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Influence of Bizerte city wastewater treatment plant (WWTP) on abundance and antibioresistance of culturable heterotrophic and fecal indicator bacteria of Bizerte Lagoon (Tunisia)

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ABSTRACT

The waste water treatment plant (WWTP) of the city of Bizerte concentrates different types of chemical and biological pollutants in the Bizerte lagoon (Tunisia). Considering four upstream and downstream WWTP discharge stations, seventy nine, culturable bacterial strains were isolated and identified from water and sediment as fecal coliforms, fecal streptococci, pathogenic staphylococci and non-enterobacteriacea. Fecal coliforms were most abundant (2.5 10^5 bacteria/mg) in sediment of WWTP discharge. *Leuconostoc spp* (23.1%) and *Chryseomonasluteola* (23.1%) were the most prevalent culturable fecal indicator bacteria (FIB) isolated at the upstream discharge stations. However, *Staphylococcus xylosus* (13.9%) was the most prevalent culturable FIB isolated at the WWTP discharge stations. Moreover, high antibioticresistance phenotypes were present in all sampling stations, but especially in WWTP discharge station in both water and sediment. Resistance levels in water and sediment, respectively were amoxicillin (58.8%; 34.8%), penicillin (50%; 31.6%), oxacillin (60%; 33.3%), cefotaxim (55.2%; 39.1%), ceftazidim (66.7%; 50%), gentamycin (42.9%; 38.9%), tobramycin (50%; 25%), vancomycin (33.3; 71.4%), amikacin (66.7%; 0%) and ciprofloxacin (100%; 100%). Interestingly, ß-lactam antibiotic resistant FIB were mostly isolated from water as well as from sediments of upstream and WWTP discharge station. Canonical correspondence analysis CCA correlating antibiotic resistance profile with the abiotic data showed that, in water column, culturable bacterial strains isolated in upstream WWTP discharge stations were interestingly correlated with the resistance to amikacin, oxacillin, cefotaxim, ciprofloxacin and gentamycin, however, in sediment, they were correlated with the resistance to amoxicillin, oxacillin, céfotaxim and vancomycin. Serious ß-lactams and aminoglycosides acquired resistance appeared mainly in fecal streptococci and pathogen staphylococci groups.

1. Introduction

Aquatic ecosystems receive various types of contaminants which come mainly through WWTP discharges of urban and industrial effluents (Soudan, 1968; Stellman, 2000), with direct discharges as a secondary source (Kim et al., 2009). The presence of enteric pathogens in aquatic environments can cause human health risks and risks are aggravated when bacteria are antibiotic resistant (Servais and Passerat, 2009). Fecal bacteria can also transmit the resistance to indigenous bacteria through lateral transfer,[1] thus contributing to the spread of antibiotic resistant bacteria (Davison, 1999). The sewage discharge is coupled with the direct discharge of antibiotics or their metabolites to the environment (Hijosa-Valsero et al., 2011), increasing selective pressure on the bacteria, and thus favouring resistance. The positive correlations between the degree of seawater contamination and frequency and variability of bacterial resistance indicate that polluted aquatic environments are sources of resistant bacteria (Fernandes Cardoso et al., 2010). Sewage effluent entering coastal waters contains a variety of harmful substances including viral, bacterial and protozoan pathogens, toxic chemicals such as antibiotics, organochlorines and heavy metals, and a variety of other organic and inorganic wastes (HMSO, 1990; Kümmerer, 2009). Moreover, hospital wastewater inflows significantly increased the prevalence of antimicrobial-resistant bacteria in WWTP and could strongly influence the toxic effects

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[1] The movement of genetic material between bacteria other than by the vertical transmission of DNA from parent to offspring.

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mediated through transcription factor-related gene expression (Akiba et al., 2015; Guruge et al., 2015).

The problems of this kind of aquatic pollution are likely to exacerbate and pose significant ecological and public health risk, especially in developing countries (Shahidul Islam and Tanaka, 2004) and recently, much attention has been paid to the safety of the treated wastewater because of water scarcity and the need to reuse waste water (Kim et al., 2009). In developing countries restricted local budgets, lack of local expertise, and lack of funding, result in inadequate operation of wastewater treatment plants (Paraskevas et al., 2002). Therefore, the problem of water pollution in the developing countries is an emergency. Organic pollution, as the most important aquatic pollution due to sewage releases, leads to disturbance of the natural environment caused by an overdevelopment of microorganisms (Bonnieux and Desaigues, 1998) and pathogenic microorganisms such as Viruses, protozoa and bacteria,which means that diseases transmitted by water remain a major safety concern throughout the world (Besassier et al., 2006). Indeed, treated and untreated wastewater, are often rejected directly in the aquatic ecosystems affecting the life of the aquatic flora and fauna and their quality that may be hazardous for the consumers (Akpor and Muchie, 2011). An environmental classification of the excreta-related diseases (Feachem et al., 1983; Mara and Alabaster, 1995) described different categories of diseases transmitted by wastewater: non-bacterial feco-oral diseases (e.g hepatitis A and E), bacterial feco-oral diseases (e.g Cholera, Salmonellosis…), geohelminthiases, taeniases, water-based helminthiases, excreta-related insect-vector disease and excreta-related rodent-vector disease.

