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AN INVESTIGATION ON PATHOGENIC VIBRIOS DISTRIBUTION IN DOMESTIC WASTEWATER

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ABSTRACT

Municipal wastewater is one of the most important pollution sources for water supply resources. Identification and enumeration of pathogenic agents particularly pathogenic *Vibrios* are beneficial for controlling and prevention planning of the infectious diseases. This research was carried out to identify the distribution of the recognized pathogenic *Vibrios* with emphasizing on identification of *Vibrio cholera* in the wastewater of Kermanshah city western Iran in 2002. The method of study was cross sectional descriptive. There were 8 discharge outlet domestic wastewaters, which had been chosen as sampling sites. Samples were collected weekly in randomized manner in daytime. Three hundred and thirty nine samples were collected and analyzed. The results indicated site 7 with 5 positives, sites 4 and 8 each with 3, site 5 with 2, sites 2, 3 and 6 each with one positive, whereas, there was not any *Vibrio* detected in site 1. The most positive samples were seen in spring, late summer and early autumn. The positive results were detected on May, June, September, and October. Among positive samples, *Vibrio parahemolyticus*, could be regarded based on differentiation tests. *Vibrio cholera* was not seen. It seems that the presence of *Vibrio parahemolyticus* was due to some food store deal with distribution of seafood. Hence it is suggested that this relationship could be considered through analytical study using PCR for detection of V*ibrios.*

Key words: Pathogenic *vibrios*, domestic wastewater, kermanshah

INTRODUCTION

In the recent years, besides the *Vibrio cholera* and *Eltour*, the role of different types of *Vibrios* in creating cholera diarrhoea and pseudocholera has been known. V*ibrio parahaemolyticus, V. volnificus, V. flavialis, V. frinsis, V. dansla, V. alginolyticus* and *V. mechini* are a group of *vibrios*, which cause human diseases (Huq *et al*., 1983; Ale-Mohamad, 1986; Chakraborty *et al*., 1997, Montanari *et al.*, 1999; Anonymous, 2000 and Park and Park 2002). From the viewpoint of their epidemiology, they could be found in the warm and wet area. There is a possibility of the prevalence of these diseases during the whole seasons in a year, but the incident of the diseases in Iran is from May to October (Anonymous, 2000). Several studies have been conducted on the role of different types of *vibrios* in the sea, and their significance in the nutrients as well as biorecycling of contained organic compounds in coastal areas (Ducklow, 1983; Jorgensen, 1983 and Colwell, 1994). Most of the studies have been done focused on the presence of different types of *vibrios* in edible shelves, seafood and fish, as well as water, benthic material and plankton (Montilla *et al*., 1995 and Macian *et al*., 2000a). There is abundant of *vibrios* in the gut of fish, probably due to the enrichment of its environment (Ugagin 1979 and McFarlane *et al*., 1986). These microorganisms are the flora of the coastal waters and their habitats, which are related to bioenvironmental parameters. Their numbers are increased due to the increasing of temperature. In fact, during summer with the increase of water temperature, their number goes up (Miles *et al.*, 1997 and Motes *et al*., 1998). According to a study in the North Union in Italy from December 1999

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to November 2000, *vibrio parahemolyticus* after Mediterranean *vibrio* had the most distribution in September and only in December and January was not found. *Vibrio parahemolyticus* may cause disease in different ways, including intestinal infections, other illnesses, such as ear infection and so on. This microorganism has been found extensively in sea beaches and estuarine. It is distributed throughout the world, in the temperate and warm seasons in polluted waters, where can be multiplied (Glosa, 1981; Ale-Mohamad, 1986; De-paola *et al*., 1990; Macian *et al*., 2000b; Mizuno *et al*., 2000 and Ellen and Finegold, 2002).

In an epidemiological study, which was carried out in Ukraine (1994-5), by applying the method of PCR, 37 types of *vibrio cholera* and 4 types of nonvibrio cholera serotypes were found. During the period of this epidemic, nontoxicogenic *Vibrio cholera* were not found, *vibrio parahemolyticus* and *vibrio alginiticus*, which apparently did not have any relationship to *vibrio cholera* were detected in this study (Clark *et al*., 1998). Some reports show the epidemic of *vibrio parahemoliticus* infections via eating raw shelves (Mortal and WEEKLY., 1998). There is an association of some types of *vibrios* in the aquatic environment, which depends on temperature and biochemical oxygen demand.

