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Occurrence, Antibiotic Resistance and Pathogenicity of Non-O1 *Vibrio cholerae* in Moroccan Aquatic Ecosystems: A Review

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1. Introduction

The problem of water scarcity is becoming more pronounced especially in countries with arid and semi-arid climates such as Morocco. Wastewaters discharge into different aquatic ecosystems (groundwater, sea water, river, lake water...), are draining different types of microorganisms and hazardous chemicals. The microbiological risk is not negligible, especially in areas where wastewater or other contaminated water, are reused for irrigation without preliminary treatment or for direct consumption by human and animals.

The emergence of bacteria resistant to antibiotics is common in areas where antibiotics are widely used, but the occurrence of antibiotic-resistant bacteria is also increasing in aquatic environments. Some pathogenic bacteria may occur naturally with the spread of resistance genes.

Vibrio cholerae is a natural inhabitant of the aquatic environment where water plays an important role in its transmission and epidemiology (WHO 1993; Chakraborty et al. 1997). This bacterium plays a role in ecological ecosystems and it is widely distributed in bays, estuaries, coastal water, reservoirs, rivers and possible water supplies for human consumption (Pathak et al., 1992; Caldini et al., 1997; Isaac-Marquez et al., 1998; Dumont et al., 2000).

The interest in examining the non-O1 serogroup of *V. cholerae* has been accentuated at an international level, given that some recent epidemic outbreaks in India and Bangladesh have been caused by non-O1 *V. cholerae* isolated in aquatic environment (Ramamurthy et al., 1993). Currently, it is recognized that non-O1 *V. cholerae* plays an important role as the causative agent of sporadic cases of cholera-like disease and isolated outbreaks linked to the consumption of contaminated water (Yamamoto et al., 1983; Chakraborty et al., 1997; Bag et al., 2008). Non-O1 *V. cholerae* has also been implicated in extra intestinal infections, including wounds, ear, sputum, urine and cerebrospinal fluid (WHO, 1993).

Resistance of *V. cholerae* to commonly used antimicrobials is increasing both in the farm animal and public health sectors and has emerged as a global problem.

This review present a synthesis of our research works on non-O1 *V. cholerae* since 1992, in comparison to faecal indicator bacteria, in some Moroccan aquatic ecosystems especially in wastewaters and groundwaters. We will discuss and compare our works with some other studies over the world.

2. Occurrence, pathogenicity of non-O1 *V. cholerae* in some Moroccan aquatic ecosystems

2.1 Occurrence and ecology of non-O1 *V. cholerae*

The use of untreated wastewater for agriculture irrigation poses serious health problems over the world. Several treatment systems of wastewater were developed to reduce the load of pollution. The stabilization pond system was tested in Marrakech region (Mezrioui et al., 1995; Mezrioui & Oufdou, 1996; Oufdou et al. 2004). It is composed of two oval ponds linked in series, each is of 2500 m^2 in area. The first pond is anaerobic (depth of water 2.3 m) and the second pond is facultative aerobic (depth : 1.5 m). The raw sewage flow to the system is maintained at 5.4 L/sec. The total hydraulic retention time was set at about 18 days with 10.5 days in the first pond and 7.5 days in the second pond.

Non-O1 *V. cholerae* was quantified using the most probale number (MPN) method using three tubes or flasks per inoculated volume and a series of 100, 10, 1 mL, and dilutions of water. They were inoculated into the three tubes with 3 stages: (i) enrichment by culture of 100, 10 or 1 mL of the sample from the series of three tubes of alkaline peptone water (1% peptone, 1% NaCl, pH 8.6) incubated at 37 °C for 18 h. (ii) Isolation was performed by culture of 0.1 mL taken from the surface in each enrichment tube or from one of its dilutions on thiosulfate-citrate-bile-sucrose agar (TCBS), incubated at 37 °C for 24 h. (iii) Identification of the colonies assumed to be those of non-O1 *V. cholerae* was carried out according to the methodology described by Lesne *et al*. (1991), Mezrioui and Oufdou (1996), Lamrani Alaoui et al. (2008) and Lamrani et al. (2010).

