## Review Article

## Aeromonas-Associated Infections in Developing Countries

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#### Abstract

Although their role in gastroenteritis is controversial, *Aeromonas* species are recognized as etiological agents of a wide spectrum of diseases in man and animals. In developing countries, potentially pathogenic *Aeromonas* sp. are 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* sp. 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 cephalosporins and the fluoroquinolones, is found among *Aeromonas* sp. isolated from clinical sources in some developing countries in Asia. Isolating and identifying *Aeromonas* sp. to genus level is simple and requires resources that are available in most microbiology laboratories for processing common enteric bacteria. The present review will cover the epidemiology, clinical syndromes, low-cost diagnostic methods, and antimicrobial resistance and treatment of *Aeromonas* infections in developing countries.

**Key Words**: *Aeromonas*, Developing countries, Diarrhea, Extraintestinal infections, Laboratory diagnosis, Antibiotic resistance.

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#### Introduction

Although their role in gastroenteritis is controversial, *Aeromonas* species are recognized as etiological agents of a wide spectrum of diseases in man and animals [1]. The literature has indicated that some motile *Aeromonas* sp. are emerging food- and water-pathogens of increasing importance [2]. These organisms have been associated with several food-borne outbreaks [3,4] and are increasingly being isolated from patients with traveler's diarrhoea [5,6].

Since 1980, more than 2,000 studies and more than 10 reviews on *Aeromonas* sp. have been reported in the English literature and the large majority of these reports were from developed countries. To date no reviews are published on these organisms in developing countries. The present review will cover the epidemiology, clinical syndromes, low-cost diagnostic methods and antimicrobial resistance and treatment of *Aeromonas* infections in developing countries.

## **Taxonomy and Serology**

Until 1984, only four species of Aeromonas were known and these are A. hydrophila, A. caviae, A. sobria (present name A. veronii biovar sobria) and A. salmonicida [7]. The latter is a nonmotile fish pathogen and rarely reported from clinical sources [8,9]. Since then the genus Aeromonas has evolved with the addition of new species and the reclassification of existing taxa. Previously Aeromonas sp. were placed together with Vibrio sp. and Plesiomonas shigelloides in the family Vibrionaceae. However, genetic studies provided enough evidence to support the placement of aeromonads in a family of their own, Aeromonadaceae [10]. In the last edition of Bergy's Manual [11], 17 hybridization groups (HG) or genospecies and 14 phenospecies described (Table 1).

Assignment of hybridization groups is based on DNA-DNA reassociation techniques. While much of the confusion surrounding the taxonomy of the genus *Aeromonas* has been dispelled, still there is lack of harmony between phenotypic and

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genotypic characteristics, and multiple methods are required for accurate classification [12-14].

There are more than 96 distinct serogroups of *Aeromonas* on the basis of presence of unique somatic antigens and they are not species specific [15]. Several studies reported a strong association of aeromonads from clinical sources and

serogroups O11, O16 and O34 [16,17], while other investigators found no association [15].

The three most common serogroups of *Aeromonas* sp. reported from different developing countries are shown in Table 2.

**Table 1.** Hybridization groups (genospecies) and phenospecies of *Aeromonas* and their isolation from different sources in developing countries.

			Isolation source <sup>b</sup>					
HGª	Genospecies	Phenospecies	Clinical specimens	Food	Water	References		
1	A. hydrophila	A. hydrophila	С	С	С	40,48,64,65, 120,121,124		
2	A. bestiarum	A. hydrophila-like	NR	R	NR	53		
3	A. salmonicida*	A. salmonicida	R	С	R	8,53,132		
3	Unnamed	A. hydrophila-like	NR	NR	NR	=		
4	A. caviae	A. caviae	С	С	С	8,40,42,43,48, 52,64,65,122, 125		
5A	A. media	A. caviae-like	NR	NR	NR	-		
5B	A. media	A. media	R	R	R	133, 138,139		
6	A. eucrenophila	A. eucrenophila	NR	R	R	138,140,142		
7	A. sobria	A. sobria	NR	R	NR	138		
8X	A. veronii	A. sobria	NR	NR	NR	-		
8Y	A. veronii	A. veroni bv sobria	С	С	С	40,48,52,64, 65,80		
9	A. jandaei	A. jandaei	R	NR	R	132,137,141		
10	A. veronii	<i>A. veronii</i> bv veronii	R	R	NR	78,137,138		
11	Unnamed	Aeromonas sp.	NR	NR	NR	=		
12	A. schubertii	A. schubertii	R	NR	NR	43,137,141		
13	Unnamed	A. schubertii-like	NR	NR	NR	-		
14	A. trota	A. trota	R	R	R	137, 138,141		
15	A. allosaccharophila		NR	NR	NR	-		
16	A. encheleia		NR	R	NR	53		
17	A. popoffii		NR	NR	NR	=		

<sup>a</sup>HG= hybridization groups, <sup>b</sup>C=common, R=rare, and NR=not reported. \*Nonmotile; most do not grow at 37℃.

#### **Habitat and Bacteriology**

Aeromonads are primarily aquatic organisms occurring naturally in different freshwater bodies that include rivers, water streams and lakes. They are predominant in estuarine waters and easily isolated from seashores but not from deep sea. They also occur in raw sewage, treated sewage and activated sludge [18]. However, these organisms do not occur in water with a very high salinity, geothermal springs or extremely polluted rivers [19]. Aeromonas sp. can also be found in chlorine-treated municipal drinking water. Massa et al. [20] reported that certain strains of Aeromonas sp. are resistant to the usual chlorine concentrations used for purified drinking water.

The wide diversity of aeromonads' habitat can clearly be seen by the recently isolated cytotoxic and hemolytic strain of *A. caviae* from an explored sulfur spring in Orissa, India [21]. The growth temperature of this strain ranged from 12° to 43°C and the optimum was 30°C.

Members of the genus *Aeromonas* are Gramnegative, facultative anaerobic, catalase- and oxidase-positive, rod-shaped bacteria. Like members of the genus *Vibrio*, motile *Aeromonas* sp. possess mainly a single polar flagella; however, lateral and peritrichous flagella may be formed by some species [13, 22]. Several extracellular hydrolytic enzymes are produced by these organisms that include, but are not limited

to, amylase, deoxyribonuclease, elastase, and lipase [23]. The motile *Aeromonas* sp. grow at a wide temperature range between 0° and 45°C for some species with optimum temperature range of 22° to 35°C [24]. They grow well at alkaline pH (optimum pH 5.5-9.0), a character that is employed in the alkaline-peptone enrichment medium (pH 8.5-9.0), which is used for the isolation of *Aeromonas* species from stool and other samples rich in enteric bacteria.

