



## First Detection of *Mycobacterium* sp. in Wastewater in Abidjan (Yopougou) Cote d'Ivoire

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### Authors' contributions

This work was carried out in collaboration among all authors. Author VNS managed the literature searches, performed the analysis and wrote the first draft of the manuscript. Author CKJ designed the study, wrote the protocol and corrected the first draft of the article. Authors ALN and BN collected the sample and performed the analysis. Author DM supervised the study and corrected the article. Author DAJ supervised the study and corrected the final manuscript of the article. All authors read and approved the final manuscript.

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### ABSTRACT

Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment and worldwide, under certain environmental conditions, they could be responsible for skin diseases or pulmonary lesions. In Ivory Coast, Buruli ulcers endemic country, very little information concerning the epidemiology of NTM in Cote d'Ivoire is known, this ignorance has led to an underestimation of its prevalence in the environment.

**Aims:** The objective of this study was to characterize strains of environmental mycobacteria in wastewater located in urban areas in Abidjan (Yopougou) Côte d'Ivoire.

**Place and Duration of Study:** The study was conducted at Yopougou (Selmer). This site is considered to be Buruli ulcer non-endemic zone according to the national program on fight against Buruli ulcer in Côte d'Ivoire. However, all biological aspect of this work was carried out at the Pasteur Institute of Côte d'Ivoire.

**Methodology:** Wastewater samples were collected at the surfaces at Yopougou using the technique described by Britton and Gresson. In the laboratory, these collected samples were

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treated according to the method described by Stinear et al. and Kankya et al. consisting of decontamination, neutralization followed by culture.

Classification of species was done according to the method described by Runyon and collaborators. Biochemical identification of mycobacteria was done according to the method described by Metchock.

**Results:** A total of 32 samples were collected, 8 water samples at each sampling site. After decontamination and culture, specimens grew on Lowenstein-Jensen medium after 7 days (at 37°C and 32°C) and revealed small, smooth, nonpigmented colonies. Smears confirmed the presence of variably acid-fast organisms. Biochemical traits of the isolates revealed, colony grown on Middlebrook agar 7H11 (at 37°C and 32°C); catalase (+); agar (+) (at 37°C and 32°C); citrate (-); mannitol (-).

**Conclusion:** This work is one of the first study focusing on Mycobacteria in wastewater in Abidjan. The isolation of *Mycobacterium sp.*, an environmental pathogenic organism, supports the hypothesis of possible colonization of urban wastewater by pathogenic mycobacteria. Further studies are needed to verify the involvement of this specie in mycobacterial infections in Côte d'Ivoire.

**Keywords:** *Mycobacteria*; *wastewater*; *Abidjan*; *Cote d'Ivoire*; *non-tuberculosis*; *Mycobacterium sp.*

## 1. INTRODUCTION

Atypical mycobacterial infections are recognized as causes of symptomatic disease in developed countries, the role of non-tubercular Mycobacteria (NTM) in the lung, skin or systemic diseases are not well described in the developing countries. Mycobacteria have been shown to concentrate in microdroplets forming aerosols from natural waters after desiccation in the air and wind transport [1], which could provoke a risk of contamination by inhalation [2].

In Côte d'Ivoire, studies on mycobacteriosis have focused more on *Mycobacterium ulcerans*, which is responsible for Buruli ulcer and *Mycobacterium Tuberculosis* for respiratory's infection [3,4]. NTMs can cause certain diseases like pneumonia, lung abscess, pleural infection, meningitis, lymphadenitis and many infections of the skin and soft tissue. In cases where symptoms and signs occur, they are often indistinguishable clinically and radiographically from those caused by *Mycobacterium tuberculosis*, responsible for Tuberculosis (TB) [5]. Consequently, many NTM-infected patients in TB endemic countries receive unnecessary long treatment with anti-TB drugs that are not only expensive but also toxic [6]. These bacteria are also all acidic. The differential detection of NTMs from *M. tuberculosis* by staining and through microscope procedures is very complex. Therefore, the control and treatment of the underlying disease is very complicated. It is therefore very difficult, in practice, to identify NTMs, their environmental resource, their population and their persistence in ecosystems.