The seasonal abundance of non-O1 *V. cholerae* in wastewaters before and after treatment in stabilization ponds in an arid Mediterranean climate has been undertaken. A series of stations along the two stabilization ponds were sampled during two periods. The cold (or hot) period corresponds to months when the water temperature is below (or above) 22°C. This temperature was the average water temperature for the whole period of study (16 months).

Results showed that high abundances of non-O1 *V. cholerae* were noted during the hot months and low abundances during the cold months. In treated wastewaters, high abundances of non-O1 *V. cholerae* were recorded during hot period with an average abundance of $1.7x10^3$ MPN/mL. During cold periods, these densities were calculated to be $2.5x10^1$ MPN mL^{-1}. These seasonal dynamics were confirmed by the autocorrelation coefficient showing the cyclic nature of non-O1 *V. cholerae* abundances (Mezrioui et al., 1995).

In contrast, the spatial-temporal dynamics of faecal coliforms (FC) were the inverse of those of non-O1 *V. cholerae* abundances. Average FC abundances at the system's inflow point were $1.7x10^5$ cfu/mL, while at the seconf pond's outflow, they were $8.3x10^3$ cfu/mL.

The average seasonal variation of FC abundances at the second pond's outflow point was evaluated to $8.3x10^3$ cfu/mL at the cold period and $1.8x10^3$ cfu/mL at the hot period (Mezrioui & Oufdou, 1996). The inverse relationship between non-O1 *V. cholerae* and FC was more pronounced at the outflow point of the second pond (R^2= 0.68) than that of the first pond (R^2= 0.51).

As for removal efficiency, stabilization pond system of Marrakech led to 97.97% average overall reduction in FCs, whereas this system treatment is not efficient in removing non-O1 *V. cholerae* abundances (Mezrioui et al., 1995; Mezrioui & Oufdou, 1996).

We have also followed the dynamics of non-O1 *V. cholerae* in Marrakech groundwater (in supplying well waters) in comparison with other bacteria of sanitary interest. Sixteen wells covering two regions (Tensift and Jbilet) were studied. They are situated at the North of Marrakesh city (31°36' N, 08°02' W, Morocco) (Lamrani et al., 2010).

Detectable non-O1 *V. cholerae* was present in 81% of samples and the average abundances ranged from 0 to 11100 MPN/100 mL. Detectable *P. aeruginosa* was present in 88% of samples and its abundances ranged from 0 to 1670 cfu/100 mL. The total occurrence of FC and Faecal Streptococci (FS) during the period of study was 94% and their densities varied respectively from a minimum of 0 cfu/100 mL to a maximum of 10200 cfu/100 mL for FC and 6700 cfu/100 mL for FS. The annual average densities of non-O1 *V. cholerae* were 4903 MPN/100 mL in all samples. Whereas, the annual average densities of *P. aeruginosa*, FC and FS were respectively 206 cfu/100 mL, 1891 cfu/100 mL and 1246 cfu/100 mL (Lamrani et al., 2010).

Our results demonstrated that non-O1 *V. cholerae* and the other studied bacteria, occurred in the majority of the studied wells water. These wells serve as an important natural resource for drinking water, domestic water supply and recreation for rural and suburban populations. This fact could be responsible for potential health effects on populations using this groundwater. According to WHO standards, the studied wells are completely unsuitable for drinking water and other domestic uses.

The highest abundances of studied bacteria were detected at the wells located near malfunctioning septic systems or beside a high number of pollution sources such as infiltration of wastewater, septic tanks seepage, discharge leachates or human and animal faecal materials nearby the studied wells. Moreover, the majority of the studied wells are situated at 0 m to 400 m from pollution sources. These factors led to the contamination of the groundwater.

Based on the results of the present study, it is possible to conclude that groundwater can play an important role as a transmission vehicle of non-O1 *V. cholerae* and the other studied bacteria. Isaac-Marquez et al. (1998) considered that the presence of non-O1 *V. cholerae* in water supplies might be responsible for a proportion of diarrheic diseases among population of the city of Campeche and the rural locality of Becal (Mexico). Several reports have demonstrated that gastrointestinal and extra-intestinal infections caused by non-O1 *V. cholerae* are linked with contaminated water and other activities in aquatic environments, and this bacterium could therefore pose a problem for public health (WHO, 1993; Chakraborty et al., 1997).

