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# Potential Pathogenic Bacteria of Wastewater Collectors from Abidjan (Côte d'Ivoire)

**Coulibaly-Kalpy Julien[1]*, Kouamé Y. Cyr-Kévin[1, 2], Ouattara Koffi Nouho[2], Kouadio Kouamé[3], Amon Lydie Nina[1], Ehuié Pierre[1], Yéo Kadjowely[1], Bamba Aboubacar[1], Gourène Germain[2] and M. Dosso-Bretin Mireille[3]**

[1]Unité de chimie et de microbiologie environnementales, Département environnement et santé, Institut Pasteur de Côte-d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire
[2]Laboratoire Pôle de Recherche Pêche et Aquaculture, UFR-SGE, Université NanguiAbrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire
[3]Unité d'éco épidémiologie, Département environnement et santé, Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte-d'Ivoire
**Corresponding author*

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**A B S T R A C T**

The production of wastewater caused by human activities exposes urban areas of Côte d'Ivoire to major health risks linked to the presence of any pathogens. This study aims to detect target pathogens in order to evaluate potential risk for local population and the influence of external factors. Samples of wastewater were collected in sterile bottles in 14 stations during 11 months. So, 833 wastewater samples were analyzed. Temperature and pH were measured in situ. Microbiology analysis were performed for the counting and the identification of *Escherichia coli*, *Vibrio* sp and *Pseudomonas aeruginosa*. The bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio* sp) were isolated. The results revealed that pH and temperature were not comply with current standards but favorable to the development of microorganisms. The presence or absence of *Escherichia coli*, *Vibrio* sp, and *Pseudomonas aeruginosa* was better correlated by the temperature of the collected wastewater. The wastewater in Abidjan is contaminated by fecal indicator bacteria pollution but also by some pathogens due to the outside temperature action. Their treatment before been rejected or eliminated should be useful for population health or public health.

## Introduction

Most urban areas in developing countries have major health risks facing the failure or lack of adequate sanitation, poor disposal of liquid waste, solid and insufficient drinking water (Collignon *et al.*, 2000). Watercourses in urban areas or located within large cities, are particularly affected by physical, chemical and microbiological pollution (Grabow *et al.*, 1996; Cabral *et al.*, 2010). However, bacterial pollutants can have various microbiological sources (domestic sewage, industrial wastewater, etc…) (Dosso *et al.*, 2012) and their presence in

aquatic environments is a real public health problem for people using water resources (OMS, (1997); Koffi-Nevry *et al.,* (2012)). The immediate consequences are the deterioration of living and this canincrease morbidity and mortality (Collignon *et al.,* 2000). Indeed, waterborne diseases cause each year the deaths of several million people in the world and particularly in developing countries(Ouattara, 2000). There are 1.8 million people, 90% of children under five, mostly living in these countries that die each year from diarrheal diseases(OMS, 2004).

Moreover, the situation is accentuated in the slums of big African cities where population growth is between 3.4 and 9.4% (Collignon *et al.,* 2000), causing an important wastewater production and huge sanitation problems. This wastewater may contained many pathogens (bacteria, viruses, protozoa, helminthes) responsible of many diseases (Cissé (1997); El Guamri *et al.,* 2007)). If they are rejected without adequate treatment in the receiving environment, they will create many health problems including diarrhea, cholera and typhoid fever (OMS (1989); Dufour *et al.,* 1994)).

The microorganisms are responsible of waterborne diseases mainly from the feces of humans and warm-blooded animals. These microorganisms are brought into surface waters, firstly, via discharges of raw sewage or treated, secondly, via runoff and leaching of agricultural soils contaminated by animal feces and spreading, on crops, manure or sewage sludge (sources) (Ouattara (2012)). Among the potentially pathogens bacteria transmitted by sewage, Enterococci Intestinal (EI) and *Escherichia coli* (*E. coli*), are considered as the indicators to evaluate the microbiological quality of surface water (Edberg *et al.,* 2000; Fewtrell *et al.,* 2001)). These indicators of fecal contamination are not pathogens but their presence indicates the existence of a contamination with feces and their abundance is indicative of the eventual presence of pathogenic microorganisms such as *Vibrio* sp. and *Pseudomonas aeruginosa* (Rose *et al.,* 2004; Bennami *et al.,* 2012)).

