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SHORT REPORT

First cases of *Mycobacterium leprae* (Hansen's disease) detection in Côte d'Ivoire using molecular diagnosis (PCR)

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Summary
Background Leprosy is a skin disease caused by *Mycobacterium leprae*. It is the second mycobacterial disease after tuberculosis still presenting a public health problem in many countries today. With the advent of multidrug therapy in 1982, much progress has been made in the fight against this disease, which causes severe social consequences. Côte d'Ivoire, like many African countries, reached the elimination threshold of disease and MDT is available throughout the country. However, Côte d'Ivoire has not managed to break the chain of transmission of *M. leprae*. Thus, in the country where leprosy is endemic, the number of Grade II disabilities observed remains significant.
Methods and results The diagnosis of infection is often made by default, based only on clinical and microscopic evidence; we are committed to implementing PCR, a previously unavailable diagnostic tool, to help confirm suspected leprosy cases. Samples consisting of nasal mucus and slits skin smears were collected from 39 suspect cases for confirmation by PCR. DNA was extracted and amplified, targeting *M. leprae* repeated elements (RLEP). Results showed a PCR positivity rate of 38.5%. PCR products of the repetitive elements were sequenced and BLAST*n* analysis confirmed that the amplified products obtained were part of the *M. leprae* genome.
PCR is now available for confirmation of leprosy cases in Côte d'Ivoire. This will help to reduce the consequences of leprosy and promote its elimination.

Keywords: Côte d'Ivoire, *M. leprae*, PCR

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Introduction

Leprosy is a chronic infectious tropical disease caused by an obligate intracellular pathogen, *Mycobacterium leprae* (*M. leprae*). *M. leprae* infection results in peripheral neuropathy and permanent progressive deformity, leading to the social consequences of discrimination and stigma.[1] Despite the efforts of WHO to eradicate the disease, the transmission chain has not been interrupted. More than 200,000 new leprosy cases are registered annually, according to official figures from 161 countries from the six WHO Regions.[2]

Until now no vaccine is available so early diagnosis and treatment with multidrug therapy remain the basic strategy for leprosy control. The diagnosis is generally based on clinical findings, but the complexity of clinical presentation with other skin diseases has prompted the development of molecular test to confirm cases. The main molecular test used for case confirmation is polymerase chain reaction (PCR). Many molecular targets have been suggested for *M. leprae* genome amplification but the main targets are the *M. leprae* repetitive elements which can be detected from many types of clinical samples[3–5] and also from environmental samples.[6]

Côte d'Ivoire has reached the threshold of elimination of the disease and MDT is available nationwide. But the country has not managed to permanently break the chain of transmission of *M. leprae*. Thus, in the country where leprosy is endemic, from 1,169 cases detected in 2013 to 891 cases reported in 2015, the number of Grade II disabilities is increasing. The diagnosis of leprosy in Côte d'Ivoire is essentially clinical and microbiological approaches were restricted to skin smear microscopy. In this context, the diagnosis of infection is often made by default. Effectively, based only on clinical arguments, we could miss specific clinical features of the disease and the threshold detection limit (10^4 bacilli) of microscopy suffers from low sensitivity.

The eradication of a disease being conditioned by the performance of diagnostic measures implemented, we have undertaken to implement *M. leprae* PCR detection in Côte d'Ivoire, a diagnostic tool unavailable in the country up to now, to contribute to case confirmation and help the National Leprosy Eradication Program to fight against the disease.

Materials and methods

STUDY POPULATION

Thirty-nine patients were enrolled for this study. All patients were diagnosed as cases of leprosy by clinicians or leprologists at the Raoul Follereau Institute of Côte d'Ivoire, a treatment center for leprosy in the south of the country.

Clinical and demographic details were recorded at the time of diagnosis and patients were classified as either paucibacillary or multibacillary leprosy (Table 1).

ETHICS STATEMENT

This study was approved by the National Ethics committee for research of Côte d'Ivoire: "Comité National d'Ethique de la Recherche de Côte d'Ivoire (CNER)" under the approval number N/Réf: N°140/MSHP/CNER-km, which also included a parallel study on the detection of drug resistance mutations by PCR.[7] All participants signed the informed consent form after reading the study information notice.