Vibrio parahemolyticus, vibrio alginiticus and *vibrio cholera* could be coincided (Watkins and Kabelli, 1985 and Rajcowsk and Rice, 2001). In a study from September 1983 to November 1984 in Jeddah, Saudi Arabia, 23 samples from the effluent of wastewater treatment plant of Jeddah were analyzed microbiologically. Totally, 395 species of fermentative gram negative bacteria were separated and typed. *Vibrio*, *Shigella*, and *Salmonella* were not detected (Muler *et al*., 1985). One of the reasons that they were not detected might be lack of using enrichment media, which was mentioned in the method of study.

This study aimed to describe and determine the probability of *cholera* and *pseudocholera* incidences, which are caused by pathogenic *vibrios*. The other goal was the determination of type differentiation, time and place distributions of pathogenic *Vibrio*s in domestic wastewater. The distribution of different types of *vibrio*s in the environment, especially, in the polluted waters and domestic wastewater, was taken into account in the frequent incidence of *cholera* and *pseudocholera* in Kermanshah province, which urged the researcher to design and operate this investigation. Furthermore, the detection of pathogenic *vibrios* in domestic wastewater results in optimizing quality assurance and management of water resources.

MATERIALS AND METHODS

This study was descriptive cross sectional research, which is designed and carried out in order to find the distribution of the pathogenic *vibrios* in the sewage of Kermanshah city in 2004. At the beginning, all discharge points of sewerage; which are ended to Gharesou River, were found. Eight points have been chosen as sampling stations, sampling was then carried out from each station. The samples were collected in a day, once a week, randomly according to Standard Methods for the Examination of Water and Wastewater (Anonymous, 1995). In addition, while sampling, pH and temperature were measured. The predicted samples number was 288 that with variations in the process of sampling reached 339. As a result, each station included 42 samples in a year, except the station 1 with 45 samples.

To identify the pathogenic *vibrios*, experiments were carried out based on Diagnostic Microbiology book (Ellen and Finegold, 2002). The process of sample preservation was carried out with sample enrichment media as a specific volume, which was 10 mL of raw wastewater centrifuged. It is appropriate to add 1-2 mL of the sediment in the lab tube containing alkaline peptone water with pH=8.5. The tube was incubated at 30 °C for 24 h, then the tube turnd to turbid and under the aseptic conditions, some of its contents were inoculated on TCBS cultural media. After 24 hr in temperature of 35 °C, if colony was formed, smear provision and gram staining were performed in order to identify the *vibrios*. If gram negative bacilli curve shaped organism was found, it was examined by KOH 30%, for assuring of the accuracy of the process. The organism should be studied by the microscope, in order to confirm the

growth of gram negative bacteria and apparent motility of colony generator microorganisms.

Differentiation tests were carried out using Oxiddase and Catalase tests: If these two tests were positive the next step of transferring bacteria from differentiation media containing glucose, succors, and lactose, had to be done. The media triple sugar iron agar (TSI) and kigler iron agar (KIA) were used. If after 24 to 48 hr. fermentation of the oases in the lab tube was in the form of ALK/A or A/A in TSI media and ALK/A in KIA media, then the organisms could be recognized and the next step of the process would be identifying the unknown sashes.

Identifying sashes, using chemical differentiation tests, such as VP, citrate, ONGP, hydrolyze, lysine, decarboxilase, Indol, Reduction and nitrate reduction to nitrite tests and also observing haemolyse on Blood Agar (B.A.).If the result of the mentioned tests were positive, then the final tests were done by implying NaCl tolerance tests in various concentrations, in which, microorganisms, transmitted on mediacontaining sodium chloride in different concentrations (0%, 3%, 6%, 8% and 10%) may grow or survive; after that, the produced results were identified with the differentiation table of *vibrios* and *entrobacteriae.*

pH and temperature of samples were measured in the field according to the Standard Methods for Examination of Water and Wastewater.

RESULTS

Total analyzed sample number was 339 and in 16 collected samples and 7 sampling stations, pathogenic *vibrios* were found. Tables 1 to 3 show the results of experiments in domestic wastewater with place and time distribution of purposed organisms in the field study. Time distribution of pathogenic *vibrios* are de-picted by Tables 2 and 3. The results indicated site 7 with 5 positives, site 4 and 8 each with 3 positives, site 5 with 2 positives, sites 2, 3 and 6 with one positive, whereas there was not any *vibrio* detected in site 1.