**Table 2.** Common serogroups of *Aeromonas* sp. reported from different developing countries.

Country	Most 3 common serogroups	Source of isolates	Reference	
Brazil	O3, O17, O38	Stool	42	
Brazil	O16, O35, O54	Freshwater	64	
Brazil O11, O19, O34		Stool, extraintestinal, freshwater	133	
India O11, O16, O34		Stool and environment	134	
India	O18, O64, others*	Stool, domestic water	15	
India O16, O83, O85		Stool	135	
Thailand	O16, O34, O83	Stool, blood, discharge	136	

<sup>\*</sup>More than one serogroup.

#### **Virulence Factors**

Virulence of Aeromonas sp. is multifactorial and not completely understood [13]. Aeromonas sp. have been reported to elaborate exotoxins (hemolysins, cytotoxins, enterotoxins), hemagglutinins, adhesins, several hydrolytic enzymes, and invade tissue in culture [25-28]. The hemolysin produced by some Aeromonas sp. (also known as aerolysin) has been shown to have both hemolytic and enterotoxic activity [29, 30]. Burke et al. [31] found that 97% of the hemolysin-producing strains were able to secrete enterotoxins. Other investigators also reported a correlation between hemolysin and cytotoxin production [32]. The hemolytic enterotoxin shares significant homology with the cytotoxic enterotoxin (Act), and two cytotonic toxins (Alt and Ast)[33]. Rahim et al. [34] tested 32 act gene probe-positive and 31 randomly selected act gene probe-negative Aeromonas

isolates for enterotoxicity in a suckling mice assay (SMA), for haemolytic activity on sheep blood agar plates, for the presence of CAMP-like factors, and for cytotoxicity in a Vero cell line. The act gene probe-positive isolates significantly differed from the toxin gene probe-negative ones with respect to in the enterotoxicity SMA (P=0.009) and haemolytic activity (P=0.005).The CAMPhaemolysin phenotype was significantly associated with the rabbit ileal loop assay (P=0.08), Vero cell assay (P=0.064), and haemolysin production under the microaerophilic conditions (P=0.056) of the act gene probe-positive isolates of Aeromonas sp. There findings indicated the role of Act in the pathogenesis of Aeromonas infections and that the enterotoxic potential of Aeromonas sp. could be assessed by simply performing a CAMPhaemolysin assay.

Enterotoxigenic isolates of *A. hydrophila* showed hemagglutination (HA) which was not sensitive to mannose (i.e. mannose-resistant [MRHA]) and fucose, but *Aeromonas* strains that were HA-sensitive to mannose or showed no hemagglutination (NHA) were non-toxic strains of *A. caviae* commonly isolated from nondiarrhoeal infection or the environment [25]. In enteric bacteria, hemagglutination of erythrocytes is associated with the ability to adhere to human epithelial cells. In *Aeromonas fimbriae*, outer membrane proteins, the lipopolysaccharide O-antigen, motility and the polar flagella have been shown to aid *in vitro* adherence of *Aeromonas* sp. to human and fish cell lines [35-39].

# **Epidemiology of** *Aeromonas* in **Developing Countries**

Hybridization groups (genospecies) and phenospecies of *Aeromonas* and their isolation from different sources in developing countries is shown in Table1. Only *A. hydrophila*, *A. veroni* biovar sobria and *A. caviae* are commonly isolated from clinical, food and water sources in developing countries, which is similar to what has been reported from developed countries [1].

#### Aeromonas in Human Cases of Diarrhoea

Nearly all the studies in developing countries on the prevalence of *Aeromonas* sp. in cases of diarrhoea have been reported from children and very few from mixed populations (children and adults). Table 3 shows the prevalence of Aeromonas sp. in diarrhoeic and non diarrhoeic children in some developing countries in Africa, Asia, and Latin America. Isolation rates of Aeromonas from diarrhoeic children ranged between 1-88%, and from controls 0.0-45%. Some studies found statistically significant differences in the isolation rates of the organism from diarrhoeal cases and controls, while others did not. Actual studies carried out in the same country gave different outcomes (Table 3). However, it should be noted that these studies were done in different cities of the same country and at different times.

Similar to what has been reported from developed countries, *Aeromonas caviae*, *A. hydrophila*, followed by *A. veronii* biovar sobria are the three dominant species associated with diarrhoea in children in developing countries. Using molecular techniques, Abdullah *et al.* [40] identified 8 *Aeromonas* isolates from Libyan children with diarrhoea to genospecies and found that 4 of the 8 were *A. caviae*, 3 *A. veroni* and 1 *A. hydrophila* HG1.

**Table 3.** Prevalence of *Aeromonas* sp. in diarrhoeic and non-diarrhoeic children in some developing countries in Africa, Asia and Latin America.

	%positive/n	o examined			Reference	
Country	Diarrhoeic children	Non- diarrhoeic children	P value	Dominant species		
Bangladesh	9.2/814	3.0/814	<0.0001	NA	118	
Brazil	19/100	13/100	NS	A. caviae	42	
Brazil	21.9/91	0/72	< 0.0001	A. caviae	8	
China	5.9/221	9.3/108	NS	NA	119	
Cuba	9.0/300	ND	-	A. hydrophila	120	
Egypt	88/52	45/38	P<0.00001	A. hydrophila	121	
India	4.7/341	0/147	< 0.01	A. caviae	122	
India	9.7/216	ND	-	NA	123	
Iran	4.5/310	0/310	< 0.0001	A. veronii bv sobria	80	
Libya	15/157	18/157	NS	A. caviae	43	
Nigeria	1.0/100	ND	-	A. hydrophila	124	
Venezuela	11.8/397	5.8/121		A. caviae	125	
Vietnam	15/291	8.0/291	NS	NA	126	

ND= not done.