Our findings (Lamrani et al. 2010) are in agreement with those reported by Nogueira *et al*. (2003) and Isaac-Marquez *et al*. (1998). These authors investigated water quality at sources

and points of consumption of urban and rural communities. According to these authors, water distribution system, spring water and private wells samples had high coliforms positive and high percentages of non-O1 *V. cholerae*.

The comparison of non-O1 *V. cholerae* and FC abundances, using the Spearman correlation test, has showed that there is generally a positive relationship between these bacteria in the studied wells. FC can be used to detect the presence of non-O1 *V. cholerae* in Marrakesh groundwater. However, no significant relationship was observed between the presence of non-O1 *V. cholerae* and *P. aeruginosa* (Lamrani et al., 2010).

The ecological role of *V. cholerae* in environment implies a direct influence of environmental conditions and climate on the presence, persistence and abundance of bacteria in the aquatic ecosystem. To explain this difference of behavior of these bacteria, we have established the correlation of some of these factors with non-O1 *V. cholerae* abundances. We also tested some experimental studies on the effects of some environmental factors (temperature, pH, sunlight and algae) on survival of non-O1 *V. cholerae* compared to faecal indicator bacteria (Oufdou et al., 1998; Oufdou et al., 1999; Lamrani et al., 2009; Oufdou & Oudra, 2009).

The correlation of non-01 *V. cholerae* abundances in Marrakech stabilization ponds (Spearman correlation) was carried out. A positive and very significant correlation ($p<0.01$) between water temperature and pH was observed at the system's outflow point. At this point, Spearman coefficients values were respectively 0.91 and 0.76. In the system's inflow, an extremely significant correlation was observed only with temperature (Mezrioui et al., 1995).

The experimental effects of pH, temperature and sunlight were carried out. The strains of non-O1 *V. cholerae* and *E. coli* tested were isolated from the first pond of Marrakech stabilization pond. The survival of these bacteria was studied in experimental microcosms of 500mL flasks, each contained 200mL of filtered outflow water. Each microcosm was seeded separately with a standard inoculums (approximately 10^5 cfu/mL) prepared from a bacterial suspension (non-O1 *V. cholerae* or *E. coli*) in physiological water (0.9% NaCl).

The pH values tested (6.6, 7.3, 8 and 8.8) and the temperature values tested (8, 15, 23 and 30°C) corresponded to those measured at the stabilization ponds over the year.

The effect on bacterial survival was evaluated after calculation of the die-off coefficient *k* which is determined in accordance with the formula:

$$N_t = N_0 e^{-kt}$$

Where N_0 and N_t are respectively the initial bacterial number and the number of bacteria at time t. *k* is the die-off coefficients expressed in hourly terms (/h) (Crane and Moore, 1986).

The effect of pH on the behaviour of non-O1 *V. choleare* differed from the effect on *E. coli*. The greatest survival of non-O1 *V. cholerae* was at pH 8 (k = 0.0164/h) followed by the pH 8.8 (k= 0.0170/h). Whereas at the pH values of 6.6 and 7.3, the die-off coefficient were respectively 0.0197/h and 0.0195/h. The alkaline pH of 8.8 promoted survival of non-O1 *V. cholerae* (k=0.0170/h) and reduced that of *E. coli* (k=0.0232/h). At neutral pH (7.3), non-O1 *V. cholerae* did not survive as well (k= 0.0195/h) as *E. coli* (k=0.0124/h).

The minor variations in pH occurring in natural environments, making pH a relatively unimportant variable compared with other environmental factors, such as sunlight, temperature... However, in aquatic ecosystems such as stabilization ponds, phytoplanktonic blooms appears systematically and increase pH values (Oufdou et al., 2004; Oufdou & Oudra, 2008). The pH is a parameter that was used to improve the isolation of *V. cholerae* environmental samples by enrichment (using alkaline peptone water at pH 8.6) (Lipp et al., 2002).

