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Characterization of Strains of *Plesiomonas shigelloides* and Motile *Aeromonas* Isolated from Raised Fish in Iran

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Abstract: *Plesiomonas shigelloides* and *Aeromonas* Species are gram negative organisms which are the members of the expanding group of known water and food borne pathogens and have been increasingly recognized as enteric pathogens. In present study we attempted to map the occurrence and pathogenesis of these bacterias in fish raised pools. We took seventy eight samples from eight fish raised pools and studied their hemolytic, enterotoxin production, motility and antibiotic resistance properties of these bacterias. Frequency of bacterias in these pools were: 5.19% *Plesiomonas shigelloides*, 51.94% *vibrio*, 9.09% *pseudomonas*, 23.07% *Aeromonas hydrophila*, 15.38% *A. Soberia*, 38.46% *A. kavieh*, 23.07% *A. trota*. In entrotoxin production 50% of *P. shigelloides* and 62.38% of *Aeromonases* were positive and in hemolytic activity 50% of *P. shigelloides* and 19.23% of *Aeromonases* were positive. Most strains were resistant to penicilline; ampiciline and carbenicillinand were sensitive to tetracycline, Toberamycine and cotrimoxasol. In conclusion, our results indicate that *P. shigelloides* and *Aeromonases* are able to product a variety of potential virulence markers which may be involved in the pathogenesis of their associated infections and should be given due attention as they might play an important role in the etiology of human gastroenteriticity.

Key words: *Plesiomonas shigelloides*, *Aeromonas*, pathogenicity, fish, gastroenterite

INTRODUCTION

Plesiomonas shigelloides and *Aeromonas* species are gram negative, oxidase positive, facultative anaerobic organisms that reside in the family vibrionaceae but *Plesiomonas shigelloides* is more closely related to the family enterobacteriaceae (Bardon, 1999; Holt *et al*., 1994; Ruymi *et al*., 1994). They are the members of the expanding group of known water and food borne pathogens (Krovacek *et al*., 2000; Hernandez and Ridriquez, 1997). *Plesiomonas shigelloides* has been increasingly recognized as an enteric pathogen (Bravo *et al*., 1998) and as a causative agent of various extra-intestinal infections such as septicemia, meningitis, septic arthritis, pseudoappendicitis and cellulites (Theodoropoulos *et al*., 2001; Tseng *et al*., 2002; Jonsson *et al*., 1997), motile *Aeromonas* Sp. have been implicated as agents of human gastroenteritis (Kuhn *et al*., 1997).

Library expositions on the occurrence of *P. shigelloides* inveterinary microbiology are less frequents then those in human microbiology (Bardon., 1999). The primary reservoirs of *P. shigelloides* are fresh water and estuarine water in temperate climates throughout the world while fish and different kinds of sea foods can act as secondary reservoirs in these environments (Khordori and Fainstein, 1998; de Mondino *et al*., 1995). This bacterium has also been isolated from domestic animals such as dogs, cats, goats, sheep and cows (Khordori and Fainstein, 1998). Most *P. shigelloides* strains secrete a β- hemolysin which may be the major virulence factor associated with this reputed enteropathogenic species (Janda and Abbott, 1993), it possess several other pathogenic properties such as production of heat stabile and heat labile toxin, cytotoxin, haemagglutinin and other potential virulence factors (Ruymi *et al*., 1994; Gardner *et al*., 1990). In this study we attempted to map the occurrence of *P. shigelloides* and other bacterias in the raised fish in the fish raising pools of Zanjan region of Iran, to evaluate its pathogenic function and to attempt a statement of the factors of pathogenicity in isolated strains.

MATERIALS AND METHODS

Eight fish raising pools were examined. Samples obtained from water, fish intestine and gill, then examined by direct culturing on tryptic soy agar. The identification proper of the isolated strains were performed by biochemical tests and only those isolates which were confirmed as gram negative, motile rods, oxidase positive,

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indol, maltose and glucose positive were recognized as presumptive *Plesiomonas* and those which were monitol and Dnase positive and melted gel and hydrolyzed starch were recognized as *Aeromonas* (Krovacek *et al*., 2000; Barron and Fingold, 2003). The strains of *P. shigelloides* and motile *Aeromonases* were examined for different putative virulence factors, such as enterotoxin and hemolysin.

Two methods were used to detect the hemolytic activity presented in both bacterias. In the first procedure a blood agar with 5% sheep blood was used. A 3 μL suspension of an overnight trypton culture of each strain was spotted onto the surface of blood agar plate and the plate was then incubated for 24 h and hemolytic activity investigated. In second procedure sheep blood washed with 8.5% normal saline and mixed with bacterias toxins and incubated in 37°C for 45 min. For enterotoxin production study, bacterias cultured in brain heart infusion broth with 0.6 % W/V yeast extract and incubated in 37°C for 24 h, after centrifuge (15000 g) liquid medium filtered by 0.45 sarterious for purification of toxin and separation of bacterias and 1 mL of liquid injected to intestine loop of rabbits, BHIB (with yeast extract without bacteria) used as negative control. In strains which produced enterotoxin, supernatant absorbed water and electrolytes and volume of loops increased (Nishikava and Kishi, 1987; Rahimz *et al*., 1984). Other virulence factors, such as adhesive and invasive properties were investigated using methods described in previous studies (Krovacek *et al*., 1994; Krovacek *et al*., 1995).