Aeromonas sp. (reported as A. hydrophila) has been found to be the most common enteric pathogen in adults with diarrhoea in Bangkok, Thailand [41]. A study from Brazil examined stool samples from 69 adults with diarrhoea and 17 adults without diarrhoea [42]. Aeromonas sp. were detected in 6 (8.7%) diarrhoeic adults (5 A. caviae and 1 A. hydrophila) and from none (0.0%) of the controls.

The isolation of aeromonads from non-diarrhoeic children in developing countries is not uncommon. For this reason the detection of virulence factors in *Aeromonas* sp. isolated from diarrhoeic and non-diarrhoeic children is important. Ghenghesh *et al.* [43] isolated *Aeromons* sp. form 15% of diarrhoeic and from 18% of non-diarrhoeic children in Libya. However, testing the *Aeromonas* strains from both groups for hemolysin production and mannose-resistant hemagglutination (MRHA), no significant difference in the hemolytic activity of *Aeromonas* from diarrhoeal and healthy children

was found, but a significant difference (p<0.002) was noticed in MRHA by diarrhoeal isolates of *Aeromonas* (26%) as compared to the healthy controls (3%).

Aeromonas in Animals and in Foods of Animal Origin

A study from Libya examined rectal swabs from 120 domestic dogs and 15 domestic cats for *Aeromonas* sp. using alkaline peptone water (pH 8.6) as the enrichment medium and blood agar containing 15 mg/l ampicillin as the plating medium [44]. Aeromonads were isolated from 13 (10.8%) dogs and from 1 (6.7%) cat. Only 9 *Aeromonas* isolates were available for speciation and testing for production of haemolysin. Of these 5 were *A. veronii* biovar sobria (including one from a cat), 2 were *A. hydrophila* and 2 were *A. caviae*. Six were positive in the haemolysin assay; 4 *A. veroni* biovar sobria (one from a cat) and 2 *A. hydrophila*. Ceylan *et al.* [45] in Turkey, using alkaline peptone

water, detected *Aeromonas* sp. in 5.5% of rectal swabs taken from 55 dogs. In both studies mentioned above the samples examined for aeromonads were taken from healthy animals. The presence of haemolysin producing-*Aeromonas* species in the feces of domestic dogs and cats may pose a public health problem for humans who come into contact with such animals.

A study by Ghenghesh et al. [46] has shown that livestock used for production of meat do not harbor Aeromonas sp.; however, they found meats from these animals sold at retail outlets are highly contaminated with the organism. They examined rectal swabs from 36 cows and 200 camels raised for meat production in Libya. None of samples from cows and only 0.5% of samples from camels were positive for Aeromonas sp. On other hand, they found that 38% and 67% of samples of meats from cows and camels sold at retail outlets were positive for aeromonads, respectively [46]. A study from Turkey reported the isolation of Aeromonas sp. from 20 (86.9%) out of 23 chicken and 40 (67.7%) out of 59 minced meat samples examined [47]. In India, Kumar et al. [48] isolated Aeromonas sp. from 33 (13.4%) out of 246 food samples of animal origin examined. They found Aeromonas in 16.7% of samples from poultry meat, in 12% from goat meat, and in 7.7% from buffalo meat. They also reported the predominance of A. hydrophila (51.5%), followed by A. veronii biovar sobria (39.4%) and A. caviae (9.1%). In addition, they reported that 70.6% of A. hydrophila, 69.2% of A. veronii biovar sobria and 33.3% of A. caviae isolated from food samples of animal origin induced enterotoxigenic reaction in mouse paw oedema test. However, a study from Libya identified 32 Aeromonas isolates from chicken carcasses to the genospecies using molecular techniques and found that A. veroni was the most predominant species seen in 30 isolates followed by A. caviae and A. hydrophila [40]. Contamination of meats sold at retail outlets with Aeromonas sp. may result from post-slaughter handling of carcasses that include washing with water contaminated with Aeromonas and manipulation of the meat at the point of sale (e.g. chopping and mincing). Although isolation of Aeromonas sp. from pork meat has not been reported from developing countries, a food-borne outbreak among 115 individuals resulting from consumption of pork contaminated with A. hydrophila was reported by Zeng-Shan and co-workers [49] in China and cited by Joseph and Carnahan [12].

Aeromonas sp. are not uncommon in seafood, particularly fish and prawns. Thayumanavan et al. [50] examined 514 samples of seafood (410 finfish and 104 prawns) for the presence of Aeromonas sp. (reported as A. hydrophila). Aeromonads were detected in 37% (37.3% of finfish and 35.6% of prawns) of samples. Of the 255 strains of Aeromonas isolated, more than 78% of them were haemolysin-producers. Vivekanandhan and coworkers [51] examined 536 samples of fishes and 278 prawn samples from the major fish market of Coimbatore, South India, over a period of 2 years for the presence of aeromonads (reported as A. hvdrophila). Aeromonas sp. were detected in 33.6% and in 17.6% of fishes and prawns samples respectively. During the period of the study, seasonal variation was observed in the prevalence levels of Aeromonas sp. in fish and prawns with a higher prevalence during monsoon seasons. Investigators in Ankara, Turkey, reported higher rates of isolation of Aeromonas from fish [52]. They examined 132 market fish (64 freshwater and 68 seawater) samples and found aeromonads in (80.3%). However, they reported predominance of A. caviae in freshwater fish and A. veroni biovar sobria in seawater fish samples. Hemolytic activity was detected in more than 80% of the 132 Aeromonas isolates. Using molecular techniques, Castro-Escarpulli et al. [53] detected A. bestiarum and A. encheleia in 20.9% and 3.9%. respectively, from 250 samples of frozen fish (Tilapia, Oreochromis niloticus) purchased in local markets in Mexico City. Using such techniques, species of Aeromonas other than A. hydrophila, A. caviae and A. veroni biovar sobria undoubtedly will be reported from different types of foods in the future.

## Aeromonas in diary products

In developing countries, Aeromonas sp. appears to be common in milk and milk products including pasteurized milk. Yucel and colleagues [52] in Turkey found aeromonads in 49.2% of 132 bulk raw milk samples, 40% of 25 raw milk samples sold in the street, 16% of 31 pasteurized milk samples, and in 8% of 150 white cheese examined. samples Speciation of isolated that 90.2% Aeromonas showed were hydrophila, 4.3% A. veroni biovar sobria and 5.4%

A. caviae. They tested the isolates for some virulence properties and found 84% and 100% of A. hydrophila and A. veroni biovar sobria were positive for hemolysin production, and 54% and 100% with protease activity, respectively. None of the A. caviae isolates were positive for both virulence properties tested. These findings indicate that motile Aeromonas sp. are common species in raw milk, and also the presence of Aeromonas strains in pasteurized milk and cheese indicates the postprocessing contamination with motile Aeromonas species as a result of unhygienic conditions during manufacturing [52].

