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Pathogenicity of different *Aeromonas* spp and theit antibiotic susceptibility, the diagnosis of different *Aeromonas* spp - REVIEW

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INTRODUCTION

Aeromonas spp are ubiquitous Gram negative bacilli, now a day classified within the Aeromonadaceae family. The species of this genus have long been known to cause different type of infections in fish, reptiles and amphibians, and some species mainly, *A. hydrophila, A sobria* and *A. caviae* have been described as emergent food borne pathogens implicated in human gastroenteritis ranging from mild diarrhea to chlora-like illness. *Aeromonas* have been reported in untreated and chlorinated drinking water, fresh food, seawater, milk, vegetable, ice cream, and several meats, including pork, beef and poultry (Albert *et al.,* 2000, Abbott *et al.,* 2003).

It appears as coccobacilli or with filamentous forms cells are 0.3-1.0X1.0-3.5µm and occur singly, in pairs or short chains. *Aeromonas* species are mainly motile, facultative anaerobic, oxidase positive and glucose fermenting bacteria. The genes *Aeromonas* comprise several species of oxidase negative and catalase positive and non-motile bacteria.

Members of the *Aeromonas* genus are widely prevalent in the aquatic environment and are frequently isolated from various foods, mainly seafood (fish, clams, shrimps etc), meat, milk and vegetables. The number of motile mesophilic *Aeromonas* spp organisms in foods varies from $<10^2$ cfu/g to 10^5 cfu/g. This is also confirmed by, demonstrating that *Aeromonas* spp counts in lamb, veal, pork and minced beef ranged from 10^2 to 10^5 cfu/g. Enumerated the counts of *Aeromonas* spp in foods and found variations from 1×10^2 to 5×10^5 cfu/g. By the 7th day of storage at 5°C, the counts of aeromonads increased by 1–3 log or 10–1000 times isolated *Aeromonas hydrophila* from a variety of foods (red meat, poultry meat, eggs and raw milk) stored in the fridge.

The main source of *Aeromonas* infection is water, even chlorinated water as well as sea food. Food product like poultry, raw meat and vegetables also may contain enteropathogenic *Aeromonas* spp. The incidence of *Aeromonas* spp in food is high but the majority of *Aeromonas* spp. Isolates from food are not enteropathogenic strains are mainly represented by *Aeromonas veroni, Aeromonas sobria* and *Aeromonas hydrophila.* Food borne gastroenteritis associated with *Aeromonas* spp has been reported in humans from all age groups and is particularly severe in risk populations like very young children and old immune compromised patients. It is important that *Aeromonas* spp, found in food are able to produce different exotoxins, some of which are clearly enterotoxins.

Broiler carcass and carcass parts have been contaminated to important level with motile *Aeromonas* species and it has been risk for public health. Furthermore, isolated *A. hydrophila* in nearly 3500 wild and pet birds provide statistically significant evidence that the composition of the intestinal flora may depend on dietary habits. The infection was found in 1.9 of the carnivorous and herbivorous species, in 7.1% of the omnivorous and in 12.4% of the carnivorous and insectivorous birds. The broad spectrum of infection with *A. hydrophila* is paralleled by a range of virulence factors including adhesions, cytotoxins, haemolysin, and various enzymes. However, most strains of *A. hydrophila* produce enterotoxins, regardless of the source.The presence of several genes encoding for putative virulence factors and phenotypic activities that may play an important role in *A. hydrophila* infection.

Contaminated poultry products are widely accepted as a major source of enteric *Salmonella* and *Aeromonas* infections. Foods of animal origin like fishes and other sea foods, meat and meat products, poultry, eggs, milk and milk products have been reported to be contaminated by these organisms. *Salmonella* and *Aeromonas* have been implicated as potential food poisoning agents and have been responsible for various human infections including gastroenteritis and extra intestinal infections.

Aerolysin is a major factor in the pathogenicity of *A. hydrophila.* The protein crosses the inner and outer membranes of the exporting bacteria as well as the plasma membrane of the exporting bacteria membrane of eukaryotic cell, which it punctures by forming discrete channels.

Organisms of the *A. hydrophila* group occur widely in the environment, especially in water. They are found in both raw and chlorinated water supplies and often exhibit a temporal (seasonal) appearance, their numbers increasing with temperature. Previous work from this

laboratory determined that organisms of the *A. hydrophila* group occurred widely in retail fresh foods of animal origin (fish and seafood, raw milk, red meats, and poultry).Other surveys indicated that these organisms also occurred in retail fresh produce. Although the link between food sand human illness has not yet been established definitely, we sought to characterize the biochemical reactions and virulence-associated factors of these organisms isolated from foods of animal origin and compare their properties with those of organisms of clinical origin.

