Acidic butt and/or slant, with or without gas and Hydrogen Sulphide (H2S), was recorded as characteristic of *Aeromonas.*
- **Citrate Utilization Test:** Inoculated Simmons citrate agar slants were incubated and monitored for color change. Conversion of the medium from green to blue denoted positive citrate utilization.
- **Lactose Fermentation on MacConkey Agar:** Isolates were streaked on MacConkey agar to differentiate lactose fermenters from non-fermenters. *A. caviae* exhibited pink colonies due to lactose fermentation, whereas *A. hydrophila* appeared as pale, non-lactose-fermenting colonies.

All biochemical test results were interpreted following standard bacteriological guidelines for identification of *Aeromonas* spp.

Molecular Identification

DNA was extracted from confirmed isolates using the Qiagen DNA extraction kit according to the manufacturer's protocol. The concentration and purity of extracted DNA were assessed using a Nanodrop spectrophotometer. Multiplex PCR was performed to confirm the identity of *Aeromonas* isolates and to differentiate species within the genus. The following genes were targeted:

- 16S rRNA: Genus-specific confirmation of *Aeromonas* spp.
- gyrB: Species-specific identification of *A. hydrophila, A. mediae*, and *A. caviae*.
- rpoB:Species-specific identification of *A. veronii.*

PCR reactions were carried out using a thermal cycler with the following conditions:

- Initial denaturation at 95°C for 2 min
- 6 cycles of denaturation at 94°C for 40 sec, annealing at 67°C for 50 sec, and extension at 72°C for 40 sec
- 30 cycles of denaturation at 94°C for 40 sec, annealing at 65°C for 50 sec, and extension at 72°C for 40 sec
- Final extension at 72°C for 2 min followed by a hold at 4°C.

PCR amplicons were analyzed by agarose gel electrophoresis using a 1.7% agarose gel prepared in 1× TAE buffer. Electrophoresis was carried out at 10 V/cm for approximately 1 hour, or until the tracking dye migrated to about two-thirds of the gel length.

Following electrophoresis, the gel was visualized and documented using a Gel Doc™ XR+ system (Bio-Rad, USA). The size of the amplified PCR products was determined by comparison with a standard molecular weight 50bp DNA ladder.

This isolation and identification approach allowed for the accurate detection of *Aeromonas* species from meat samples using a combination of selective culture, biochemical tests, and molecular methods. The integration of molecular techniques helped to confirm species-level differentiation, ensuring precise identification for epidemiological and food safety assessments.

RESULTS AND DISCUSSION

Isolation of *Aeromonas* species

Conventional method of isolation was performed by initial inoculation in nutrient broth (Fig.2) followed by enriched cultures were selectively plated on Ampicillin Dextrin agar (ADA). On Ampicillin Dextrin agar, the colonies of Aeromonas species exhibited the characteristic yellowish green colonies (Fig.3). A total number of 95 presumptive isolates of Aeromonas species were obtained based on colony characters on selective agar viz. 22 from fish, 18 from chicken, 24 from mutton and 31 from pork meat (Table 1).

Gram's staining

The phenotypically identified colonies on Ampicillin Dextrin agar were suspected for of Aeromonas species and were stained by Gram's staining and visualized the morphology of the isolates. For which the isolated colonies from Ampicillin Dextrin Agar were selected and smeared. All the presumptive isolates were gram negative (Fig. 4).

Biochemical test for *Aeromonas* species

Biochemical tests were done as per the procedure mentioned in Bacteriological Analytical Manual protocol. All the presumptive isolates were positive for catalase, oxidase and citrate tests (Fig. 5).

However, growth on Mac Conkey agar (Fig. 6), hemolytic pattern on 5 % sheep blood Agar (Fig.7), fermenting reaction

in Triple Sugar Iron (TSI) agar produced highly variable results. Accordingly, the *Aeromonas* species were co-related with molecular tests and identified.

Aeromonas hydrophila isolates were showing acid butt and alkaline slant with Hydrogen sulphide production in TSI agar slant. The hemolytic pattern on sheep blood agar was variable but 14 out of 20 isolates were shown complete hemolysis. All the isolates produced a pale or colourless non lactose fermenting colonies on Mac Conkey agar (Fig.8a).

Aeromonas veronii isolates produced acid butt and alkaline slant without Hydrogen sulphide production. On sheep blood agar, only 29 isolates out of 59 showed hemolysis (Fig.8b).