In West African sub-region, Abidjan is one of the few capitals with functional drainage systems for sewage and rainwater. However, only 30% of the Abidjan population is connected to the existing sewerage system and over 25% have no sanitation equipment (Métongo *et al.,* 1993). In addition, among the existing sanitation facilities, the large open sky collectors, assigned exclusively to the storm waters which should be spilled into the lagoon Ebrié, is dysfunctional (Métongo *et al.,* 1993). These collectors carry domestic sewage, storm water and effluent of various natures (Cissé *et al.,* 2011). They receive wastewater from many municipalities: Abobo, Adjamé, Yopougon, Attécoubé, Marcory and Cocody. But this water is directly discharged without treatment into the lagoon Ebrié which is now in an advanced state of pollution (Scheren *et al.,* 2004). They therefore constitute an environmental and health risk to aquatic organisms that live there and especially for local communities.

Indeed, the city of Abidjan knows since January 2011, a re-emergence of diarrheal diseases (including cholera) after 6 years of relative disappearance. Microbiological and epidemiological studies have confirmed cholera cases in Attécoubé, Adjamé, Cocody, Port-Bouet and Kumasi (Dosso *et al.,* 2012). In addition, climate change, with warming temperatures could affect the persistence of some pathogens even some epidemics.

This study was established to evaluate the influence of physic-chemical parameters such as pH and temperature on the overall

quality of wastewater, more particularly the microbiological quality. More specifically, (1) fecal contamination indicator bacteria (*E. coli*) and target pathogenic bacteria (.) will be detected, (2) physical and chemical parameters on the basis of pH and temperature will be determined, (3) and the relationship between pH, temperature and the detection of bacteria will be established.

## Material and Methods

Equipment of the biological material used for the realization of this study consists of waste water collected from different districts of Abidjan namely Cocody, Yopougon, Attecoubé, Abobo and Marcory. The programming language R and Multiple Correspondence Analysis enabled the statistical treatment of data.

## Sampling

The wastewater samples were collected in the different municipalities, every three weeks (Figure 1). Samples were collected at the water surface in sterile bottles of 500 mL with a seal which was attached to a rope. Once collected, the samples were stored in a cooler with ice packs in order to maintain them at a temperature of about 4 °C. Every withdrawals of operations were performed by the methods of Rodier (Rodier, 1996) and standards of AFNOR (Afnor, 1990). The samples were routed to the Chemistry Unit and environmental Microbiology laboratoryfor being analyzed according to the scheme defined by (Rodier, 1996; Servais *et al.*, 2009a; Rodier *et al.*, 2009) for isolation of *Escherichia coli*, *Vibrio* sp. and *Pseudomonas aeruginosa*.

## Physicochemical Parameters Analyses and Germs Isolation

The physico-chemical parameters (pH and temperature) were measured in situ using a mixed pH electrode 323 / S and B, and analyzed by standard methods of the French Association for Standardization(AFNOR, 1990; AFNOR, 1986) and those of Rodier (Rodier, 1996).

The studied bacteria including *E. coli*, *Pseudomonas aeruginosa* and *Vibrio sp* were sought according to the methods of classical bacteriology. In 225 ml of Buffered Peptone water was seeded 25 ml wastewater sample to revive any *E. coli* and Pseudomonas present. Water Alkaline peptone was used for *Vibrio* sp research.

After 24 hours of incubation at 37 ° C, Rapid'E.coli agar (REC2), Cetrimide agar and TBCS agar were seeded for the isolation of *E. coli*, and *Vibrio* sp. and *Pseudomonas aeruginosa* respectively. Biochemistry tests adapted of each bacteria genera were then used for species identification.

## Statistical Analysis

Descriptive statistical techniques including strip charts were used for exploratory data analysis. In addition the analysis were deepened through factorial methods in particular, using the technique of Multiple Correspondence Analysis (MCA). This exploratory analysis was performed with R. programming language

## Results and Discussion

A total of 833 wastewater samples were collected as follows: Cocody (n = 145), Yopougon (n = 307), Attécoubé (n = 176), Adjamé (n = 52) Abobo (n = 61) and Marcory (n = 92).