Araujo and co-workers [2] examined a total of 45 samples of soft cheese from three different brands marketed in Rio de Janeiro, Brazil, Thev detected A. hydrophila and A. caviae in 17.7% of the samples examined. Similarly, a study from Libya identified Aeromonas sp. in 18% and 20% of 111 open and 49 packed ice cream samples examined, respectively [54]. Aeromonas sp. were found in 18% and 20% of samples respectively. However, 100 samples of different types of locally produced and imported yogurts in Libya were examined and none were found positive for Aeromonas sp. (Ghenghesh KS, unpublished observation). High rates of isolation of Aeromonas from ice cream and other dairy products in developing countries may be related to low standards of hygiene practiced and the quality of raw materials used in different dairy industries. On the contrary, studies from developed countries reported isolation rates of aeromonads of less than 5% from certain dairy products [55].

Detection of enterotoxigenic *Aeromonas* sp. from ice cream is not uncommon. Yadav and Kumar [56] in India examined 285 food samples comprising fish (100), milk (85) and ice cream (100). *Aeromonas* sp. were found in 40 (14%) samples examined with predominance of *A. hydrophila*. More than 50% of isolated aeromonads produced enterotoxin by ligated rabbit ileum loop technique.

## Aeromonas in Vegetables

In Rio de Janeiro, Brazil, Alcides *et al.* [57], examined parsley and watercress samples acquired in a street market for the presence of *Aeromonas* on the day of purchase without refrigeration and after 7 days of storage at 5°C. Aeromonas strains were isolated from both

refrigerated and nonrefrigerated samples with predominance of A. caviae. Of the 39 isolates of Aeromonas strains, 51.5% were enterotoxigenic, 48.5% hemolytic, and all (100%) showed protease activity. Because the vegetables are usually consumed raw, they can pose a risk of infection with pathogenic aeromonads, especially for immunocompromised individuals [57]. Dhiraputra et al. [58] examined the bacterial contamination of vegetables served in hospitals in Bankok, Thailand. They detected Aeromonas in 14 (3.5%) of 403 fresh vegetable samples (including lettuce, onions, parsley, celery and tomato) before being washed and in 17 (4.3%) of 396 ready-to-serve vegetable samples. These findings support the view that food rather than water might be the source of aeromonads associated with outbreaks occurring in hospitals and in the community.

#### Aeromonas in Other Animals and Foods

Aeromonas sp. were detected in 2 (0.8%) and 8 (5.3%) of 253 hospital and 150 household German cockroaches in Tripoli, Libya [59]. Also, Rahuma et al. [60] in Misurata, Libya, detected Aeromonas sp. in 16 (10.7%) of 150 houseflies collected from the city central hospital, city streets, and the city abattoir. Since cockroaches and houseflies are clearly very mobile, it seems quite likely that they can carry Aeromonas sp. from hospitals into neighboring communities, and vice versa.

Other foods may also become contaminated with aeromonads including foods with acidic pH. A study from Libya reported the isolation of *Aeromonas* sp. from 3 (2.1%) of 146 different fruit juices manufactured locally and sold at retail outlets [61]. In India, Kumar *et al.* [48] found *Aeromonas* sp. in 12.5% of samples from poultry eggs.

#### Aeromonas in Water

Earlier studies have reported that the drinking of untreated water is the most probable manner of acquiring *Aeromonas* [62, 63]. In developing countries, these organisms appear to be very common in such waters. Pitaransi *et al.* [32] in Thailand found that water samples from 17 of 23 canals and 15 of 15 water jugs (ongs) were positive for *Aeromonas.* Isolation of enteropathogenic aeromonads is common in untreated waters including those used for drinking

in developing countries. A total of 70 freshwater samples from different sites along the Cambe Stream in the State of Parana, Brazil, were examined for Aeromonas sp. (Gibotti et al.) [64]. Aeromonads were isolated across all sampling sites, representing 12 A. hydrophila, 12 A. caviae and 8 A. veroni biovar sobria belonging to different serogroups. More than 90% of the isolated aeromonads were hemolytic on sheep blood agar plates and showed mannose-resistant hemagglutination using human erythrocytes (group O). Furthermore, 16% (5/32) of the Aeromonas isolates were enterotoxin producers by the suckling mouse test. A study from Libya found nearly 50% of 1,000 water samples from untreated wells and other miscellaneous sources used for drinking positive for Aeromonas sp. [65]. Speciation of 382 strains resulted in 225 (59%) being A. hydrophila, 103 (27%) A. caviae, 42 (11%) A. veroni biovar sobria and 11 (3%) other species. In addition, nearly 50% of strains tested were producers of hemolysin using human and horse erythrocytes.

In developed countries aeromonads are isolated in higher numbers during the summer [66] and Aeromonas-associated diarrhoea was found to be high during the summer months [25, 67]. A study from North Africa found the isolation of Aeromonas sp. to be highest during the months of winter and lowest in summer [65]. Pathak et al. [68], in India, investigated the seasonal distribution of aeromonads in river water and reported similar findings. The lack of seasonal variation in the isolation rates of Aeromonas sp. in developing countries may be due to mild weather throughout the year in most of these countries.

Obiri-Danso et al. [69] assessed the microbiological quality of bottled and plastic-bagged drinking water sold on the streets of Metropolitan Kumasi, Ghana. Aeromonas sp. was detected in some of the water samples examined. These investigators concluded that Ghanaian bottled water is of good microbiological quality but some factory-bagged sachet and hand-filled hand-tied polythene-bagged drinking waters are of doubtful quality. A study from Libya examined 216 water samples of locally produced and imported bottled water and none were positive for Aeromonas sp. (KS Ghenghesh, unpublished observation).