Non-01 *V. cholerae* and *E. coli* survived longer at low temperatures. The survival of both bacteria was noticeably reduced at 23 and 30°C. This low survival rate of non-01 *V. cholerae* did not explain the high positive correlation between the non-01 *V. cholerae* abundances and temperature. Indeed, it would appear that the effect of temperature is a function of other factors such as nutrients. In microcosms such as flasks, where there is considerable confinement, nutrients are heavily depleted at 23 or 30°C, with a resultant decrease in bacterial survival. In the environments like wastewater, where there is no lack of nutrients, high temperatures lead to a multiplication of bacteria.

Solar radiation had a much greater effect on *E. coli* than it did on non-O1 *V. cholerae* (Mezrioui et al. 1995). This difference in bacterial survival as a result of sunlight factor could be explained by a difference in the bacterial's reaction to sunlight. Indeed, sunlight is absorber by a sensitizer that reacts with oxygen to form peroxides or hydroxyl radicals (Curtis et al., 1992). These authors indicated that damage to the membrane of an organism is ecologically important, since it makes the organism more sensitive to the effects of other factors such as the high pH values encountered in stabilization ponds. The obtained results by Mezrioui et al. (1995) showed that alkaline pH values inhibit the survival of *E. coli*, and its survival is thus less after exposure to sunlight. Non-O1 *V. cholerae*, on other hand, which survived better at pH 8 than at pH 7.3, is less sensitive to sunlight.

The effect of the cyanobacterium *Synechocystis* sp. on the survival of non-O1 *V. cholerae* was carried out (Oufdou et al., 1998 ; Oufdou et al., 2000). Blooms of this cyanobacterium occur during hot periods in wastewater stabilization ponds of Marrakech. Oufdou et al. (1998) have studied the effect of the picocyanobacterium Chroococcale: *Synechocystis* sp. on the behaviour of non-O1 *V. cholerae* in comparison to those of *E. coli* and *Salmonella* sp.. *Synechocystis* sp. was isolated from this ecosystem and cultivated in laboratory at controlled conditions of light and temperature.

Extracellular and intracellular products released by this microalga were tested on studied bacteria. Extracellular products obtained at the supernatant of algal culture in stationary phase, reduced *E. coli* and *Salmonella* sp. growth and stimulated non-O1 *V. cholerae* growth. Intracellular products obtained after lysing algal cells by ether, reduced *E. coli* and *Salmonella* sp. growth. The effect of products released by *Synechocystis* sp. was compared for axenic and non axenic strain alga. Obtained results showed that the presence of heterotrophic bacteria increased the reduction of *E. coli* and *Salmonella* sp. growth by extracellular and intracellular products of *Synechocystis* sp..

Blooms of this picocyanobacterium in Marrakech waste stabilization ponds, is among the important factors that affect the dynamics and survival of studied bacteria in this aquatic ecosystems which functions under a Mediterranean arid climate.

2.2 Pathogenicity of non-O1 *V. cholerae*

Several virulence factors such heat stable toxin (ST) (Arita et al., 1986), hemolysin (Yoh et al., 1986; Bag et al., 2008) and other cell-associated hemagglutinins (Banerjee et al., 1990) have been identified in non-O1 *V. cholerae*. Production of hemolysin and surface hemagglutinins of pathogenic bacteria, are important virulence determinants as they may serve as recognition and invasion molecules in cell-cell interaction affecting the host-pathogen relationship (Guhathakurta et al., 1999; Singh et al., 2001; Chatterjee et al., 2009). It has been demonstrated that non-O1 *V. cholerae* adheres and invades the epithelial cells of gut mucosa and starts its multiplication (Nishibuchi et al., 1983). This situation occurs only with expression of certain virulence factors as previously cited (Nishibuchi et al., 1983; O'Brien et al., 1984; Ichinose et al., 1987).

To characterize the virulence factors of the bacterial isolates in our study, hemolysis and hemagglutination with human erythrocytes were realized.

The hemagglutination and hemolytic activities of non-O1 *V. cholerae* strains isolated from wastewater and suburban and rural groundwater supplies of Marrakech region were carried out. Non-O1 *V. cholerae* strains isolated from Marrakech wastewater showed a hemagglutination rate of 55%. The distinction between the degrees of hemagglutination showed that 42.5% of non-O1 *V. cholerae* strains are able to agglutinate with a high level, red cells of human blood O group, while the percentage of strains showing hemagglutination reaction with low level is only 12.5%. As for the production of hemolysins, non-O1 *V. cholerae* strains showed 37.5% of β-hemolytic activity whereas no hemolytic activity α was noted.