The analyzed samples showed the presence of the main target bacteria (*Escherichia coli*, *Vibrio sp*. and *Pseudomonas aeruginosa*) in the collectors of different districts of Abidjan. Furthermore, there is a correlation between the detection of bacteria and the

temperature of the collected wastewater.

## Descriptive Analysis

Variation of the temperature and pH of wastewater sampling station

Figure 2 show the average values of the temperature and pH of wastewater sampling station.

The results show that the lowest average temperature per station for the waters studied are of the order of 25 °C (25 °C (Lycée Technique station), 25,10 °C (Andokoi station) and 25,85 °C to the Corniche station). The highest average temperatures are close to 28 °C (28,03 °C or (Cocody station), 28,05 °C (Boribana station 2) 28,30 °C respectively in stations like Gouro market and Phoenix pharmacy, 28,36 °C (station CHU Yopougon) and 28,80 °C (Remblais station).

Lowest temperature (20, 40 °C) was recorded at the Lycée Technique station in July and the highest temperature (34,40 °C) is from the Phoenix pharmacy station in May. The largest temperature variation was observed at the Phoenix pharmacy station (minimum and maximum temperatures of 22,20 and 34,40 °C, respectively). The lowest temperature variation was observed at the Remblais station (minimum and maximum temperatures of 27,90 and 29,30 °C, respectively).

The average pH range between 7,75 and 11,90. The lowest and highest pH were recorded respectively in the Boribana station 2 (4,01) in the month of September and the station Gouro Market (13,14) in August. The biggest change in pH was observed at the station Andokoi (minimum and maximum pH of 4,13 and 10,36 respectively). The lower pH change was observed at the Lycée Technique station (minimum and maximum pH of 7, 15 and 9,68 respectively).

Detection of target bacteria in wastewater collection in the city of Abidjan

Samples of positive wastewater by bacteria are illustrated by Figure 3.

For all samples analyzed in Cocody, the highest attendance percentages were observed for *P. aeruginosa* with minimum of 54,90% (Corniche station) and a maximum of 60% (Lycée Technique stations and Deux plateaux). The presence of *E. coli* percentages vary between 11,76% (station CHU Cocody) and 33,33% (in Deux Plateaux station), those of *Vibrio sp*. vary between 10% (Lycée Technique station) and 27,45% (Corniche station). For samples analyzed in Yopougon, the highest attendance percentages were observed also for *P. aeruginosa* with a minimum of 17,65% (Phoenix pharmacy station) and a maximum of 38,10% (CHU Yopougon). The presence of *E. coli* percentages vary between 17,65% (Phoenix pharmacy station) and 34,52% (CHU Yopougon station) and those *Vibrio sp*. Range from 5,88% (Phoenix pharmacy station) and 17,65% (Gouro Station Market) in the municipality of Attécoubé, for *P. aeruginosa* detected with a minimum of 36,62% (Boribana station 2) and a maximum of 52,50% (Boribana station 1). For *E. coli*, the presence is between 15,38% (mosque Boribana station) and 20% (Boribana station 1). For *Vibrio sp*., presence is between 6,15% (Mosque Boribana station) and 19,72% (Boribana station 2).

Finally, in samples provided from Adjamé and Abobo municipalities, the highest attendance percentages were also observed for *P. aeruginosa* with 63,46%, respectively (Fraternité Matin station) and 67,21%

(station Zoo) against 17,39% at the station Marcory Remblais. The presence of *E. coli* in Fraternité Matin is estimated at 30,77%, while the Zoo station level is 36,07% against 18,48% in Marcory Remblais. However, in the latter three stations, the percentage of presence for*Vibrio sp*.is highest at Zoo station (44,26%) while the lowest was recorded in Remblaisstation (6,52%) against 15,38% in the station Fraternité-matin.