The *Aeromonas* and *Enterobacteriaceae* share many biochemical characteristics but are easily differentiated by oxidase test for which the *Aeromonas* are positive. Generally, members of the genus are characteristically divided into three biochemically differented group (*Aeromonas hydrophila, Aeromonas caviae*, and *Aeromonas sobria*), and these contain a number of genomospecies, and recently, new species have been added (Abbott *et al.*, 1998, Janda *et al.*, 2010).

The short period of shelf life of poultry meat at refrigerator temperature can be associated with its composition, but also with spoilage microorganisms present during poultry rearing and primary production. These microorganisms can multiply at a relatively low temperature, and the result of their metabolic activity is manifested as product spoilage,(Singh,1993) and consequently, they are the most important factors of chicken meat shelf life. The shelf life of poultry meat depends on the initial number of microorganisms, which emphasises the importance of hygienic conditions and control during various stages of the production process. Decomposition processes are manifested by a change in specific sensoric properties of meat. In a majority of cases, the sensoric changes and the degree of contamination with microorganisms, and their biochemical activity, are in correlation with the meat ammonia content (Zivkovic, 1986).

Aeromonas hydrophila has been associated with several disease conditions, including tail and fin rot and haemorrhagic septicaemia. Fish pathogenic isolates produced various toxin of largely undermined function. Interestingly, the presence of literal flagella has been considered to be related to the presence of toxigenic properties. Surface structures of *A. hydrophila* have been associated with autoaggregation, hydrophobicity and heamagglutination. The surface of such strains has been shown to be completely covered by a regularly arrayed surface layer(s-layer) that may provide production for microorganisms in their natural environment, composed of tetragonally arranged subunits. S-layers are present on the number of human pathogenic bacteria,

including *Campylobacter* spp, as well as many non-pathogenic bacteria.

Foodborne diseases are an important public health problem in both developed and developing countries. *Aeromonas* have emerged as foodborne pathogen with worldwide distribution. There are currently 15 identified and 2 non-identified species that can be categorized into at least 17 DNA hybridization groups. *Aeromonas* are isolated worldwide from drinking, estuary and wastewater and also from different kinds of food (Merino *et al.,* 1995).

Motile *Aeromonas* species were present in all samples including retail lamb meat and offal; so it was concluded that meat products were potentially significant sources of virulent *Aeromonas* species and might play an important role in the etiology of *Aeromonas gastroenteritis* (Majeed *et al.,* 1989).

Since meats products are important source of nutrition and could act as a factor in transfer of pathogenic strain and since there is no any published report as to the prevalence and patterns of *Aeromonas* species isolated from raw meat in Iran, *Aeromonas* strains isolated from retail raw chicken samples and minced meat in regions under supervision of TUMS (Avadisians*et al.,* 2012).

Most of the *Aeromonas* species isolated from either clinical or environmental sources are being classified with multitest systems such as API-20E. However, it was reported that the commercial miniaturized systems are not always adequate for the identification of environmental strains devised a multitest medium for the rapid presumptive identification of *A. hydrophila* (AH medium).

The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 20 years. Valera and Esteve (2002) have reported that it currently constitutes a new family, Aeromonadaceae, and that the number of recognised species in the genus *Aeromonas* increased from 4 to 16. In spite of this progress, many questions concerning the taxonomy of this genus remain unresolved, among them the identification of new isolates to the species level.

Only 5 species of *Aeromonas* were recognised twenty years ago and out of the concerned *A. hydrophila, A. sobria,* and *A. caviae* existed as phenol species, which means a named species containing multiple DNA groups, and whose members could not be differentiated from one another using simple biochemical characteristics, which were no longer adequate. In recent times, molecular and chemotaxonomic methods have been devised in order to identify *Aeromonas* spp and these represented some improvement. However, remarkable discrepancies

were observed in association with DNA/DNA homology data and16S rRNA sequencing data. On the other hand, there is still some confusion regarding the determination of the appropriate assignment of *Aeromonas* strains to the recognised species using biochemical characters, and further assessment is needed to overcome this confusion. It has been reported that the use of available diagnostic kits and phenotypic schemes was not advisable for making such precise identifications.

The emergence and dissemination of antibiotic-resistant bacteria are a serious concern worldwide. The dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria, and acquisition of antibiotic resistance genes is facilitated by mobile genetic elements such as integrons.

Although tissue reaction against *A. hydrophila* infection was studied in details at different aquatic species the available literatures did not cover the pathogenicity of this microorganism in chickens either in tissue or ultra structural level. Due to public health significance of *A. hydrophila* and little information regarding incidence, pathogenicity and ultra structural cellular changes of *A. hydrophila* in chickens. The biochemical reactions of the isolates and the ability of the isolated strains to exotoxin assays, Congo red (CR) binding test, Crystal violet (CV) binding and antibiogram. Experimental infection of broiler chicks with the isolated *A. hydrophila* was done to study the postmortem, histopathological and ultrastructural changes in this avian species in case of *A. hydrophila* infection (Yogananth, 2009).