Aeromonads can also be isolated from exotic and sometimes unexpected sources. Sanval et al. [70] found Aeromonas in 98 of the 100 tropical aquaria sampled. In Libya, Aeromonas sp. were isolated from 9 (18%) of 50 water samples collected from clay and stainless steel water containers used for drinking in mosques [71]. Of the isolated aeromonads, 5 were A. hydrophila, 3 A. veroni biovar sobria and 1 A. caviae. Isolation of Aeromonas sp. from samples of holy water from a church in a developed country (UK) has been reported previously [72]. In developing countries, people often drink untreated water, which may help to explain the frequent occurrence of Aeromonas in feces of people with or without diarrhoea. Also, in such countries it is common to use water obtained from wells and other untreated sources, in addition to drinking, for bathing and other purposes and this may be hazardous to individuals with wounds, lacerations or abrasions, and to the immunocompromised [73-77].

Water-associated outbreaks due to *Aeromonas* sp. have not been reported from developing countries. Recently, Hofer *et al.* [78] reported an acute diarrhoea outbreak, with 2,170 cases, in Sao Bento do Una, Pernambuco, Brazil. They detected aeromonads in 19.5% of 582 stool samples examined. Other enteropathogens were detected in 5.3%. Although the source of the outbreak was not determined, the authors suggested that the use of microbiologically unmonitored drinking water and poor hygienic living conditions may be responsible for the *Aeromonas*-associated acute diarrhoea outbreak [78].

## **Clinical Infections**

A wide spectrum of infections has been associated with *Aeromonas* sp. in developing countries that include gastroenteritis, wound infections, septicemia and lung infections. As in developed countries [1,79], the large majority of all *Aeromonas* clinical isolates are caused by *A. hydrophila*, *A. caviae* and *A. veroni* biovar sobria. It should be noted that *Aeromonas* infections in the immunocompromised are usually more severe than in the immunocompetent individuals.

#### Gastroenteritis

There is a consensus among different investigators that certain strains of *Aeromonas* which carry required virulence factors are likely

human enteric pathogens while others are not [75]. Diarrhoea due to Aeromonas presents with varied clinical manifestations. Watery and self-limited diarrhoea is common. However, some patients may develop fever, abdominal pain, and bloody diarrhoea. Dehydration may accompany the above mentioned symptoms in sever cases. Frank mucus and blood can be seen in more than 25% of stools of children with Aeromonas-associated diarrhoea and nearly 35% of patients exhibit symptoms of fever and vomiting [43]. Presence of blood in stool is an indication of dysentery. A study from Iran [80] found dysentery was the dominant clinical feature in children positive for Aeromonas sp. Diarrhoeic children with Aeromonas may have up to ten episodes of stool passages per day and diarrhoea may last from 2-10 days [43].

Aeromonas-associated cholera-like diarrhoea, have been reported from several developing countries. Symptoms include non-bloody ricewatery diarrhoea with some patients dehydrated. A Thai woman from Bangkok traveling to France was admitted to a hospital in Paris for a cholera-like diarrhoea illness [81]. The patient's ""rice water" stool was found positive for A. veroni biovar sobria (reported as A. sobria) and negative for Vibrio cholerae and enterotoxigenic Escherichia coli. The isolated organism was positive for enterotoxin, hemolysin, cytolysin, proteolysin and a cellrounding factor. Acute- and convalescent-phase sera showed an increase in neutralizing antibodies enterotoxin. hemolysin and cvtolvsin. Furthermore, the enterotoxin induced accumulation of fluid in the rabbit ileal loop test, but was not neutralized by antiserum to cholera toxin.

Three patients with symptoms of cholera-like diarrhoea and rice watery stools have been reported from Libya [82]. One of the patients was a Japanese woman who had diarrhoea and was initially treated with ampicillin. Her symptoms remained for several days and she developed cholera-like diarrhoea and was dehydrated. All patients drank untreated well water. Stools from the three patients were positive for *A. caviae* and negative for enteropathogenic *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio cholerae* O1, and rotavirus.

Immunocompromised patients may have severe symptoms. A case of severe acute diarrhoea in a 26-year-old male with cholera-like

clinical symptoms due to A. veroni biovar sobria was reported from Cuba [83]. The patient was suffering from Crohn's disease and was previously colectomized. Isolation of Aeromonas sp. from cases of cholera-like diarrhoea strongly supports the view that some strains of Aeromonas sp. are enteropathogens of humans. Diarrhoea due to Aeromonas sp. may also be chronic and lasting for months, particularly in the immunocompromised. Obi and Bessong [84] reported the isolation of Aeromonas sp. from 8 (13.3%) of 60 HIV patients with chronic diarrhoea in rural communities of the Limpopo Province. South Africa. Immunocompetent individuals can also suffer from Aeromonas-associated chronic diarrhoea. In Saudi Arabia, Ibrahim et al. [85] reported two cases of chronic colitis from immunocompetent patients associated with A. hydrophila. The first case had a nine-month history of chronic inflammatory bowel disease. Bacteriological cultures of the rectal secretions grew A. hydrophilia with no other enteric pathogen isolated. The second case had a one-year history of chronic inflammatory bowel disease, confirmed clinically and histologically. A. hydrophilia was isolated in pure culture from his stool.

#### Extraintestinal Infections

It is important to suspect Aeromonas as a pathogen in wounds sustained in a fresh water environment. Soft tissue infections following waterrelated injuries are the most common. After the tsunami that occurred in December 2004 in southern Thailand, nearly 800 patients were transferred to hospitals in Bangkok. More than 500 of these patients had skin and soft-tissue infections with Aeromonas sp. being the most common organism isolated from them [86]. Neardrowning pneumonia (aspiration pneumonia) due to Aeromonas has been reported from several developing countries. In one case reported from Cuba, a patient who fell into an irrigation canal suffered from an incomplete drowning syndrome [87]. He was admitted in the Intensive Care Unit with acute inflammatory pneumonia and a strain of A. hydrophila was isolated from his blood. In Indonesia during the Andaman Nicobar earthquake and tsunami in 2004, Guha-Sapir and van Panhuis [88] found Aeromonas sp. to be one of the major pathogens causing aspiration pneumonia with symptoms appearing in less than 24 hours and a 63% case fatality rate. Rodriquez and co-workers [89] reported the isolation of *A. hydrophila* from the blood of a 3-year-old Venezuelan boy, exposed to a recreational water source, who had diarrhoea, pneumonia and sepsis. They indicated that their report supports the importance of bacteriological diagnosis of *Aeromonas* respiratory tract infection, as well as the epidemiological relevance of stool investigation for certain cases, namely, those in which ingestion of contaminated water is suspected [89].