Table 1: Ferequency of positive samples in the stations in 2004

Stations	Number of positive samples	Percentage %
1	0	0
2	1	6.25
3	1	6.25
4	3	18.75
5	2	12.50
6	1	6.25
7	5	31.25
8	3	18.75
Sum	16	100.00

Table 2: Frequency of positive samples within the seasons

Seasons	Number of positive samples	Percentage %
Spring	10	62.50
Summer	1	06.25
Fall	5	31.25
Winter	0	100.00

Table 3: Frequency of pathogenic *vibrios* in different months of a year

Months	Number of positive samples	Percentage %
Feb-Mar.	0	0
April-May	4	25.00
June-July	6	37.50
Sept-Oct.	1	6.25
November	5	31.25
Dec.-Jan.	0	0
Sum	16	100.00

DISSCUSION

The obtained results showed that 4.72% of the analysed samples contained pathogenic *vibrios*. Station 7 with 5 positive samples (April-May 2 cases, June-July one case and September-October 2 cases) showed the highest pollution. The mean pH of samples was 8.05 (±0.5) with a minimum of 7.5 and maximum of 8.6. The most of the positive samples had pH equal or more than the mean. The mean temperature of samples in the field of sampling was 21.6 ^{o}C with minimum and maximum of 11 ^{o}C and 26 ^{o}C, respectively; the positive samples had temperature more than 18 ^{o}C.

According to the differentiation tests, *vibrio cholera* and *Eltour* were not observed. However, the results showed that the positive samples had most adaptation with *V. parahemolyticus*, this result seems to be adjusted with the study of Watkins and Kabelli (1985), which reported a project result on the Naragan B.R.I estuarine, this report revealed the population density of *V.*

parahemolyticus related to the faecal pollution level. A significant association between the population level of *V. parahemolyticus* and *Escherichia coli, Clostridium perfrengence* as well as *Entrococci* was found. The most densely of *V. parahemolyticus* was found near the water surface of the polluted areas. Their habitat decreased while distance increased from the source of pollutants of the site of wastewater discharge. In addition, with the increasing of water depth, the population of this microorganism decreased rapidly. An indirect impact of the pollution induced by wastewater on the presence and abundant of *V. parahemolyticus* was noticed. Probably this situation was due to the biostimulation and the diversity of biological food chain, such as development and growth of the micro flora; because; it does need chitin materials in its food chain, which could be produced as a result of crustaceans and other planktons (Watkins and Kabelli, 1985). *V. parahemolyticus* in the aquatic environment with the biochemical oxygen demands more than 2 mg/L and in temperature of 4 to 50 °C may survive, however, within six days its population decreases rapidly (Rajcoski and Rice, 2001). According to Moller *et al*., (1985), no *vibrios* were detected in wastewater treatment plant of Jaddeh in Saudi Arabia. Of course in this research the enrichment media, which were necessary for growth of *vibrios*, were not used.
Station number 1, Dolat Abad, during the sampling did not show any positive results, because, this part of the city is an industrial and commercial area. Station number 7, Abshoran, which receives a large volume of wastewater of the south and center of the city, had the most number of positive cases (33.3%). Regarding the confirmation of the existence of *V. parahemolyticus*, which is one of the pathogenic *vibrios* and the relatively high number of the positive cases in this station, a focal investigation on the causality of this fact is inevitable. There is a possibility of seafood supplement and distribution or other related sources in this area.
The probability of the existence of *V. parahemolyticus* in polluted water and wastewater effluents is in agreement with Watkins and Kabelli studies (1985).

From seasonal distribution point of view, the positive samples have been obtained in spring, late summer and the beginning of fall. This finding is in accordance with other studies (Watkins and Kabelli, 1985; Mortal. Weekly., 1998 and Rajcoski and Rice, 2001). *Vibrios* in the temperate conditions between 15 to 25°C had the most rates on the epidemiology of its causality. The time distribution of *vibrios* in the seasonal and monthly periods could be related to the temperature variations. Incidence and prevalence of shiglloid diseases and *pseudocholera* could be due to this microorganism while rejection of *Shigellae* and *cholera* agents are confirmed. This health problem in such months and seasons can not be irrelevant to the mentioned microorganism in Kermanshah.
One of the considerable points in this study was the presence of the correlation between survivals of *vibrios* with pH of the environment, so that all samples with pH<8 were negative and all positive samples were with pH≥8. Although, this subject was not matter as an issue in this study, however, it could be one of the confirmation aspects from the viewpoint of the working parameters in the process of growth and multiplication of Vibrios. The expected pathogenic *Vibrio* as a result of differentiation tests, in accordance with the standard table, the expert opinion and the specialists, adjusted with *V. parahemolyticus* and other types of *Vibrios*, *vibrio cholera* and *Eltour* were not detected.

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