In the groundwater, bacterial strains were found to be adhesive (hemagglutination), with percentages of 63.09%, 65.09%, 84.06% and 87.98% respectively for non-O1 *V. cholerae*, FS, FC and *P. aeruginosa*. Non-O1 *V. cholerae* strains had the highest percentage of hemolytic activities (production of hemolysin: α+β) (71.29%), in comparison to FS (20.71%), to FC (16.88%) and to *P. aeruginosa* strains (9.13%).

Analysis of a total of 1183 strains isolated from the studied wells, revealed that non-O1 *V. cholerae* had the highest β hemolytic activity (33.12%), while only 3.44% of FC and 4.44% of FS strains have this type of hemolysis. As for *P. aeruginosa*, β hemolytic activity was very low (1.44%). FC, FS and *P. aeruginosa* strains isolated from Marrakech groundwater expressed significantly lower hemolytic activity compared to non-O1 *V. cholerae* ($P < 0.05$, test of two proportions). Hemolysin of *V. cholerae* is suggested to be a virulence factor contributing towards pathogenesis (Nagamune et al., 1995). Guhathakurta et al. (1999) purified a bifunctional hemolysin-phospholipase C molecule from non-O1 *V. cholerae* (O139) showing enterotoxic activity, as shown by fluid accumulation in the ligated rabbit ileal loop and in the intestine of suckling mice (Pal et al., 1998).

The percentages of hemolytic isolates observed in this study are comparable to those reported by Begum et al. (2006). These authors found that 80% of the total non-O1 and non-O139 *V. cholerae* isolates were hemolysin positive. However, our results were lower than those obtained by Amaro et al. (1990). These authors showed that 97% of environmental non-O1 *V. cholerae* strains displayed hemolytic activity for human blood.

Adhesion to the intestinal mucosa represents the first step in the infectivity of bacterial pathogens such as *V. cholerae* (Booth and Finkelstein, 1986). This process is mediated by non-specific (mainly hydrophobic) and specific (binding of the bacterial adhesin with its receptor on the epithelial cell) interactions (Kabir and Ali, 1983). Agglutination of erythrocytes is among the most useful assays to test the attachment ability of potential pathogens.

Bacterial strains isolated from Marrakesh groundwater were found to be adhesive, with a range of hemagglutination activities varying from 63.09% for non-O1 *V. cholerae* to 65.09% for FS, 84.06% for FC and 87.98% for *P. aeruginosa*.

Among 317 strains of non-O1 *V. cholerae*, 60 strains (18.93%) were strongly adhesive (+2) and 140 (44.16%) were partially agglutinated (+1) to erythrocytes. On the other hand, 69.06% of FC strains and 62.02% of *P. aeruginosa* expressed complete agglutination (+2) capacity, and respectively 15% and 25.96% of them agglutinated partially (+1) to erythrocytes.

Our findings are in agreement with previous studies on hemagglutination distribution in *V. cholerae* (Amaro et al., 1990). These authors showed that 109 (78%) of the environmental non-Ol *V. cholerae* strains assayed, possessed agglutinating capacity.

Determination of several potential virulence factors in *Vibrio* spp. by Baffone et al. (2001) demonstrated that species were adhesive, with percentages ranging from 40% for *V. fluvialis* to 55-80% for *V. alginolyticus*, non-O1 *V. cholerae* and *V. parahaemolyticus*.

2.3 Antibiotic resistance of non-O1 *V. cholerae*

Among the 240 non-O1 *V. cholerae* strains isolated from Marrakech stabilization ponds, 89 (37.1%) isolates were resistant to at least one of 14 tested antibiotics (Mezrioui et al., 1995; Mezrioui & Oufdou, 1996). The levels of antibiotic resistance at the inflow and outflow points of the system were respectively 40 and 34% and were not significantly different. This antibiotic resistance level was lower than that obtained by Amaro et al. (1988). These authors showed that among 146 non-O1 *V. cholerae* strains isolated from the environment and tested for antibiotic resistance, 93% were resistant to at least one antibiotic.