Aeromonas lung infections may occur in immunocompromised patients not related to contact with water. Bravo et al. [90], in Cuba, evaluated a case of an 87-year-old female with a history of heart disease who had been presenting dysnea and fever for 2 months and suffering from lung cancer. A bacteriological examination of sputum specimen from the patient was positive for A. hydrophila and negative for acid fast bacilli.

An eight-year bacteriological study of gas gangrene was carried out in Pondichery, India [91]. Aeromonas sp. were detected in 21 (12.4%) of 169 cases of gas gangrene. Due to the polymicrobial nature of gas gangrene, Aeromonas sp. were usually detected in association with other aerobic and anaerobic bacteria. Skin infections due to Aeromonas may also be the result of bites by reptiles. A fulminating A. hydrophila infection of the right arm of a twelve-year-old Thai boy following a snake bite was reported [92]. Surgical intervention and appropriate antimicrobial therapy resulted in complete recovery. Also, three cases of A. hydrophila soft-tissue infection as a complication of snake bite were reported from Brazil [93]. Snakes are known to be affected by aeromonads and they may carry the organism in their mouths, fangs or venom. This may explain the skin infections resulting from their bites.

Bacteremia due to *Aeromonas* may result in disseminated intravascular gas production. A fatal case of *A. veronii* biovar sobria infection with disseminated intravascular gas production in a 15-year-old girl with a 6-hour history of increasing pain and swelling in her left thigh and who had been quite healthy until the onset of illness was reported by Shiina *et al.* [94]. It is important to keep the possibility of such an infection in mind when a patient complains of severe muscle pain. A case of bacteremia due to *A. hydrophila* in a

patient recovering from cholera was reported from Malavsia [95].

Aeromonas appears to be a major pathogen for septicemia in patients with hepatic cirrhosis with a rapidly fatal outcome [96]. The main clinical manifestations include fever, chills, abdominal pain, diarrhoea and shock. In these patients nosocomial infection is the predominant way of infection [96]. Meningitis caused by Aeromonas sp. is a rare clinical entity and it may involve all age groups. A. hydrophila isolated from the CSF of a 3-month-old male child with history of fever, of not sucking the breast and deviation of eyeball towards the right side has been reported from India [97]. The same organism was also isolated from blood samples and well water of the patient's dwelling.

Aeromonas sp. organisms rarely cause urinary tract infections; however, recently Al-Benwan et al. [98] in Kuwait reported a case of cystitis due to A. caviae in a 39-year-old Bangladeshi male who presented to the emergency department with a 2-month history of increased frequency of urination, dysuria, hematuria, and weight loss of 7 kg.

Infection in burn patients due to *Aeromonas* is not common. In Singapore, Chim and Song [99] reported 4 cases of *Aeromonas* infection in burn patients admitted to the burn intensive care unit with one mortality in the series. Interestingly, there was no history of exposure to soil or fresh water in all patients.

Aeromonas-associated outbreaks of nosocomial infections

Several outbreaks of hospital-acquired diarrhoea have been reported from developing countries. One report from India described six children admitted to the hematology-oncology unit who developed acute watery diarrhea during a period of four weeks [100]. Stool samples from patients were positive for *A. veroni* biovar sobria (reported as *A. sobria*) with similar biotype and antibiogram. Interestingly, *A. veroni* biovar sobria with a similar profile was also isolated from one sink that was being used by the patients' attendants for washing utensils.

Aeromonas veroni biovar sobria (also reported as A. sobria) was also isolated from 28 patients involved in an outbreak of acute gastroenteritis that affected 69 patients during a one-month period in Benghazi, Libya [101]. All isolates had

the same phenotypic profile using the API 20E identification system. However, the source of outbreak could not be traced.

Although water is usually suspected to be the source of *Aeromonas* associated with nosocomial outbreaks, certain foods may also be the source of such outbreaks. Suthienkul *et al.* [102] examined bottle milk samples obtained randomly from 500 infants under 6 months of age who came to the outpatient clinic at Children's Hospital in Bangkok. *Aeromonas* was detected in 14.4% of samples.

## **Laboratory Diagnosis**

Due to lack of resources in many developing countries, we will describe procedures used in our laboratories that require the minimum resources possible for isolation and identification Aeromonas sp. Laboratories with more resources can use the references provided at the end of the references list. The oxidase test is used to separate Aeromonas sp. from members of the family Enterobacteriaceae to which the latter are negative. Tests required to differentiate aeromonads from the oxidase-positive Vibrio sp. and Plesiomonas shigelloides are shown in Table 4 below.

**Table 4.** Differential characteristics of *Aeromonas* sp., *Vibrio* sp. and *Plesiomonas shigelloides* 

Test*	Aeromonas sp.	Vibrio sp.	Plesiomonas shigelloides	
Oxidase	+	+	+	
Growth in nutrient broth with:**				
No added NaCl	+	-	+	
1% NaCl	+	+	+	
6% NaCl	_	+	_	
Gelatin hydrolysis	+	V	-	
Acid production from inositol	-	V	+	

\*Incubation temperature at 35-37°C. V= variable result. \*\*From reference number 143.

## Culture media used

Several types of media can be used and it should be noted that there is no single media that is preferred as each media has its pros and cons.

- Ampicillin blood agar (ABA):

This is a widely used medium and simple to prepare. Trypticase soy agar (or any other blood

agar bases) with 5% sheep blood supplemented with 10 gm/L ampicillin is used as plating medium. Plates kept in sealed plastic bags at 4° to 8°C can be used for up to 1 week. This is a selective medium that will inhibit some of the normal fecal flora but most of Aeromonas sp. particularly A. hydrophila, A. caviae and A. veroni biovar sobria will grow well on this medium. Aeromonas trota and some strains of A. media are sensitive to ampicillin and will not grow on this medium. However, these organisms are rarely isolated from fecal specimens (See table 1). If sheep blood is not available, expired human blood (group O) can be obtained from a local blood bank and used in this medium. We obtained excellent results with ABA using expired human blood when compared with sheep blood (KS Ghenghesh, unpublished observation). It should be noted that the blood should still retain its red color when used in ABA. Use of blood with lysed erythrocytes will not show the beta hemolysis, a characteristic that assists in identifying Aeromonas sp.