All the *Aeromonas mediae* isolates produced acid butt and acid slant without Hydrogen sulphide production. On sheep blood agar, only two isolates out of five showed hemolysis and on Mac Conkey agar, no growth was observed (Fig.8c).

Among the 11, only two *Aeromonas caviae* isolates produced acid butt and acid slant without Hydrogen sulphide production, remaining nine produced acid butt and alkaline slant. On sheep blood agar, only 2 isolates out of 11 showed hemolysis and on Mac Conkey agar, all the 11 produced a clear lactose fermenting colony (Fig. 8d).

DNA extraction from presumptive colonies

DNA extraction was performed by Qiagen kit method as described in materials and methods. Good concentration of DNA was obtained and optimized to 50ng/µl and the A260 to A280 ratio was between 1.8 and 2.0 which showed no contamination of protein or RNA. The optimized DNA was used as template for the PCR assay.

Confirmation of Aeromonas species by PCR

Molecular confirmation of all the 95 *Aeromonas* species isolates which were presumptively confirmed by conventional culture and biochemical test were further confirmed by multiplex PCR assay targeting 16S rRNA, *gyr*B and *rpo*B gene. All the 95 isolates amplified universal genus specific 16s rRNA gene with a specific product size of 461bp. Further, with species specific *rpoB* primer *A. veronii* amplified 224bp product and with *gyr*B primers, a product size of 144bp and 70bp respectively for *A. hydrophila*, and *A. caviae* and the same primer amplified 99bp amplicon for *A. mediae*. The PCR products were confirmed by Agarose gel electrophoresis (1.7%) with a 50bp DNA ladder and were visualized under Gel Documentation system (Fig. 9).

Occurrence of Aeromonas species in various meat samples

The percent occurrence of *Aeromonas* species in various meat samples viz. Fish, Chicken, Mutton and Pork were

44%, 36%, 48% and 62% respectively. *Aeromonas* species wise percent occurrence resulted in *A.hydrophila, A. veronii, A. mediae*, and *A. caviae* were 21.05% (20), 62.11% (59), 5.26% (5), and 11.58% (11) respectively (Table 2).

CONCLUSION

This study demonstrates a high prevalence of *Aeromonas* species in retail meat sold in Chennai, with pork showing the greatest contamination, followed by mutton, fish, and chicken. The integration of selective culture, biochemical assays, and multiplex PCR provided reliable identification of *A. hydrophila, A. veronii, A. mediae*, and *A. caviae*. Among these, A. veronii was the most frequently detected species, indicating its dominant distribution in meat environments. The presence of potentially pathogenic Aeromonas species in commonly consumed meats underscores a significant public health concern, especially considering the organism's ability to survive refrigeration and its association with virulence and antimicrobial resistance.

These findings highlight the need for routine monitoring of meat hygiene, improved sanitation at slaughterhouses and retail points, and strict adherence to food safety regulations. Public awareness on proper handling and cooking of meat is essential to reduce the risk of Aeromonas-associated foodborne infections. Future studies should focus on whole-genome characterization, assessment of virulence gene expression, and antimicrobial resistance profiling to better understand the epidemiological significance of Aeromonas in the food chain.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

Table.1. Sample collection Details

Place of sample collection	Sampling location (Chennai)	No. of Samples			
		Fish	Chicken	Mutton	Pork
	Outlet 1 (Light house)	33	-	-	-
	Outlet 2 (Chintadripet)	17	8	3	-
	Outlet 3 (Vepery)	-	4	13	-
	Outlet 4 (Choolai)	-	16	8	-
	Outlet 5 (Anna Nagar)	-	12	7	20
	Outlet 6 (Aaduthotti,Pulianthope)	-	-	19	-
	Outlet 7 (Moolakadai)	-	-	-	21
	Outlet 8 (Koyambedu)	-	10	-	9
	Total	**50**	**50**	**50**	**50**

Table.2. Number of *Aeromonas* Positive Isolates - Species Wise

	Fish	Chicken	Mutton	Pork	Pork
A. hydrophila	10	1	5	4	20 (21.05%)
A. veronii	3	17	17	22	59 (62.11%)
A. mediae	2	-	1	2	5 (5.26%)
A. caviae	7	-	1	3	11 (11.58%)
Total	**22 (44%)**	**18 (36%)**	**24 (48%)**	**31 (62%)**	**95 (47.5%)**