It appears that in wastewater treated by Marrakech stabilization ponds treatment, non-O1 *V. cholerae* antibiotic resistance was not significantly modified. However, in the same treatment system, Hassani et al. (1992) have showed that the antibiotic resistance increased in 693 *E. coli* strains as they passed through the ponds. Levels of *E. coli* antibiotic resistance on the inflow and the outflow were 21% and 34% respectively.

Mezrioui & Oufdou (1996) have noted that non-O1 *V. cholerae* showed high resistance to ampicillin, amoxicillin and mezlocillin at all sampling points of Marrakech stabilisation pond system, followed by resistance to cefalexin, cefoperazone and amikacin.

Combined resistance to ampicillin and amoxicillin or to ampicillin and mezlocillin were the most frequently observed resistance pattern. Few isolates were resistant to cefalexin, cefoperazone or amikacin (less than 9%).

More importantly, some strains of non-O1 *V. cholerae* were found to be capable of receiving and stably maintaining plasmids conjugally transferred from *E. coli*. Antibiotic resistance can be transferred from non-O1 *V. cholerae* to other members of the *Enterobacteriaceae* family such as *E. coli* K12. Transfer frequencies in nutrient broth and filtered wastewater were respectively 3×10^{-5} and 2×10^{-8} (Mezrioui & Oufdou, 1996).

As for antibiotic resistance in groundwater of Marrakech-Tensift-Al Haouz region, antibiotic susceptibility testing revealed that the overall resistance (resistance to at least one antibiotic) of non-O1 *V. cholerae* strains was 79%, while it was 100% for *P. aeruginosa*, faecal coliforms (FC) and Faecal streptococci (FS) strains (Lamrani et al. 2010). 317, 208, 320 and 338 strains were respectively tested. The multiresistance level of non-O1 *V. cholerae* strains (69%) was significantly lower than that of FC and FS strains (95%), whereas 100% of *P. aeruginosa* strains were multiresistant. The monoresistance (resistance to one antibiotic) of non-O1 *V. cholerae* was 10% while it was 5% for FC and FS strains. Sixty six strains (21%) of non-O1 *V. cholerae* were susceptible to all antibiotics tested, while none of the isolates *P. aeruginosa*, FC and FS was susceptible to all antibiotics tested. Our results showed that among non-O1 *V. cholerae* strains resistance was most commonly observed towards sulfamethoxazole (75%), followed by streptomycin (62%) and cephalothin (60%) and trimethoprim (49%). A smaller proportion of these isolates were resistant to erythromycin (18%), kanamycin and polymyxin B (12%), cephotaxim (8%), gentamycin (7%) and tetracycline (2%). All the 317 non-O1 *V. cholerae* isolates were susceptible to chloramphenicol, nalidixic acid and novobiocin.

The obtained results showed correlation between bacteriological pollution and their antibiotic resistance and virulence.

The dominant multiresistant profiles noted for non-O1 *V. cholerae* were to seven antibiotics; of 220 strains resistant to at least two antibiotics, 53 strains (24.09%) were resistant to seven antibiotics. The maximal multiresistance was to ten antibiotics with two profiles: "Gm, Str, Km, Tpm, Smx, Amp, Amx, Cfl, Cfm, Ery" and "Gm, Str, Km, Tpm, Smx, Tc, Amp, Amx, Cfl, Cfm".

Of the antimicrobial resistant FC strains isolated, 80% were resistant to five or more antibiotics. The dominant multiresistant profile noted for FC was to eight antibiotics (11.6%). The maximal multiresistance was to fourteen antibiotics with two profiles: "Amp, Amx, Amx-clav, Cfl, Cfm, Cft, Chl, Gm, Na, PB, Smx, Str, Tc, Tpm" and "Amp, Amx, Amx-clav, Cfl, Cfm, Cft, Gm, Km, Na, PB, Smx, Str, Tc, Tpm".