Virulence of *Aeromonas* spp is multifactorial and incompletely understood. Factors contributing to virulence include toxins, protease, haemolysins, lipase, adhesions, agglutinins, and various hydrolytic enzymes. These virulence factors are useful in distinguishing between potentially pathogenic and non-pathogenic strains. Some investigators observed that *Aeromonas* induced gastroenteritis is due to an enterotoxin which is cytotoxin in nature but Stelma *et al*, 1986 reported aerolysin to be the main virulence factor involved in intestinal disorders. About 6.5% of diarrhoeal cases in the southern part of India have been attributed to *Aeromonas.* This indicates an urgent need for information on the casual role of this pathogen in other parts of the country.

Aeromonas sobria strains produced more intensively haemolysin and enterotoxin than *Aeromonas hydrophila*, whereas *Aeromonas caviae* isolates were not haemolytic and enterotoxigenic. Data reported by evidence that 48% of isolated *Aeromonas hydrophila* strains

were haemolytic and 92% – cytotoxic. Strains isolated from meat were mainly cytotoxigenic, and then came enterotoxigenic and haemolytic *Aeromonas hydrophila*. All *Aeromonas hydrophila, Aeromonas sobria* and *Aeromonas caviae* strains detected by and were enterotoxigenic. The *aerA* gene coding for aerolysin was present only in 3 *Aeromonas hydrophila* isolates but wasnot present in any of *Aeromonas caviae* and *Aeromonas veronii, A. biovars, A. sobria* strains. None of 17 strains in another studypossessed the haemolysin coding gene.All strains isolated by possessed virulence genes, coding for haemolysin and enterotoxin, while the isolates of were enterotoxigenic.

The contamination of meat with *Aeromonas* spp, according to is due to washing of carcasses with water containing *Aeromonas* spp and poor hygiene during processing and realisation of meat (cutting and mincing). Speculate that the causes for meat products contamination with *Aeromonas* spp were the neglected control of raw meat and fat and inadequate sanitary measures during their handling and processing.

Ability of local isolates of *A. hydrophila* in haemolysin production as a major virulence factor was studied. Haemolytic activity was examined by measuring the diameter of hydrolysis zone around each bacterial isolate on blood agar medium containing 5% human blood after 24 hours of incubation at 35°C.

The virulence of *Aeromonas* is multifactorial, including adhesions, S-layer, lipopolysaccharides, siderophores, and an array of exoenzymes and exotoxins, i.e. aerolysin/haemolysin, lipases, proteases among others. Genes encoding these virulence factors have been isolated and sequenced allowing the detection of signature regions of these genes and the evaluation of their presence in *Aeromonas* clinical and environmental isolates. Moreover, the regulation and secretion processes of virulence factors, as well as the host response alter the pathogenicity of *Aeromonas.* Antimicrobial resistance among enteric pathogens is a serious problem in developing countries where there is a high frequency of gastroenteric illness and many antibiotics fall routinely into inadequate use. Antibiotic resistance is particularly relevant in pathogenic *Aeromonas* species in which, besides the classical resistance to β-lactamic antibiotics, multiple-resistance has been frequently identified. These bacteria can receive and transfer antibiotic resistance genes to other Gram negative bacteria.

A. hydrophila identified one protease from isolates of *A. hydrophila.* Purified two proteases from *A. hydrophila* ATCC 14715, whereas two and, subsequently, three proteases were

reported from *A. hydrophila* AH22 by and found several proteases in strain P50. Detected four or five zones of proteolytic activity with isoelectric focusing in crude extracts from strain B5. Unfortunately, these investigators worked on different strains of *A. hydrophila,* and in each case only a single strain was completely examined. The use of different media and growth conditions and a variety of assay and purification procedures also make the literature reports very difficult to interpret and compare. The objective in this work was to assess the extent of differences in the proteases produced by strains of *A. hydrophila*, by making direct comparisons on many isolates, grown under uniform cultural conditions. To provide a better basis for comparison of the proteases, a combination of inhibition patterns and thermo stability characteristics was used. In addition, it was recognized that serological comparisons were essential, which required purification of proteases(Amborski, 1984).

The biofilm is a complex aggregation of microorganisms growing on a solid substrate. Biofilm are the microbial communities that are attached and encased in a matrix of exopolymeric material. Biofilms have common mechanisms by which they can adhere to surface of each other. Dental plaque is a formation of biofilm. In industrial environments biofilms can develop on the interior on pipes and lead to clogs and corrosion. In medicine, biofilms spreading along implanted devices are wires can lead to pernicious infections in patients.

Different methods as DNA-DNA hybridization, SDS-PAGE analysis of cell proteins, RAPD patterns, PCR, pulsed-field gel electrophoresis, 16s rDNA-RFLP analysis and plasmid DNA have been used to type isolates. However, these methods are not generally accepted as standard systems for the evaluation of *Aeromonas* isolates, as a standard method should be simple, rapid, inexpensive, reliable, and applicable in any kind of routine laboratory.