Some bacterial groups such as fecal coliform, fecal streptococci and staphylococci pathogens can be identified by specific tests and be considered as indicators of fecal contamination and the possible presence of ecological risks (Fernández-Molina et al., 2004; Tallon et al., 2005). Fecal indicators Bacteria FIB vary according by country and subnational jurisdictions (Tallon et al., 2005). The abundance of FIB is hypothesized to be correlated with the density of pathogenic microorganisms from fecal origin and is thus an indication of the sanitary risk associated with the various water utilisations (Ouattara et al., 2011). In addition, water quality is influenced by the control of other factors like the pharmaceuticals that represent a growing concern regarding their occurrence in the aquatic environment (Heberer, 2002; Smital et al., 2004; Alzieu and Romana, 2006; Budzinski and Togola, 2006; Kümmerer, 2009). These emerging chemical contaminants have been detected in river water, groundwater and drinking water samples (Halling-Sorensen al, 1998; Kanda et al., 2003). There are also several investigations showing that pharmaceuticals are not eliminated during wastewater treatment and also not biodegraded in the environment (Ternes, 1998; Daughton and Ternes, 1999; Nakada et al., 2006; Okuda et al., 2008). Antibiotics ATB, are widely used in human and veterinary medicine (Kümmerer, 2008). One of the consequences of their presence in the receiving environment is that many bacterial species develop transferable mutations, which allow them to develop antimicrobial resistance increasingly problematic for the environment. This resistance could be an aggravating factor, assuming that it would reduce the therapeutic options in case of infection. The problem is more aggravated in semi-closed aquatic environments, where the water renewal takes a relatively long time which could be long enough to make a significant impact on the environment (Harzallah, 2003). In case of sewage water discharge, simultaneous presence of FIB and ATB could be reported and therefore the problem of the spread of FIB antibiotic resistance should be an urgent concern.

In a previous paper (Ben Said et al., 2016), we examined the sale of ATB pharmacies in Bizerte city in 2008 and we highlighted that ß-lactams are the predominant family of consumed antibiotics. The present work puts more emphasis on the diversity of the Bizerte lagoon isolated bacterial species and their relationship with a biotic parameters prevailing in the Bay of Sabra where WWTP of Bizerte city are released.

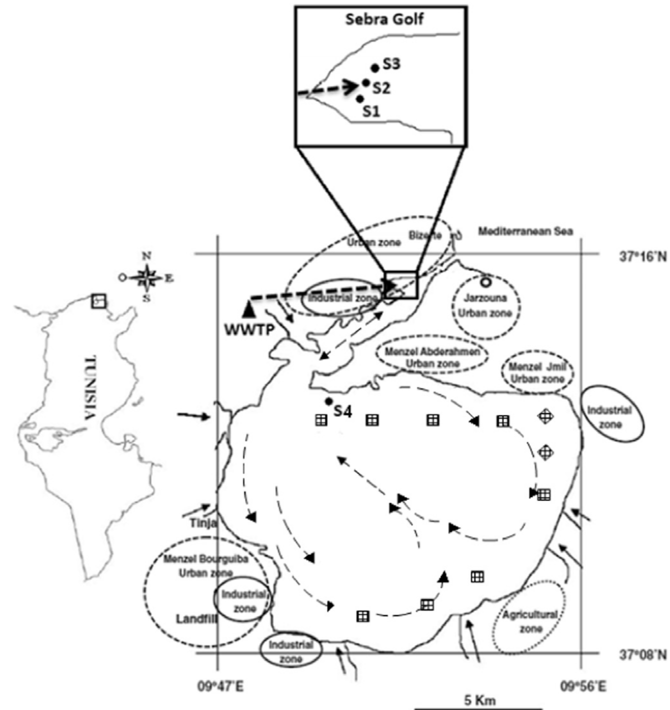

Fig. 1. Sampling sites in Sebra Golf and Chaàara in Bizerte lagoon. →: Oued; - - → water current direction. ⊞ : shellfish farm. The populated areas (dashed circles) and industrial zones (continuous circles) are indicated. S1: station upstream of the discharge; S2: discharge station; S3: downstream station of the discharge; S4: station away from discharge point (Châara). WWTP: wastewater treatment plant of Bizerte City.

2. Materials and methods

2.1. Bizerte Lagoon presentation

Bizerte Lagoon is an important ecological and economic ecosystem (fishing and shellfish activities). This semi-closed aquatic environment is located in the northern part of Tunisia and extends over 150 km^2 with a mean depth of 7 m and is linked to the Mediterranean Sea by an artificial channel and with the Ichkeul Lake through the Tinja oued (Zaaboub et al., 2015; Ben Salem et al., 2017). This area constitutes a receptor of several industrial sewages, aquaculture wastes, fertilizers, and pesticides through runoff and soil erosion, wastewaters from towns implanted around (Yoshida et al., 2002; Derouiche et al., 2004; Ben Said et al., 2010). In the northwest of the Bizerte lagoon, the wastewater treatment plant (WWTP) of Sidi Ahmed is situated between Bizerte and Menzel Bourguiba cities (Fig. 1). This plant treats the wastewater from the Bizerte city since 1997. Treated wastewater is discharged into the Bizerte lagoon, exactly in the bay of Sabra adjacent to the cement factory (Fig. 1).

2.2. Sampling and field measurements

Water and sediment samples have been collected on September 2009 from the bay of Sabra (Bizerte lagoon) in 4 stations (Fig. 1). Sites were selected based upon the extent of WWTP discharge. The first station (S1) was located upstream of the WWTP discharge, the S2 station was located at the WWTP outflow, and third S3 and fourth S4 stations were located downstream of the WWTP discharge.

Water column temperature, pH, and salinity were determined in the field with a handheld multi-parameter system (WTW Multi-197i). The water temperature was determined with a multi-parameter probe (YSI GRANT 3800). Sediments were sampled with Van Veen Grab quickly homogenized and stored at 4 °C for microbial analysis.