Disc diffusion performed for all isolates by discs of Ampicilin, Penicillin, Tetracycline, Solfometoxasol-trimetoprim, Tobramycin, Cephalotin, Carbenicillin, Nitrofurantoin and Chloramphenicol (O' Toole and Kolter, 1998).

RESULTS

Strains which isolated from pools were *Plesiomonas shigelloides*, *vibrio*, *pseudomonas, Aeromonas hydrophila*, *A. Soberia*, *A. kavieh* and *A. trota*. Frequency of all strains in pool samples showed in Table 1.

Enterotoxin production examined in *Plesiomonas* and *Aeromonases* and results showed 50% of *Plesiomonas* strains and 62.38% of *Aeromonas* strains produced enterotoxin in intestine of rabbits. In supernatant hemolysin test we used 5% sheep blood and 50% of *Plesiomonas* and 19.23% of *Aeromonas* strains were positive and in hemolytic activity study in plate, 50% of *Plesiomonases* had β-hemolytic activity and in *Aeromonas*, 11.5% had β-hemolytic activity and 34.5% had α-hemolytic activity. With use of human blood agar, hemolytic activity of *Aeromonases* enhanced and 23% had hemolytic activity and 34.5% had α-hemolytic activity. In other pathogenic activities, most strains had adhesive activity. All strains of *Plesiomonas* were resistant to Cephalotin. All *Plesiomonases* were resistant to Penicillin, Ampicillin and Carbenicillin and Most of them were sensitive to Tetracycline, Chloramphenicol, Tobramycin and Nitrofurantoin. In *Aeromonas* most of them were resistant to penicillin, carbenicillin and some were sensitive to Tobramycin, Nitrofurantoin, Cotrimoxasole, Chloramphenicol and Tetracycline.

DISCUSSION

Production of a protein with β- hemolytic activity was observed in 50% of *P. shigelloides* strains in both blood agar culture (with 5% sheep blood) and supernatant fluid analysis. In *Aeromonas*, β-hemolytic activity observed 11.5% in blood agar and 19.23% in supernatant fluid analysis and all hemolytic activity was 50% in *P. shigelloides* and 46% in *Aeromonas*. The results of present study diverge from those described previously by Janda and Abbott (de Mondino *et al*., 1995). All of their 36 strains of *P. shigelloides* were non-hemolytic on blood agar plates and 90% produced β-hemolysin in the agar ovelay assay (de Mondino *et al*., 1995).

In entrotoxin production as results showed half of strains of *P. Shigelloides* and most of *Aeromonas* (62.38%) produced enterotoxin which is an important virulence factor in these bacterias. It's well known that flagella motility is one of the possible mechanisms of bacterial translocation that is required for the initial attachment of bacteria to surfaces and thereby acts as potential virulence factor (O' Toole and Kolter, 1998) our results showed that motility of the investigated strains were relatively high.

In fish frequent clinical and pathoantomical finding are death of fish, hyperemia and inflammation of the

Table 1: Frequency of batteries isolated from fish raised pools

Strains :	*P. shigelloides*	A. Hydrophila	A. Soberia	A. Kavieh	A. Trota	Vibrio	Pseudomonas
Place of Isolation:							
Intestine	-	-	-	2	4	11	-
Gill	-	2	4	3	-	15	1
Water	4	4	-	5	2	14	6
Total (%)	4(5/19)	6(23.07)	4(15.38)	10(38.47)	6(23.07)	46(51.94)	7(9.09)

intestines (catarrhal and hemorrhagic), degeneration of hepatopancreas, bleeding in the ventricle and internals edema of the kidney, dilatation of gall bladder and skin lesions (Bardon, 1999). The results of our study indicate, production of enterotoxin and hemolytic and motility activities of these bacterias can act an essential role in pathogenesis of the disease, Clinical symptoms and occurrence of *P. shigelloides* and *Aeromonas* will enhance with extension of immunodeficiency disease. In *P. shigelloides* and *Aeromonas* infections, because of mild and self limited symptoms, antibiotics are given for more severe symptoms (Tseng *et al*., 2002; Wong *et al*., 2000). *P. shigelloides* is naturally resistant to several antibiotics, mainly to a variety of β-lactamase (Penicillin and some Cephalosporins) (Stock and Wiedemann, 2002), as in our study observed, but aminoglycoside antibiotics decrease surface hydrophobicity and motility of *P. shigelloides* and *Aeromonas* and in this study there were susceptibility to Tobramycin and other aminoglycosides, also these results confirmed previous study by Hostacka and Ciznar in this subject (Hostaka and Ciznar, 2003).

CONCLUSION

Our results indicate that *P. shigelloides* and *Aeromonas* are able to produce a variety of potential virulence markers which may be involved in the pathogenesis of their associated infections and should be given due attention as they might play an important role in the etiology of human gastroenteritis, further epidemiological and pathogenicity studies are necessary to elucidate the public health significance of *P. shigelloides* and *Aeromonas* in sea foods and water.

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