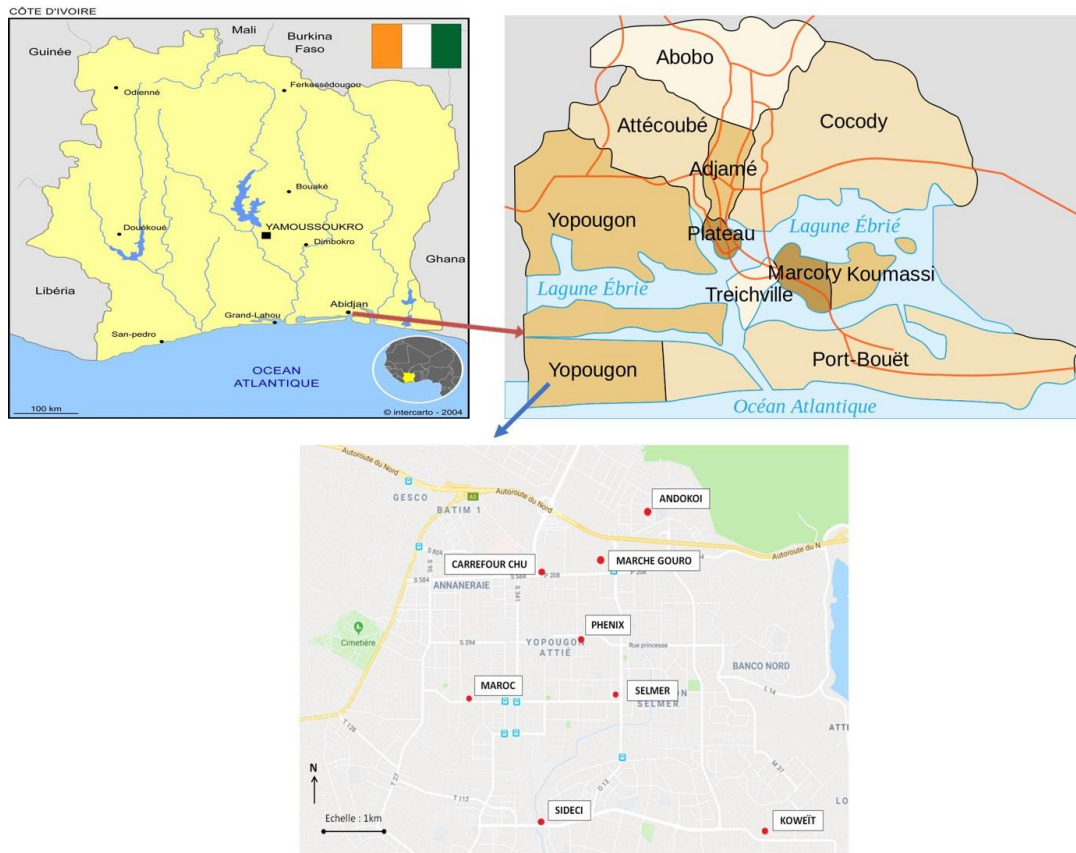
Therefore, one of the most important role of modern microbiology laboratories would be the diagnosis and identification of NTMs and their differentiation from very similar strains of *M. tuberculosis* and *M. ulcerans* [7].

At present, more than 170 species of nontuberculous mycobacteria (NTMs) have been identified [8]. Members of the genus NTM are widespread in the environment and can be found underneath the soil and in various aquatic habitats. At present, diseases caused by NTMs are increasingly recognized worldwide [9]. The authors described a rapidly growing mycobacterium (RGM) with a notable high thermos tolerance in comparison with other NTMs. No definite clinical significance was assigned to this new species so far [10] and every day, new species are emerging. Species identification of rapidly growing mycobacteria is important, because the members differ in their susceptibilities to different antibiotics [11]. To be able to understand the level of prevalence of mycobacterial infections, it is important to identify the different species and to estimate the impact that these environmental pathogens have on human and animal species. The main objective of this study was to characterize potentially pathogenic environmental mycobacteria strains in wastewaters in Abidjan (Yopougou) Côte d'Ivoire.

## 2. MATERIALS AND METHODS

### 2.1 Sites and Scope of the Study

The study was conducted at Yopougou (Selmer). This site is considered to be Buruli ulcer



**Fig. 1. Sampling sites (Photo: Vakou sabine)**

non-endemic zone according to the national program on fight against Buruli ulcer in Côte d'Ivoire. However, all biological aspect of this work was carried out at the Pastor Institute of Côte d'Ivoire.

## 2.2 Materials

The materials consisted of wastewater samples collected at Yopougon (Selmer).

## 2.3 Sampling Method

Water samples were taken at the surface using the method described in [12]. The collected samples were stored in a cooler containing accumulators of cold in order to maintain them at a temperature of about + 4°C until arrival at the laboratory, protected from light within 24 hours of sampling [13].

In the laboratory, these collected samples were processed according to the method described in [14,15]. Decontamination of the

water samples and the recovered supernatant was performed with cetylpyridium chloride (CPC), followed by neutralization with phosphate buffer.