## Multidimensional Exploration

The projection of quantitative variables in a Multiple Correspondence Analysis allows us to understand their positioning in relation to cloud variables (Figure 4).

The presence or absence of *E. coli*, *Vibrio* sp. and *Pseudomonas aeruginosa* is better correlated by the temperature of the collected wastewater.

In this study, we characterized the wastewater collectors Abidjan via the measurement of two physicochemical parameters (temperature and pH) and microbiological parameters (*E. coli*, *Vibrio* sp. and *P. aeruginosa*).

The lowest average temperatures per station for the waters studied are in the order of 25°C at stations Lycée technique, Andokoi and Corniche. The highest average temperatures are close to 28°C at stations Cocody, Boribana 2, Gouro market, Phoenix Pharmacy, CHU Yopougon and embankments.

Overall, the average temperatures recorded for wastewater are near the threshold of 30 °C

Generally indicated for the discharge of waste water (OMS, 1986; Bouzid *et al.*, 2013). The wastewater from the city of Abidjan are relatively warm with a maximum of 34,40 °C observed at the Phoenix pharmacy station during the main rainy season (GSP) in May. This same observation was made by Kadjabanga *et al.*, (2006) which have conducted similar work with an observed temperature (35 ° C) between the long dry season (GSS) in the months of March and the long rainy season (GSP) which usually begins in late May.

This value is lower than 35 °C, considered indicative limit value for water for irrigation (Belghyti *et al.,* 2009). This temperature rise can be explained by the influence of environmental conditions (rich in easily biodegradable organic matter, temperature, high light intensity, high humidity comparing to temperate regions, hydrological factors) (Houhamdi *et al.*, 2006; Rochelle-Newall *et al.*, 2015). However, the minimum temperature (20,40 °C (Lycée Technique station) recorded in the month of July at the GSP could be justified by the freshness due to the wind blowing monsoon in the months ofJuly and August. This wind promotes cooling water and gives them a lower temperature (Kouassi *et al.*, 1990).The values of temperature are consistent with those of (Djèdjiho *et al.*, 2013) who believe that the temperatures between 24 °C and 35 °C are favorable to the detection of bacteria.

The pH analysis of wastewater shows high variability in the Andokoi station (minimum and maximum pH of 4,13 and 10,36 respectively), reflecting the influence of various pollutions. This pollution from intensive human activities practiced by the local population, leaching of soil runoff and driving the waste of plant and animal origins, rich in organic nitrogen compounds which could affect the overall quality of wastewater (Close *et al.*, 1989; Hassoune *et al.*, 2006). However, the low variability

observed at the Lycée technique station (minimum and maximum pH of 7,15 and 9,68 respectively) could be explained by a weak influence of pollution sources (domestic wastewater, industrial sewage and so on) (Close *et al.*, 1989; Mbawala *et al.*, 2010). But, the average pH values of wastewater sampling stations have higher average levels in the water discharge standard in the environment (OMS, 2006), ranging from 7,75 (CHU Yopougon) to 11,90 (Gouro market). The average pH noted for such water is basic to all stations unlike the results of (47) which recorded average pH acids for raw wastewater from the city of Cotonou, Benin.