Table.3. Primers used in the mPCR method for Aeromonas species identification

S. No.	TARGET ORGANISM and GENE	PRIMER SEQUENCE (5'-3')	SIZE (bp)
1.	*Aeromonas* Universal - 16S rRNA	F - CGACGATCCCTAGCTGGTCT R - GCCTTCGCCACCGGTAT	461
2.	*A. hydrophila* - gyrB	F - AGTCTGCCGCCAGTGGC F - CRCCCATCGCCTGTTCG	144
3.	*A. veronii*– rpoB	F -CGTGCCGGCTTTGAAGTC R – GATCACGTACTTGCCTTCTTCAATA	224
4.	*A. mediae* - gyrB	F – GGCCAAGCGTCTGCGT R - CGCCCTCGTAGCAGAAGTGA	99
5.	*A. caviae* - gyrB	F - TGCTGCTGACCATCCGC R - GGTGCCTGCGGCTCG	70

***Primers designed for this research study using NCBI- Primer BLAST**

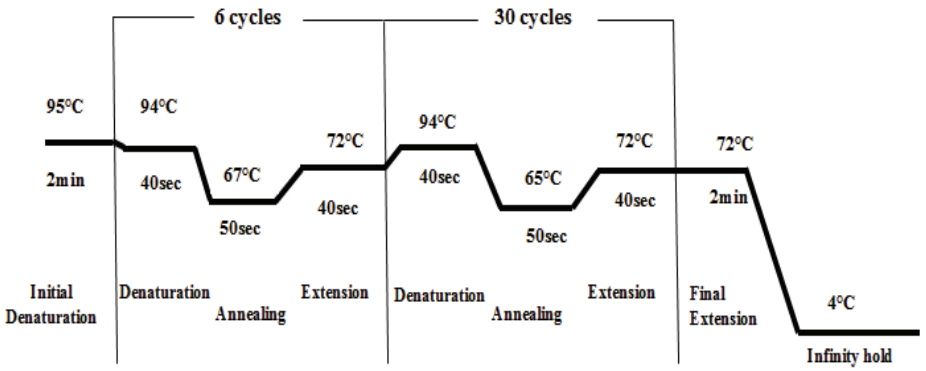


Fig.1. PCR Cycling Conditions for Amplification of Genus (16S rRNA) and Species (gyrB, rpoB) specific Target Genes.

Fig.2. Enrichment of meat samples in Nutrient broth.

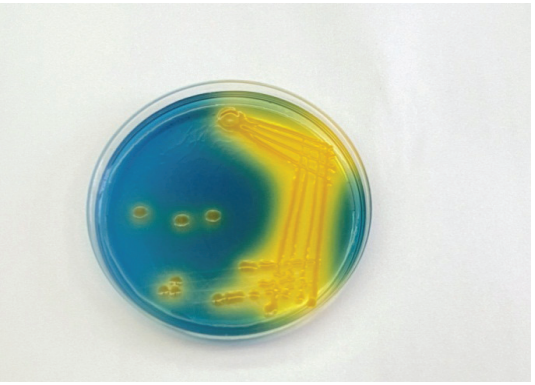


Fig.3. Growth of Aeromonas spp. on Ampicillin Dextrin Agar (ADA)