SDS-PAGE of protein pattern has been widely used for typing strains within particular bacterial species. *A. caviae* and *A. veronii* recovered from clinical and environmental samples were characterized by SDS-PAGE of whole cell proteins and all strains were typable and showed unique bandingpatterns. SDS-PAGE remains a powerful method for structural studies and for typing and classification of microorganisms (Dijkshoorn *et al.*, 2001).

The detection methods of aerA was recently proposed as a reliable approach by which to identify a potential pathogenic *Aeromonas* strain by using methods involving PCR and restriction fragment length polymorphism analysis, the virulence genes of *Aeromonas* spp. Were grouped as aerolysin haemolysins, cytolytic enterotoxins, or cytotoxinic enterotoxins.

The culminating quarter of the previous century witnessed the explosion of scientific interest in members of the genus *Aeromonas* as human and animal pathogens. Aeromonads have been implicated as potential food poisoning agents. Foods of animal origin like fishes and other seafoods, meat and meat products, eggs, milk and milk products have been reported to be contaminated by *Aeromonas* spp. Difficulty in recognizing potentially significant *Aeromonas* strains in foods poses a dilemma for public health authorities. The conventional microbiological procedures for isolating and identifying *Aeromonas* spp from foods are laborious and time consuming. Some novel approaches like polymerase chain reaction (PCR) have been used for detection and identification of *Aeromonas* spp from foods or environmental or clinical samples.

There is a need to develop rapid techniques, and to assess the best technique out of them, for detection of *Aeromonas* spp from food samples and to compare their results with the conventional cultural procedures. Thus, the present work was carried out to standardize an OMP based indirect plate ELISA, 16 a duplex-PCR and to evaluate the suitability of the developed assays for detection of *Aeromonas* from foods of animal origin. The results of the developed assays were compared among themselves and with conventional cultural method.

REVIEW

Lore Anne Mcnicol *et al.* (1980) reported that the antibiotic-resistant strains of *Aeromonas hydrophila* have been isolated, from the natural environment in the Chesapeake Bay and areas surrounding Dacca and the Mat lab region of Bangladesh. The Bangladesh strains carried resistance to Chloramphenicol, Streptomycin, and Tetracycline, and 57% of them had a multiple Streptomycin, Tetracycline resistance phenotype correlated with the presence of a large plasmid. The Chesapeake Bay strains were resistant to polymyxin B and tetracycline, but showed neither multiple resistance nor R-factor carriage. Toxigenicity showed no positive correlation with drug resistance or with plasmid carriage. Environmental areas of heavy human impact appear to be associated with a higher incidence of antibiotic resistant strains of aeromonads.

Popoff and Lallier (1984) reported that a total of 154 food samples (chicken, fish, and ready-to-eat sprouts) from various retail outlets in Mumbai, India, were analyzed for the presence of *Aeromonas* spp over a period of 2 years. Twenty-two *Aeromonas* isolates belonging to 7 different species were isolated from 18 (11.7%) food samples. The highest percentages of isolation were from chicken (28.6%) followed by fish (2%) and sprout (2.5%) samples.

Aeromonas caviae, *A. veronii*, *A. sobria*, and *A. salmonicida* were the most frequently isolated species from sprouts, chicken, and fish samples, respectively. The genes encoding for putative virulence factors, cytotoxic enterotoxin (act), hemolysin (hly), aerolysin (aer), and lipase (lip) were detected using polymerase chain reaction method in 59.1%, 4.9%, 22.7%, and 31.8% of the strains, respectively. The isolated *Aeromonas* strains were found to be positive for virulence factors, that is, amylase, DNAse, gelatinase, protease, and lipase production. More than 6% isolates were also positive for β-hemolytic activity. All these food isolates were found to be resistant to Ampicillin, Bacitracin, and sensitive to Gentamicin, 3rd-generation Cephalosporins (Ceftazidime, Cephotaxime, Ceftriaxone), and Chloramphenicol. Seventeen (77.2%) isolates harbored single and/or multiple plasmids. The XbaI digestion patterns of chromosomal DNA of these isolates, using pulsed field gel electrophoresis, showed high genetic diversity among these isolates. Our results demonstrate the presence of various *Aeromonas* spp with virulence potential and antimicrobial resistance in different food products marketed in Mumbai, India. The potential health risks posed by consumption of these raw or undercooked food products should not be underestimated.

Palumbo *et al.* (1985) reported that interest in *Aeromonas hydrophila* as a food-borne and human pathogen is increasing. Isolation media from the clinical laboratory were evaluated for food use and either did not give quantitative recovery of *A. hydrophila*. A new medium was developed which permitted quantitative recovery of *A. hydrophila* from foods. The medium consisted of phenol red agar base, soluble starch (10 g/litre), and Ampicillin (10 mg/litre). All foods survey contained *A. hydrophila.* Food samples included red meats, chicken, raw milk, and seafood. The count of *A. hydrophila* at the time of purchase ranged from 1×10^2/g (lower limit of detection) to 5×10^5/g. In most instances, the count of *A. hydrophila* increased during 1 week of storage at 5°C.