**Fig.1** Geographical location of the study area and the localities surveyed Abidjan district (Ahoussi *et al.*, 2008)

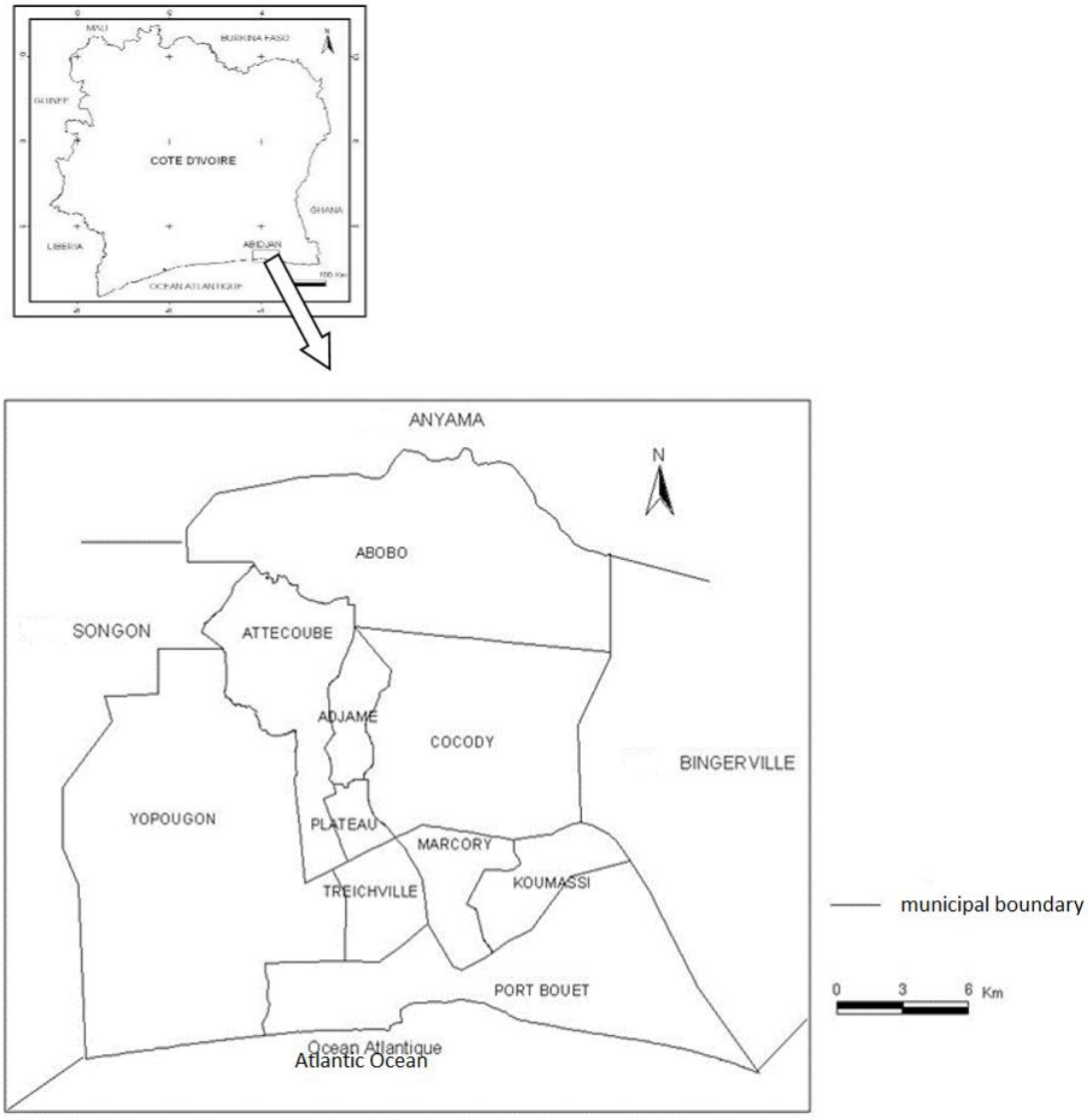


**Fig.2** Physico-chemical parameters (pH and temperature) recorded by station from January to November 2013.

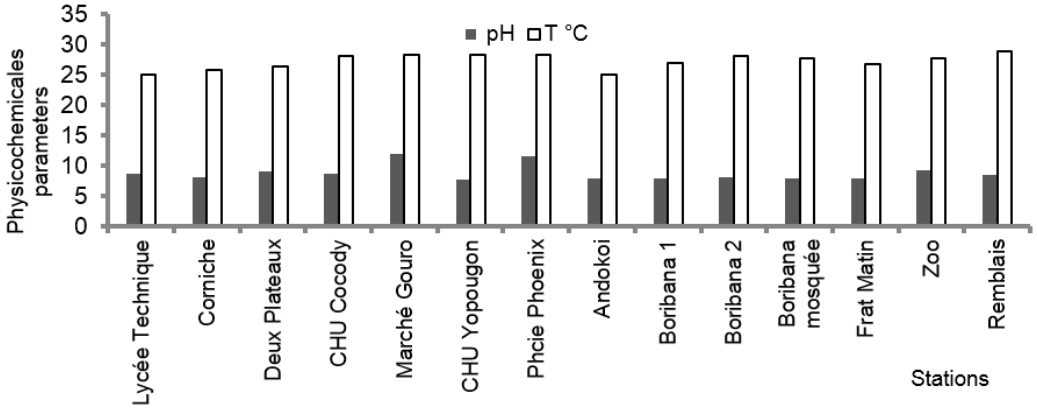



**Fig.3** Presence of bacteria by sample in each sampling stations

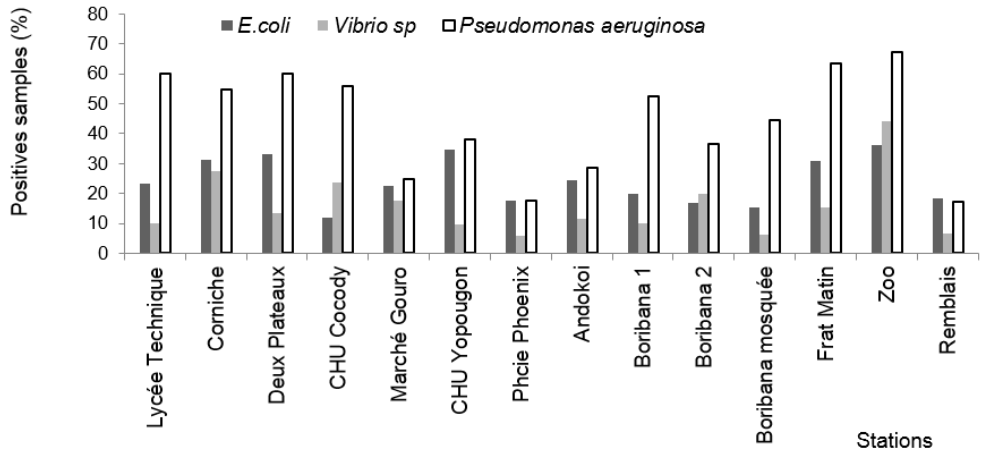
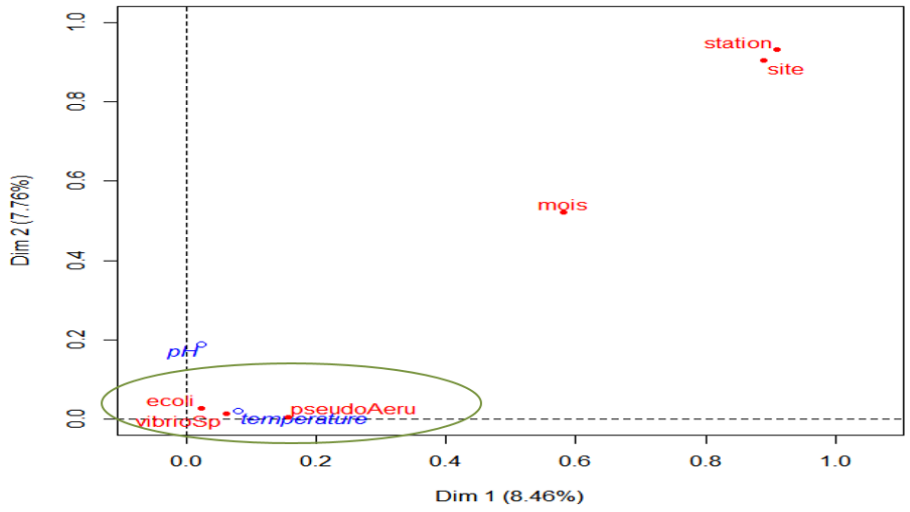



**Fig.4** Cloud active variables with positioning of illustrative variables



The results of our study are similar to those of (Abouelouafa, 2002) and (N'Diaye *et al.*, 2011) which recorded pH slightly to moderately alkaline, respectively in wastewater cities of Oujda and the Kingdom of Morocco in Nouakchott Republic of Mauritania. This character of waste water in the city of Abidjan does not respond within the set standards of the World Health Organization, which recommended pH between 6 and 9 for water releases to the environment (OMS, 2006). This is the case, for example, at Phoenix pharmacy and Gouro market station in Yopougon where the average values of pH, respectively of 11, 90 and 11,64 are beyond the safety threshold.