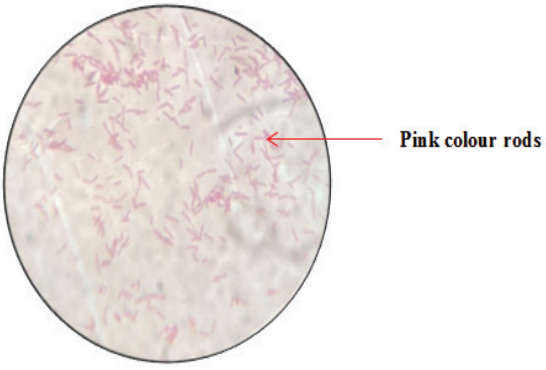


Fig.4. Gram Staining of Aeromonas spp. Showing Pink-Colored Rods

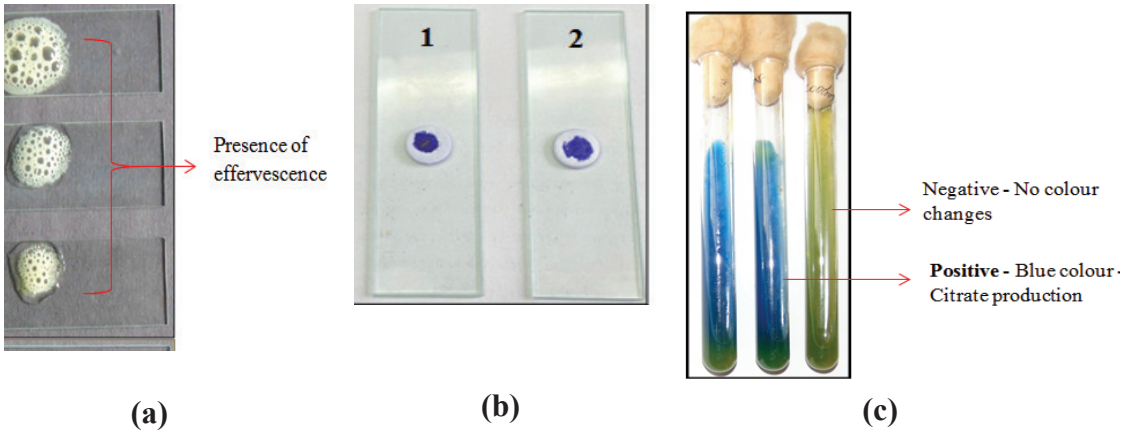


Fig.5. a) Catalase Test, b) Oxidase test, c) Citrate utilization test

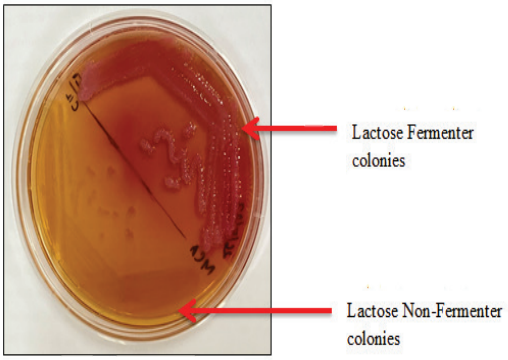
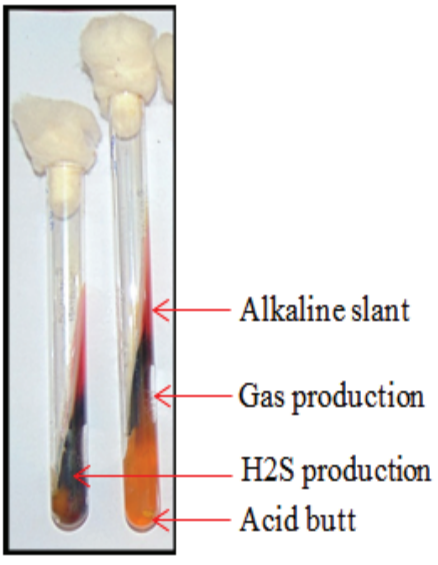
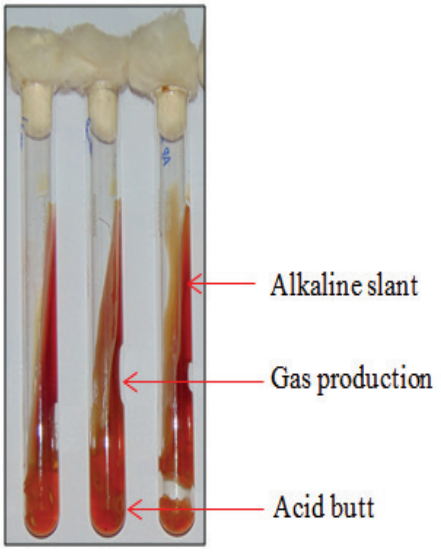


Fig.6. MacConkey agar

Fig.7. Haemolysis pattern on Sheep blood agar



8(a)



8(b)