The different culture medium: Lowenstein Jensen (LJ), Mac Conkey without purple crystal, ordinary agar and Middlebrook 7H10 agar were used for seeding. The samples in LJ and Middlebrook 7H10 agar were seeded in duplicate. One lot of each of them was packed in an aluminum foil for photoinduction test. The incubations were made at 23 and 37°C in the ovens. Daily observations were made until colonies were obtained. An optical microscope (Zeiss®) was used for the observation of Acid-Alcohol Bacillus Resistant after Ziehl-Neelsen coloration [16]. Classification of species was done according to the method described by Runyon and collaborators [17]. Biochemical identification of mycobacteria was done according to the method described by Metchock [18]. Briefly, for biochemical identification, the colonies obtained after culture were observed

under a microscope. The Acid-Alcohol Bacillus Resistant were cultured in the presence and absence of light on LJ medium for the test of photo-induction. They were then seeded on Agar at different temperatures (37, 42, 45 and 52°C), on LJ medium with 5% NaCl with the manitol test, the test in the presence of sodium citrate and test in the presence of ferric ammonium. The microscopic techniques consisted of Ziehl-Neelsen and Gram staining. The phenotypic and biochemical characters taken into account the observation in the culture will be those described by [17,19].

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

A total of 32 samples were collected, 8 water samples from each sampling site. After decontamination and culture, specimens grown on Lowenstein-Jensen medium after 7 days (at 37°C and 32°C) and revealed small, smooth, nonpigmented colonies (Fig. 2). Smears confirmed the presence of variably acid-fast organisms. Biochemical features of the isolate revealed colony, grown on Middlebrook agar

7H11 (at 37°C and 32°C) ; are catalase (+) ; agar (+) (at 37°C and 32°C ) ; citrate (-) ; mannitol (-).

#### 3.2 Discussion

The identification of mycobacteria at the species level is very important for the management of patients. This identification is generally performed through phenotypic tests which are based on a panel of biochemical tests, pigmentation and growth characteristics [20].

The incidence of disease is increasing [21] and the issue of water as a potential source of infection needs to be addressed. It has been shown that mycobacteria are present in drinking water distribution system, in hospital water [22], and in municipal water [23]; the diseases caused by these organisms have different manifestations. They have been responsible for a number of healthcare-associated infections outbreaks and pseudo-outbreaks.

Mycobacterioses differ depending on the species and hosts involved and on ways of infection, and may be present in pulmonary, skin or soft tissue lesions. There is a very little data on species distribution of these organisms in Cote d'Ivoire.



**Fig. 2. Fast growing mycobacteria strains on loweinsten jensen (photo: Vakou S); Colony with a rough, eugonic appearance, not pigmented in light (achromogenic strain)**

This is the first study to report the presence of potential pathogenic NTM in wastewaters in Abidjan (Cote d'Ivoire). In this work, our aim was to characterize strains of environmental mycobacteria in wastewater in Abidjan (Yopougon) Ivory Coast. Our results revealed the isolation of rapid growing *Mycobacterium sp.* The rapid growing mycobacteria are generally defined as nontuberculous species of mycobacteria that show visible growth on solid laboratory medium within 7 days. The species of RGM capable of causing disease in humans consist of nonpigmented and pigmented species primarily belonging to the *M. fortuitum* group, the *M. chelonae abscessus* group and the *M. smegmatis* group [24]. Previous studies in Côte d'Ivoire suggested the ability of these bacteria to cause skin ulcers or to be involved in isolation areas [25].

The reasons for the increase of mycobacteria in the environment are not clearly known, but environmental changes, the availability of better equipment for detection are probably contributing factors [26,27]. With regard to urban wastewater in Ivory Coast, this study is the first to identify the mycobacteria in it. Note that the daily quantities of wastewater disposed by the population of the city of Abidjan increased from 162,323 m<sup>3</sup> in 1998 to 193,186 m<sup>3</sup> in 2002 [28]. The most polluted water comes from the municipalities of Yopougon, Abobo, Cocody, Koumassi and Adjame with an average daily production of more than 7000 kg of BOD<sub>5</sub> [29]. These conditions could lead to many health problems with adverse consequences on families: proliferation of water-borne diseases, high infant mortality, economic difficulties etc. The isolation of *Mycobacterium sp.*, which could be a potential pathogenic organism helps, not to rule out the hypothesis of the biological risk of mycobacterial infections to the population, since according to previous studies, the presence of certain genes previously found in *Mycobacterium ulcerans* are also found in rapid-growing mycobacteria responsible for skin infections according to the literature [30]. Large gaps still exist in our knowledge of rapid growing mycobacteria. Undoubtedly further studies are required. It would therefore be wise to continue studying the distribution of these species in Côte d'Ivoire and to detect the different sources of direct contamination by the vulnerable population and indirect contamination from the drinking water distribution system.

#### 4. CONCLUSION

This work has shown the presence of nontuberculous mycobacteria in urban wastewater in Côte d'Ivoire. Our study is one of the first to identify this kind of bacteria in wastewater. Investigations must therefore continue in order to establish a mapping of mycobacteria in the city of Abidjan. All this could help prevent the proliferation of a potential pathogen due to *Mycobacterium* other than *M. ulcerans* and *tuberculosis* in Côte d'Ivoire.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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