-Alkaline peptone water (APW):

Peptone water with pH adjusted to 8.4. This is an enrichment medium that is used for isolation of *Vibrio cholerae*, too. This medium can be prepared in 10 ml volumes and can last for months in screwcapped tubes.

Isolation from stool samples

In clinical microbiology laboratories, stool specimens are the most common samples examined for *Aeromonas* sp. Liquid or semi-liquid stools from diarrhoeic patients and solid stools from non-diarrhoeic individuals collected in sterile containers should be transported to the laboratory and processed within two hours from collection. Clinical information should accompany every specimen.

A volume of 1 ml of liquid or semi-liquid stool is added to 10 ml APW and a large loopful of the stool is plated directly to ABA in a way to obtain well-isolated colonies. One gram (or 2 to 3 large loopfuls) of solid stool is added to sterile saline, well mixed (preferably by vortexing for 15 seconds) and then processed as for liquid stool. Both media are incubated at 37°C. After overnight incubation, suspected colonies on ABA plates are tested for oxidase. Oxidase-positive identified are biochemically. A loopful of the APW is plated on an ABA plate, incubated at 37°C overnight and then processed as above.

## Identification

Identifying Aeromonas to the genospecies level requires the use of molecular techniques which are not available in most developing countries, and even if they are available, they are still not practical for routine identification in many microbiology laboratories. In addition, it is recommended that laboratories that are unable to properly identify the strains by molecular methods should refer to their isolates as Aeromonas sp., or they should send their strains to reference laboratories for identification to genospecies level. especially if the identification results are intended for publication [103]. However, identification of Aeromonas sp. to genospecies using biochemical tests is possible but requires a battery of at least 18 tests [79, 104], which is probably not a justifiable for routine use in clinical laboratories because of expense, length of incubation required (72 hours) for final identifications in many instances, and technical time involved [1]. Several commercial identification systems are available, though they are usually expensive. These systems are biotyping methods and therefore will not identify aeromonads correctly to genus level. In addition, a few Aeromonas isolates can be misidentified as members of genus Vibrio by these systems [105,106]. Misidentification of Aeromonas isolates may be overcome by testing the growth of the organism at a different concentration of NaCl (Table 4).

Identification of aeromonads to genus level can be conducted using routine tests employed in the identification of other enteric bacteria. Table 5 can be used to identify aeromonads to genus level. We commonly used the API20E system (bioMerieux) for confirmation of results obtained by tests shown in Table 5. These tests can identify more than 95% of aeromonads to genus level when compared with data obtained from PCR methods (KS Ghenghesh, unpublished observation).

#### Isolation from sterile body sites

Blood is usually the most common specimen from sterile body sites. Volumes of 5 to 10 ml of blood are added to blood bottles and incubated at 35° to 37°C for 1 to 15 days until growth occurs. When growth is observed, a sample is taken from the blood bottle and plated directly on a normal

blood agar (without ampicillin) and then processed as above.

#### Isolation from water and foods

Volumes of 25 ml of water or 25 g of homogenized food are added to 225 ml of APW and incubated at 35° to 37°C overnight and then processed as above. If resources are scarcely available, reliable results can be obtained using one tenth (i.e. 2.5ml water or 2.5g food in 25 ml APW) of these volumes [65].

**Table 5.** Tests to identify aeromonads to genus level.\*

Test	Result <sup>1</sup>				
Oxidase	+				
Acid from:					
glucose <sup>2</sup>	+				
Lactose	– or +				
H2S	_				
Urease	_				
Indole	– or +				
Motility	+ or –				
Citrate	+ or –				
beta-hemolysis <sup>3</sup>	+ or –				

- 1- or + = most are negative but few are positive;
- + or = most are positive but few are negative.
- 2 some isolates will give gas too.
- 30n human or sheep blood tryptic soy agar.
- \*From references 42,73,143.

#### Interpretation of results

Still there is no general agreement on reporting *Aeromonas* sp. from stool of diarrhoeic patients. However, as mentioned before, some strains of aeromonads are enteropathogens and therefore should be reported when possible. However, the lack of definitive tests to confirm the pathogenicity of *Aeromonas* makes it difficult to take a decision on whether an isolated *Aeromonas* from diarrhoeal case is responsible for the symptoms or not. The following might assist in reporting isolated *Aeromonas* from cases of diarrhoea to the physician.

- 1. If Aeromonas is isolated from a diarrhoeic stool and no other enteric pathogen was detected, especially if the isolated Aeromonas was in pure culture.
- 2. If a physician specifically requested *Aeromonas* to be looked for in the submitted stool sample.

It should be noted that it is not recommended to report *Aeromonas* if other enteric pathogens (i.e. *Salmonella* or *Shigella*) are detected. Also, it is not recommended to do sensitivity testing for

aeromonads isolated from stool unless specifically requested by the physician. However, the final decision on including aeromonads in the list of pathogens that should be searched for depends on the available resources and should be taken by the head of the microbiology laboratory in consultation with medical staff in the hospital.

Aeromonas should always be reported if isolated from sterile body sites. It is recommended that antibiotic sensitivity testing of isolated Aeromonas should be done and reported as soon as possible. It is important that the reporting of the isolated Aeromonas should not wait until the antibiotic sensitivity testing is done. Because many physicians in developing countries lack information regarding Aeromonas sp., the microbiology laboratory may have an important role in providing such information to the medical staff and assist them in choosing the proper drugs for treatment.

Few developed countries include aeromonads in their standards and require such organisms in water and foods to be reported to authorities for the safety of water and foods. To our knowledge aeromonads are not included in the water and foods standards of any developing country. Therefore, reporting of *Aeromonas* sp. from water and foods in developing countries should only be done when they are specifically requested by local authorities.

## Tests for pathogenicity

Several virulence factors of *Aeromonas* can be detected employing methods that are simple and require materials commonly found in most microbiology laboratories of developing countries. Hemolysin production can be tested by plating the organism directly on sheep blood agar. Testing for mannose-resistant hemagglutination (MRHA) may be used as one of the virulence markers for distinguishing between *Aeromonas* isolated from diarrhoeal children and healthy controls or environmental isolates [43]. Also, the enterotoxic potential of *Aeromonas* sp. could be assessed by simply performing a CAMP-haemolysin assay [34].