Alicia Toranzo *et al.* (1986) reported that the important biochemical reactions in conventional tests were compared with counterpart reactions in two multiple test systems, API-20E and *Aeromonas hydrophila* medium, to evaluate their accuracy for the identification of motile *Aeromonas* spp. isolated from chicken. In a total of 49 *Aeromonas* spp isolates and ten *A. hydrophila* reference strains, false-negative or positive reactions were detected in the Voges-Proskauer test, Indole production, gelatinase activity, production of gas, fermentation of arabinose, and lysine decarboxylase reaction. A good correlation was found, among the three

identification systems, for the fermentation of mannitol and inositol as well as for the arginine dihydrolase and ornithine decarboxylase tests. The failure of *A. hydrophila* medium in the detection of gas indicates that this medium is not entirely suitable for defining aerogenic or anaerogenic strains.

Rita Brenden *et al.* (1987) reported that the Aeromonads had the ability to produce haemolytic activity and significantly associated with strains belonging to the *Aeromonas hydrophila* and *A. sobria* groups. Furthermore difference in the stability of the haemolysis of selected *A. sobria* and *A. hydrophila* isolates at different temperatures, and in the presence of urea or dithiothreitol were observed.

Palumbo *et al.* (1989) suggested that a recent survey of retail fresh foods of animal origin (fish and seafood, raw milk, poultry, and red meats) for organisms of the *Aeromonas hydrophila* group isolated the representative strains from the various foods. In this study, it is characterized that these isolates for biochemical properties and virulence associated factors and to compare the food isolates with clinical isolates. Hence identified all food and clinical isolates as *A. hydrophila* and found that all isolates were typical in their biochemical reactions. Examination of the isolates for various virulence-associated factors indicated that most food and clinical isolates were serum resistant, beta-haemolytic, cytotoxin positive, hemagglutinin positive, Congo red positive, and staphylolysin positive.

Kirov *et al.* (1990) reported that the possible role of *Aeromonas* spp as potential food-borne psychotropic pathogens were investigated by examining organisms isolated from processed raw chicken for their biochemical characteristics, ability to produce exotoxins and to grow at chill temperatures. These strains, in particular *A. sobria,* with identical characteristics to human diarrhoea-associated aeromonads were readily found. Chicken, human and environmental (water) strains characterized in a previous study, were investigated for their ability to grow at refrigeration temperatures (5 +/- 2°C) and, for selected strains, the theoretical minimum temperature for growth was determined from the growth pattern in a temperature gradient incubator. All enterotoxigenic chicken strains tested were typical mesophiles, with an optimal growth temperature of approximately 37°C. They were rapidly outgrown by a psychotropic *Pseudomonas* spp typical of spoilage biota found on food. Enterotoxin was not produced below 15°C by any of the toxigenic food strains tested. The *Aeromonas* strains isolated from chickens seems unlikely therefore causing significant health risk, provided the chickens are properly

stored and cooked. This would appear to be substantiated by the lack of reports of food associated outbreaks of illness from these sources.

Krovacek *et al.* (1994) determined that *Aeromonas* spp comprise a complex group of ubiquitous bacteria. They were widely distributed and often isolated from clinical environmental and food samples.

Singh and Umadatt (1997) suggested that two commercially available media, Ryan's Aeromonas medium (RAM) and Starch Ampicillin Agar (SAA), were compared for their ability to recover *Aeromonas* spp from pure culture, In all instances SAA medium proved to be superior for recovery of *Aeromonas* spp. Selectivity with SAA and GSP was better than with RAM with 100% of typical colonies confirming as *Aeromonas* spp. The incidence of motile *Aeromonas* spp in ground meat samples in Eastern Canada was determined during a 1 year period using SAA as the isolation medium. *Aeromonas* spp was found in 4 of 4 ground pork, chicken, turkey, and sausage samples and in 15 of 19 ground beef samples. Two hundred and ten presumptive *Aeromonas* isolates were characterized biochemically to the species level and were identified as *Aeromonas hydrophila.* Of the isolates from chicken and turkey, 40 and 56% respectively were found to be this latter species. The numbers of *Aeromonas sobria* and *Aeromonas caviae* isolated from these products were 30 and 20% for chicken and 8 and 16% for turkey respectively.