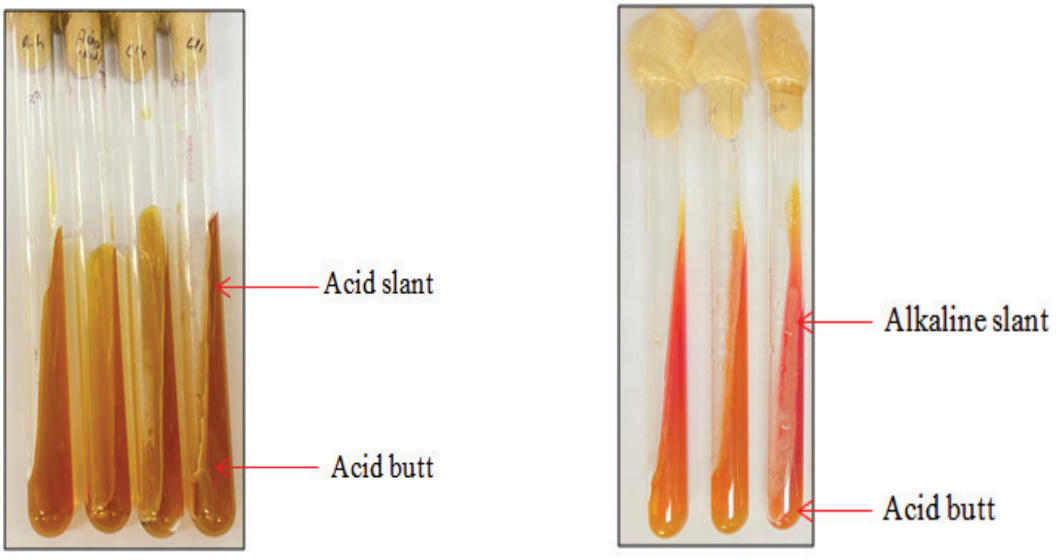
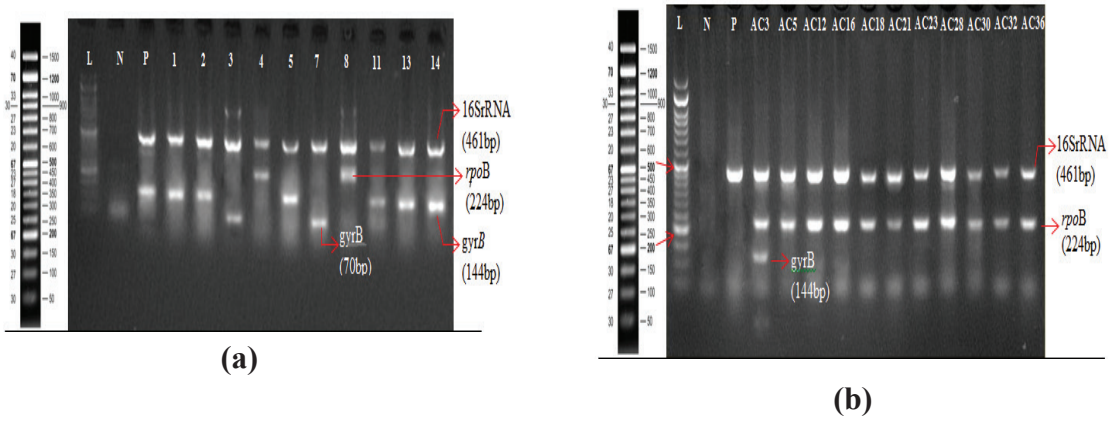


Fig.8. Biochemical tests for Aeromonas sp.



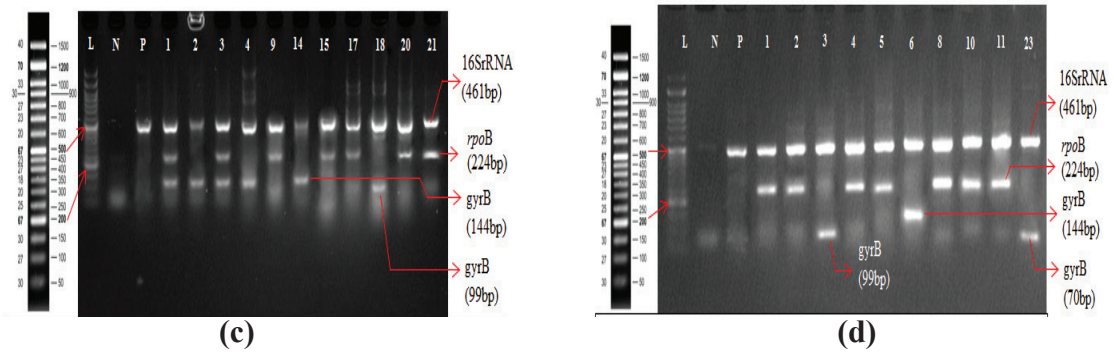


Fig.9. Fresh meat sample isolates analyzed by mPCR for Aeromonas species.Lane 1, 50-bp DNA marker, Lane N- Negative control, Lane P- Positive controla)Lane 1 to 14- Fish meat samples, b)Lane 3,5, 12,16,18, 21,23,28,30, 32 and 36 – Chicken meat samples, c)Lane 1 to 4, 9, 14, 15, 17, 18, 20 and 21 – Mutton meat samples, d)Lane 1 to 6, 8, 10, 11, 23 – Pork meat samples

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