#### **Antimicrobial Resistance and Treatment**

In the last two decade, high rates of resistance to commonly used, cheap oral antibiotics among enteric pathogens has been reported from several developing countries [107-109]. As can be seen from Table 6, the same can be said for *Aeromonas* 

sp., particularly those isolated from clinical sources and to a lesser extent from foods and water. The ease of which antimicrobial agents can be obtained in these countries has been blamed for this problem [110,111]. High resistance rates to antimicrobial agents appear to be common among aeromonads isolated from fish in developing Antimicrobial countries. agents are extensively in fish farms to treat and prevent fish diseases and also as feed additives. Such practice has been shown to increase drug resistant bacteria as well as R plasmids [112,113]. However, variation in the resistance rates of aeromonads to different antimicrobial agents in different developing countries can be observed (Table 6). Such differences in the frequency of resistance may well be related to the source of the Aeromonas isolates and the frequency and type of antimicrobial agents prescribed for treating Aeromonas infections in different geographical areas [114].

Resistance of most aeromonads to ampicillin is generally considered to be intrinsic chromosomal mediated [115]. Several studies have shown that patients taking ampicillin for reasons other than diarrhoea may predispose them to infection with Aeromonas [43,63]. Moyer [63] reported that for the susceptible host, antibiotic therapy and drinking of untreated water are two significant risk factors for infection with Aeromonas. However, gastrointestinal infections with Aeromonas are generally self-limiting. Although treatment of patients with symptoms of infectious diarrhoea with antibiotics remains controversial, antimicrobial therapy should be initiated for those who are severely ill and for patients with risk factors for extraintestinal spread of infection after obtaining appropriate blood and The current accepted fecal cultures [116]. treatment of all acute infectious diarrhoeal diseases is rehydration, antibiotic treatment (when indicated), and nutritional therapy [110].

There is a dearth of information on the treatment of extraintestinal infection due to developing Aeromonas sp. in countries. Aeromonas sp. isolated from skin and soft-tissue infections among tsunami survivors in southern Thailand were reported susceptible to amikacin, gentamicin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, imipenem, and trimethoprimsulfamethoxazole [86]. However, only 21% of

Aeromonas isolates were susceptible to cefazolin, and only 23% were susceptible to amoxicillinclavulanate. It should be noted that only patients treated in private clinics were examined and more than 90% of those were foreign tourists and may not represent the local victims [117]. Aeromonas caviae isolated from a case of urinary tract infection in an adult patient in Kuwait was found susceptible to ciprofloxacin, cefotaxime. and gentamicin and resistant amoxicillin. to ampicillin, cefuroxime. cotrimoxazole. and The patient cephalothin. was given oral ciprofloxacin, 500 mg every 12 h, for 2 weeks. A repeat urine culture after completion of the antibiotic therapy did not grow any bacteria. The patient remained well during the 3-month followup. Recently, Rodriguez et al. [89], in Venezuela, reported the isolation of *A. hydrophila* from human blood that is resistant to amikacin, gentamicin, oxacillin, piperacillin, ampicillin-sulbactam, cefotaxime, levofloxacin, and ciprofloxacin, but susceptible to imipenem and cefoperazonesulbactam. The isolate was an ESBL-producer, as determined by the double-disk technique, although this method is not standardized for this pathogen. Most of these antimicrobial agents are expensive and usually are not available in many developing countries, and if available they will be beyond the reach of the majority of the population in such countries.

Table 6. Resistance of Aeromonas species isolated from different sources in several developing countries to

antimicrobial agents.

		No.	% resistant to*								
Country	Source	tested	Amp	Сер	Cef	Na	Cip	Gen	TMS	Tet	Reference
Brazil	Clinical	20	90	60	NT	33	0.0	33	50	60	8
Brazil	Food	61	100 <sup>1</sup>	90	NT	5	NT	0.0	NT	7	127
Brazil	Food	55	NT	NT	0.0	NT	0.0	0.0	0.0	4	128
Brazil	Clinical	28	NT	NT	14	NT	0.0	0.0	29	25	128
Egypt	Clinical	72	100	79	0.0	NT	NT	0.0	0.0	0.0	121
India	Clinical	67	85	NT	0.0	0.0	0.0	0.0	15	16	129
India	Clinical	71	NT	NT	47	91.5	51	41	NT	86	130
India	Clinical	14	92	NT	23	69	36	31	36	NT	131
Libya	Clinical	62	97	64	NT	0.0	0.0	0.0	23	11	43
Libya	Water	40	100	95	0.0	0.0	0.0	0.0	0.0	5	65
Malaysia	Food	21	100	NT	NT	5	NT	0.0	9.5	48	114
South Africa	Clinical	8	37.5	37.5	NT	12.5	12.5	25	NT	37.5	84
Turkey	Water	147	55	NT	26	2	1	1	14	12	132

<sup>\*</sup>Amp=ampicillin, Cep=cephalothin, Cef=ceftraixone or cefotaxime, Na=nalidixic acid, Cip=ciprofloxacin, Gen=gentamicin, TMS=trimethoprim-sulphamethoxazole, Tet=tetracycline, NS=not tested. 1penicillin was used.

## Summarv

In developing countries, potentially pathogenic Aeromonas sp. are very common in water used for drinking and in different types of foods, particularly seafood. Although they are few, food-borne and water-borne outbreaks as well nosocomial outbreaks associated with aeromonads have been reported from these countries. Diarrhoea is the most common manifestation associated with these outbreaks. Many studies from Africa, Asia and Latin America reported significant association of Aeromonas sp. with diarrhoea supporting the reports from developed countries types of Aeromonas that some enteropathogens. These organisms are major causes of skin and soft-tissue infections and aspiration pneumonia following contact with water and after floods. A high incidence of antimicrobial resistance, including resistance to third-generation cephalosporins and the fluoroquinolones, has been found among Aeromonas sp. isolated from clinical sources in some developing countries in Asia. Isolating and identifying Aeromonas sp. to genus level is simple and requires resources that are available in most microbiology laboratories for processing common enteric bacteria. In the future, more research is needed from developing countries to determine the sources, transmission mechanisms, clinical significance, drug resistance and treatment options of Aeromonas-associated infections that suit each country. assistance from research centers in developed countries in studying aeromonads isolated from developing countries on the molecular level undoubtedly will assist greatly in the better understanding the role of these organisms in infections in the latter countries.

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