3. Conclusion

Although the stabilization ponds showed considerable effectiveness in eliminating faecal coliforms, the system's final effluent contained not inconsiderable non-O1 *V. cholerae* concentrations. Their presence in treated wastewater limits their re-use in agriculture. The risks associated with the presence of non-O1 *V. cholerae* in effluent will be greater if these bacteria are multi-antibiotic resistant. The addition of a third maturation pond to Marrakech stabilization ponds may help in the reduction of bacteria.

The experimental studies on the effects of some environmental factors (temperature, pH, sunlight and the cyanobacterium; *Synechocystis*) on survival of both bacteria, showed that the alcaline pH (>8) seems to present a more bactericidal effect on FC than on non-O1 *V. cholerae*. Thus, the Cyanobacteria blooms, occurring periodically during summer in sewage stabilization ponds of Marrakech, will be considered as one of the major factors leading to high levels of non-O1 *V. cholerae* and low abundances of FC bacteria during the hot period.

Conjugative transfer of resistance genes occurred between non-01 *V. cholerae* strains and other bacteria such as *E. coli*. The high dissemination capacity for these R-factors plasmids can occur even when intergeneric transfer frequencies are relatively low.

Effluent discharged from stabilization ponds into receptor environment or re-used for irrigation purposes should be purified by more advanced methods prior to discharge in areas of greatest human impact and where antibiotic resistance could well prove to be a serious human problem in the future.

The bacteriological quality of groundwater in Marrakech region suggested that the studied wells water were heavily contaminated with FC, FS, *P. aeruginosa* and non-O1 *V. cholerae*. Their presence could have significant health risks for local population when it is used as a drinking water. According to WHO standards for drinking water, the studied well waters were unsuitable for the consumers. The characteristics of the environment of the prospected wells and their proximity from many sources of pollution as well as the lack of rigorous protection contributed to their contamination. In these wells water, the result of the interaction network underwent a high variability. This may be at the origin of a high ecological instability of the studied bacteria and physicochemical parameters. The need for guidelines to protect groundwater quality in Morocco is imperative.

Non-01 *V. cholerae* and the other studied bacteria isolated from Marrakesh groundwater are virulent since most of them are producers of hemolysins, hemagglutinins and are multiresistant to antibiotics. These bacteria may have important public health implications. Their role in several cases of gastro-enteric and systemic pathologies noted at the local population of Marrakech area (Jbilet and Tensift region) deserve greater interest and attention.

Urgent reactions are required to apply adequate solutions such as disinfection of groundwater, protection of the wells, public awareness. This study may be considered a typical example of what is happening in other cities in the developing world and it is estimated to assist local authorities in developing plans and actions to improve groundwater quality.

4. Acknowledgment

This work is partly financed by the ifs projects n°F/2826-2 and F/2826-3F.

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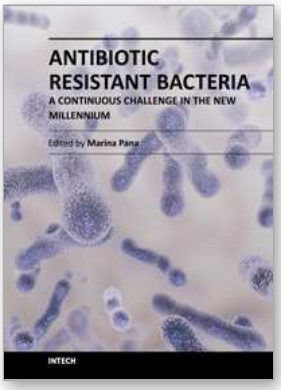

Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium

Edited by Dr. Marina Pana

ISBN 978-953-51-0472-8
Hard cover, 576 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Antibiotic-resistant bacterial strains remain a major global threat, despite the prevention, diagnosis and antibiotherapy, which have improved considerably. In this thematic issue, the scientists present their results of accomplished studies, in order to provide an updated overview of scientific information and also, to exchange views on new strategies for interventions in antibiotic-resistant bacterial strains cases and outbreaks. As a consequence, the recently developed techniques in this field will contribute to a considerable progress in medical research.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Khalid Oufdou and Nour-Eddine Mezrioui (2012). Occurrence, Antibiotic Resistance and Pathogenicity of Non-O1 Vibrio cholerae in Moroccan Aquatic Ecosystems: A Review, Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium, Dr. Marina Pana (Ed.), ISBN: 978-953-51-0472-8, InTech, Available from: http://www.intechopen.com/books/antibiotic-resistant-bacteria-a-continuous-challenge-in-the-new-millennium/occurence-antibiotic-resistance-and-pathogenicity-of-non-o1-vibrio-cholerae-in-moroccan-aquatic-ecos

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