Chamorey *et al.* (1998) stated that two polymerase chain reaction (PCR) based methods were used for epidemiological typing of *Aeromonas hydrophila* Random amplification of polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) were applied to an outbreak involving seven patients. The epidemiological situation appeared complex, with the exception of two clinical isolates; all gave unique patterns with both techniques. The coincidental clustering of patients infected with *Aeromonas hydrophila* probably resulted from an increased prevalence of aeromonads in water during summer, although no single RAPD or ERIC-PCR patterns were found both clinical and environmental samples. RAPD and ERIC-PCR proved to be effective for the epidemiological study of *Aeromonas hydrophila.*

Vasaiker *et al.* (2002) reported that a total of 67 *Aeromonas* strains were located as the bacterial pathogen from 1485 with acute gastroenteritis *A. hydrophila* (64.2%) was the predominant isolate followed by *A. sobria* (28.4%) and *A. cavia* (7.4%). Majority of the isolates were sensitive to Gentamicin, Nalidixic acid but were resistant to Ampicillin. Minimum inhibitory concentration (MIC) of resistant strains of *Aeromonas* to Ampicillin ranged from 80-1280µg/ml.

Arora *et al.* (2005) reported that the most commonly used methods for detection of aeromonads in food of animal origin. With this objective an OMP based indirect plate ELISA and a duplex-PCR using primers targeting aerolysin gene and 16S rRNA gene and yielding amplicons of 252 bp and 599 bp, respectively, were standardized. The standardized protocols and the conventional cultural method were then compared for their respective sensitivities and specificities for detecting aeromonads from chicken and milk samples. Both the standerdized assays were found to be highly specific for *Aeromonas.*

Yu-Chang Chang *et al.* (2007) reported that one hundred thirty-three *Aeromonas* spp. Isolates were examined for multiple antibiotic resistance phenotypes and prevalence of class 1 integrons sequence. Twenty four (18.0%) of these isolates contained class 1 integrons. Seven different classes 1 integrons were found among 24 strains, with a total of 10 different gene cassettes encoding for resistance trimethoprim, aminoglycosides, β-lactam antibiotics, Chloramphenicol, and quaternary ammonium amines with unknown function. Rate of antibiotic resistance was different between integron-positive and integron-negative strains. Trimemoprim and Trimethoprim, Sulphamethoxazole resistance were commonly associated with integron and all of integron positive isolates were multiple resistant to more than 3 agents.

Khalifa Sifaw Ghenghesh *et al.* (2008) reported that the roles of Aeromonas species in gastroenteritis are recognized as etiological agents of a wide spectrum of diseases in man and animals. In developing countries, potentially pathogenic *Aeromonas* spp is very common in drinking water and in different types of foods, particularly seafood. Several food-borne and water-borne outbreaks as well nosocomial outbreaks associated with aeromonads have been reported. Significant association of *Aeromonas* spp. with diarrhoea in children has been reported from several countries. These organisms are important causes of skin and soft-tissue infections and aspiration pneumonia following contact with water and after floods. High incidence of antimicrobial resistance, including to third-generation Cephalosporin and the Fluoroquinolones, is found among *Aeromonas* spp isolated from clinical sources in some developing countries in Asia. Isolating and identifying *Aeromonas* spp to genus level is simple and requires resources that are available in most microbiology laboratories for processing common enteric bacteria.

Mohmoud *et al.* (2008) suggested that seventeen isolates of *Aeromonas hydrophila* were isolated from 250 commercial broiler chicks with an incidence of 6.8 %. Most *A. hydrophila* isolates (88.24 %) were positive for exotoxin assay and Congo red binding test, while 52.94 %

were positive for crystal violet binding activity. Most strains of *A. hydrophila* were sensitive to Chloramphenicol, Ciprofloxacin and Norfloxacin followed by Gentamicin and Neomycin while Nalidixic acid, Tetracycline, Streptomycin and Trimethoprim Sulphamethoxazole had moderate effect. On the other hand, all *A. hydrophila* strains were resistant to Amoxicillin, Cephalothin, Erythromycin and Penicillin G. Two experiments were done, in the first experiment, chicks of one day old were infected with 1.5 X 10^9 organisms via subcutaneous and yolk sac. The infected chicks dead within 24 hrs. *A. hydrophila* were isolated from most organs. The lesions observed included congestion in the internal organs and few cases showed hepatic and muscular petechiae. The ultrastructural study of this group showed presence of the bacilli inside the hepatocytes and macrophages with marked cellular changes.

Kaskhedikar and Chhabra (2009) suggested that the fourteen antibacterial agents belonging to 9 different groups of antibiotics viz Aminoglycosides, Cephalosporins, Nitrofurantoin, Fluroquinolones, Chloramphenicol, Sulphonamides, Tetracyclines, Penicillin and Polymixin were used for in vitro sensitivity testing of *Aeromonas hydrophila* isolated from fifteen samples of chicken collected from retail shops in Mhow city. The sensitivity (100%) was attributed to Ciprofloxacin, Cefuroxime, Ceftriaxone, Cephotaxime, Chloramphenicol, Gentamycin, Kanamycin, Nitrofurantoin, Nalidixic acid and Ofloxacin followed by Oxytetracycline (50%). All the isolates were resistant to Ampicillin and Colistin antibiotics. That means, none of the isolates were found to be sensitive for Penicillin and Polymixin group of antibiotics. Multiple drug resistance was also observed in all *A. hydrophila* isolates. Out of total isolates, 100% were resistant to two antimicrobial drugs and 50% to three drugs.