In natural waters, the physic-chemical parameters such as acidity or alkalinity pH and temperature should not exceed a safe level, since they can cause a change in the characteristics of these waters leading to that cause a serious public health threat (Houhamdi *et al.*, 2006).

Microbiological analysis focused on the detection of target pathogenic bacteria (*Escherichia coli*, *Vibrio sp*. and *Pseudomonas aeruginosa*).

The presence of *E. coli*, specific fecal pollution indicator (Adjahouinou *et al.*, 2013; Edberg *et al.*, 2000) in the analyzed water, clearly indicates contamination by fecal micro-organisms and therefore the epidemiological potential risk is to leave untreated discharge (Adjahouinou *et al.*, 2013; Servais *et al.*, 2006). Indeed, the presence of enteric bacteria should suspect many other pathogens (Adjahouinou *et al.*, 2013; Elmund *et al.*, 1999; Ajit *et al.*, 2009). It is the same for *Vibrio sp*. and *P. aeruginosa*. It should however be noted that the positive rates recorded for *E. coli* are lower than those of raw water to the city of Oujda the Kingdom of Morocco (Adjahouinou *et al.*, 2013; Abouelouafa *et al.*, 2002) and the registered rate for wastewater in Nouakchott Republic of Mauritania (Adjahouinou *et al.*, 2013; N'diaye *et al.*, 2011) and Ouargla (Adjahouinou *et al.*, 2013; Hamdi *et al.*, 2012). This phenomenon is due to the specificities linked either to the physicochemical parameters of these waters, or their origin (domestic or industrial).

*P. aeruginosa* shows a high positivity rate on all the 14 stations. This species in waters may constitute a health risk as highlighted by the work of Manizan *et al.*, (Palleroni *et al.*, 2002; Manizan *et al.*, 2009) .The stations located in the municipality of Attécoubé receive wastewater from Adjamé municipality, crossing some homes and sites before emptying into the lagoon Ebrié. Previous studies have shown that following Adjamé, the municipality of Attécoubé, due to its proximity to Adjamé was hit by a cholera epidemic, especially in Boribana.

This case was confirmed by the Pasteur Institute of Côte d'Ivoire. Eventually the epidemic spread in 2011 to other neighboring municipalities (Yopougon, Abobo, Cocody and Marcory) (Koutouan (2012). These studies are consistent with the results from our research that signal the presence of *Vibrio sp*. in wastewater samples analyzed. The presence of bacteria in wastewater samples could be justified by the discharge of waste water into the gutters. Their presence is also justified by a number of inherent factors in agricultural activities around the stations, a development failure of these stations, proximity to pollution sources such as household waste, latrines and non-compliance basic hygiene by the population (Mbawala *et al.*, 2010). Our study provides above all the existence of a correlation between the detection of bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Vibrio* sp.) and the waste water temperature.

In conclusion, this work enabled to detect *Escherichia coli* germs *Vibrio* sp. and *Pseudomonas aeruginosa* in wastewater collection in the city of Abidjan in 2013. The presence of these pathogens is related to the temperature rise in the waters and reveals the danger to local residents dumping sites for domestic wastewater. This untreated wastewater transit in urban areas via collectors often open and spilled into the lagoon Ebrié. Also, this study reveals the lack or absence of monitoring of wastewater discharges at the Abidjan sanitation, which highlights the need for better wastewater management to protect local residents but also the lagoon Ebrié.

Considering the results of this study which shows the need to set up a short-term wastewater drainage system via cutlery collectors and effectively maintain it in connected neighborhoods.

This will prevent the presence of sewage in the streets. In the long term, it is necessary to resize the sanitation system in the new development taking into account the potential occupancy rates. The waste water will be routed to wastewater treatment plants to prevent the pollution of receiving waters. The main outlets being at the lagoon Ebrié microbial pollution is a real environmental problem.

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