Ozkan Gorgulu *et al.* (2009) suggested that the study of whole cell protein profile of 20 local strains of *Aeromonas hydrophila* isolated from different foods in Turkey and one reference strain was analysed by SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis). It was observed that there was variability among the strains. Molecular weights of proteins were found between 21-116 kDa. The protein polypeptide bands from 37.8 to 101.4 kDa were common in both local strains and reference strain of *A. hydrophila.* The results of the study indicated that there is a genetic similarity between strains of *A. hydrophila* and reference strain. These protein patterns are likely to be beneficial to differentiate between the strains in epidemiological studies.

Abigail Perez-Valdespino *et al.* (2009) reported that determined the presence of class 1 integrons related to the acquisition of resistance to antimicrobials in *Aeromonas* spp isolated from

individuals with diarrhea. Species were identified as *A. caviae, A. hydrophila*, *A. veronii* and media using PCR-RFLP of the 16S rDNA. Selected isolates were further characterized by ERIC-PCR Resistanceto Chloramphenicol, Aztreonam, Tetracycline, Trimethoprim, Sulfamethoxazole, Nalidixic acid and Streptomycin, antibiotic character was determined using the Kirby-Bauer method. Integrons were detected by PCR amplification of the 5′ conserved variable, and 3′ conserved regions. Sequencing of the variable regions revealed class 1 integrons with cassettes encoding resistance to Trimethoprim (dfra12, dfra15, dfrb4), Streptomycin/Spectinomycin (aadA2, aadA1), Oxacillin (oxa2) and Chloramphenicol (catB3, cmlA4).

Abdelraouf *et al.* (2011) reported that the interest of the occurrence of *Yersinia enterocolitica* and *Aeromonas hydrophila,* their pathogenicity and antimicrobial resistance is increasing worldwide because both were linked to acute and chronic gastroenteritis, septicemia and wound infections. Though reports on the occurrence of both pathogens all over the world, no published data are available from Gaza strip. Moreover, there is no routine testing for the detection of *Yersinia* and *Aeromonas* in clinical or environmental samples. The study was conducted to investigate the occurrence, sources of both *Y. Enterocolitica* and *A. hydrophila* in clinical, food and environmental samples.

Avadisians *et al,* (2012) reported that, *Aeromonas* spp was commonly isolated from ground meat and chicken samples at the retail level. From January to September 2009, 92 samples of chicken and 158 samples of minced meat for sale in retail outlets from regions under supervision of Tehran University of Medical Sciences were analysed for the prevalence of *Aeromonas* species. Aeromonads were isolated from 80 (32%) of the samples analysed, including 53 (57.6%) of chicken and 27 (17%) of minced meat. The isolation rate in chicken was significantly higher than minced meat. The highest contamination was found in chicken with *Aeromonas caviae* and minced meat samples contaminated with *Aeromonas hydrophila.*

Stratev *et al,* (2012) suggested that *Aeromonas* spp was isolated from hydrobionts meat, meat products, milk, dairy products and vegetables at densities of 10^2 to 10^5 cfu/g. A substantial risk is posed by the ability of microorganisms of genus *Aeromonas* to grow in foods stored in refrigerator.

Kameli Osman *et al,* (2012) reported that, the meat commonly contain the same *Aeromonas* spp which occur in human diarrhoeal and non-diarrhoeal faecal samples. Motile *Aeromonas* were isolated from 5.6% of total 302 samples. The distributions of the isolates were 5.9 and 5.2% in

fresh and frozen samples, respectively. Of the 302 samples taken of the four animal meat species investigated, the genus *Aeromonas* were isolated in 12.3% of the fresh samples collected from chicken meat, in 6.5% of the samples collected from sheep meat and, 14.0% from the cattle frozen meat samples. *Aeromonas hydrophila* was isolated as the most prevalent species with 6.8%, followed by *Aeromonas caviae* with 2.7% and *Aeromonas sobria* with 2.1% from the total meat samples. Aerolysin toxin gene was detected in 3/17 isolates of *A. hydrophila* isolated from contaminated meat. Infection due to bacterial pathogen with such virulent factor through contact with contaminated meat while handling them, poses health hazards to humans.

Arunava Das *et al,* (2012) suggested that in tropical countries like India, commercial food items are often contaminated by various food-borne pathogens. Recent research work reports the surveillance of *A. sobria* and *A. hydrophila* from commercial food stuffs and environmental sources across Tamil Nadu and Kerala. Samples were aseptically collected throughout the year and processed for isolation and identification of *A. sobria* and *A. hydrophila*. Isolates of *Aeromonas* were characterized for arrays of biochemical and phenotypic traits and finally assayed for antibiotic susceptibility test. All the isolates were 100% resistance to Ampicillin, Carbenicillin, Cephalothin and Clindamycin 100% sensitive to Colistin and moderate to Cefuroxime, Chloramphenicol, Nalidixic acid, Neomycin and Nitrofurantoin. The current research suggested that colistin could be useful for motile *Aeromonas* infection but there has also been prevalence of multi drug resistant strains of Aeromonads in the Southern states of India. The results aided the efforts to prove the strong occurrences of *A. sobria* and *A. hydrophila* as food borne pathogens in human consumable foods than in the environmental samples.

Isoken Igbinosa *et al*, (2012) reported that the *Aeromonas* species are ubiquitous bacteria in terrestrial and aquatic habitat. They are becoming renowned as enteric pathogens of serious public health concern as they acquire a number of virulence determinants that are linked with human diseases, such as gastroenteritis, soft-tissue, muscle infections, septicaemia, and skin disease. Proper sanitary producers are essential in the prevention of the spread of *Aeromonas* infection. Oral fluid electrolytic substitution is employed in the prevention of dehydration, and broad spectrum antibiotic are used in severe *Aeromonas* outbreaks.

Agniswar Sarkar *et al,* (2012) stated that, *Aeromonas hydrophila* isolated from fish *(Labeorohita),* pond water, river water, raw meat of chicken and mutton and raw cow milk were characterized through Random Amplified Polymorphic DNA (RAPD) analysis and Sodium

Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of cellular proteins. RAPD-PCR amplification of bacterial DNA was done by using ten random primers (OPA-01 to 10) and found some distinct banding pattern on agarose gel. RAPD profile was studied with each isolate and absolute polymorphism indicating its application as an ideal tool for molecular characterization. Other methods like morphological, serological and biochemical characterization gives contradictory results and total cellular protein profiling does not show any significant polymorphism for identification and discrimination.

Faby and Alexander *et al,* (2012) reported that, the work concentrates on the detection of human enteric bacteria in artificially contaminated chicken sample. The tests were performed in inoculated chicken samples using *Salmonella enterica* and *Aeromonas hydrophila,* with dilutions of 10^6,10^5,10^4 CFU/ml. Thus developed a direct metagenomic (chicken DNA, inoculated bacterial DNA and endogenous microbial DNA if any) PCR technique for detection of bacteria from this food metagenomic. Amplification of respective bacterial 16S rRNA region was performed. PCR conditions were optimized and amplification of *Salmonella enteric* specific DNA was achieved in all samples inoculated with different concentration of bacterial suspension. *Aeromonas hydrophila* infected tissues failed to reveal a specific amplification even after several modifications in gradient PCR. Interestingly, the control chicken tissues also exhibited a less intense amplification of similar size DNA to target, indicating the possible endogenous contamination of the chicken meat obtained from the retail shop.

Hind Mohammed *et al,* (2013) suggested that, *Aeromonas hydrophila* was isolated from different local sources includes fresh fish, water, and lake of fish farm in Baghdad governorate, and identified according to their morphological and cultural characteristics and biochemical tests. Virulence factors produced by *A. hydrophila* isolates were detected to select the more virulent isolate. The isolates showed that they were having the haemolytic activity, slime production and with high ability in protease production. Antibiotic susceptibility of the more virulent isolate was examined. The study showed that *A. hydrophila* H4 was resistant to many antibiotics, while it was sensitive to others. Plasmid profile *A. hydrophila* H4 was studied by extraction of plasmid DNA and electrophoresis on agarose gel. Results showed that this isolate was harbouring two small plasmids which may be responsible for the production of virulence factors and/or the antibiotics resistance. After curing of these two plasmids by using SDS (6%), it was found that cured colonies of *A. hydrophila* H4 was lost its ability to resist many antibiotics, while it was still

having the ability to produce different virulence factors, which means that genes encoding these virulence factors are chromosomally located.

Dashe *et al*, (2013) suggested that, many bacterial agents have been incriminated in cases of fowl cholera outbreaks in chickens. The study was conducted to determine the prevalence of *Aeromonas hydrophila* infection in chickens affected by fowl cholera between November, 2010 and October, 2011 in Jos, Nigeria. A total of 2000 samples consisting of bone marrow, heart, liver, lung and spleen were aseptically collected from 400 clinically sick chickens suspected to be suffering from fowl cholera and cultured for *Pasteurella multocida* and *Aeromonas hydrophila* organisms. Four hundred oropharyngeal swabs were also collected from 400 apparently healthy chickens for bacteriological analysis. Swab from each sample was cultured on 7% defibrinated sheep blood, Mac Conkey and casein sucrose yeast agar. Presumptive colonies of *P. multocida* and *A. hydrophila* were subjected to biochemical characterization and antimicrobial *P. multocida* 20 (1.0%) was isolated from all the tissue samples of clinically sick chickens, while *A. hydrophila* 11 (0.5%) was recovered from bone marrow, heart and liver of the sick chickens. *P. multocida*5 (1.25%) was isolated from oropharynx of apparently healthy chickens, while *A. hydrophila* was not isolated from the healthy chickens.

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2.

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