Table 1. Clinical and demographics characteristics of leprosy patients

Characteristics	Types	Patients (n = 39)	
		No	%
SEX	Male	20	51.28
	Female	19	48.72
Age	9–22	11	28.20
	23–33	13	33.33
	37–49	9	23.07
	>50	6	15.38
WHO Classification	PB	26	66.66
	MB	13	33.33

BI = Bacteriological Index, PB = Paucibacillary leprosy, MB = Multibacillary leprosy, WHO = World Health Organization, NM = Nasal Mucous, TF = Tissue fluid.

BIOLOGICAL SAMPLES

The biological samples to be analyzed were obtained from all 39 patients. These consisted of nasal swabs and tissue fluids (dermal pulp fluid). Tissue fluids were collected from the right and left ear lobes. For microscopy, smears were performed from nasal swabs and dermal pulp fluid (slit skin smears), while for the molecular analyses, the nasal swabs and the dermal pulp fluid were collected in 2.5 mL microtubes containing 500 μL of phosphate buffered saline (PBS).

MICROSCOPY

Detection of acid fast bacilli (AFB) by microscopy was carried out by the coloration method of Zielh Neelsen[8] and the bacteriological index was determined according to the WHO acid fast bacilli counting scale.

DNA EXTRACTION AND MOLECULAR CONFIRMATION BY PCR

DNA was extracted from samples, as previously described.[7] Leprosy cases were confirmed by conventional PCR, targeting the *Mycobacterium leprae* repetitive element RLEP as described by Woods and Cole.[9]

PCR was carried out in a GeneAmp 9700 PCR System (Applied Biosystems) with the following program: an initial denaturation at 94 °C for 5 min following by 35 cycles consisted of denaturation: 94 °C for 30 s, annealing: 57 °C for 30 s, extension: 72 °C during 60 s and a final extension at 72 °C for 10 min.

SEQUENCING

To confirm that the 545bp PCR products were sequences from the *M. leprae* genome, 6 of them were sequenced. They were first purified and sequences were obtained on a 24 capillary ABI 3500 XL Genetic analyzer (Applied Biosystem).

Bioinformatics analysis was done using MEGA 7.0 software[10] and the BLAST*n* program[11] was used for identity search in the NCBI database.

Results and discussion

Among the 39 patients were 20 males and 19 females. Ages varied from 9 to 72 years with a mean of 32 years old. According to the WHO classification, 13 patients presented the

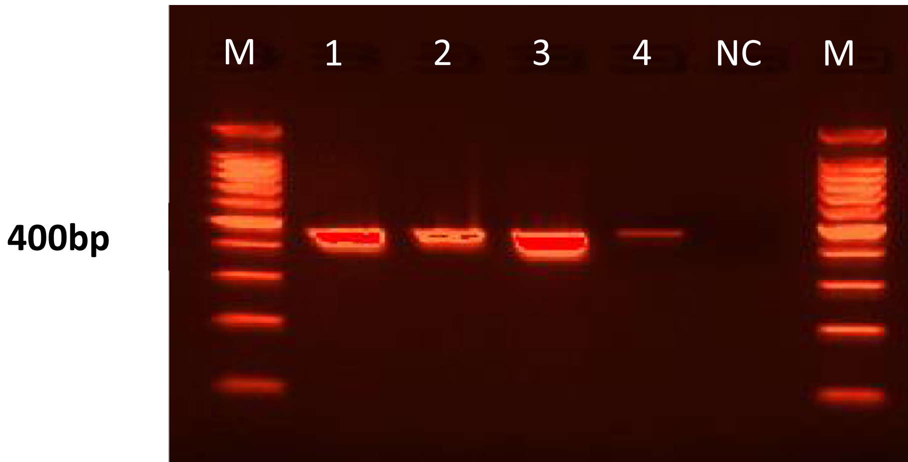

Figure 1. RLEP PCR product amplification on 1% agarose gel (ETBr staining). M: molecular weight marker 100bp, 1 to 4: samples, NC: negative control.

Table 2. *M. leprae* detection results by PCR RLEP

		PCR		Total
		Positive (%)	Negative (%)	
Leprosy form	PB	3 (7.7)	23 (59)	
	MB	12 (30.8)	1 (2.5)	
Total		15 (38.5)	24 (61.5)	39
Sample type	NM	9 (23.1)	30 (76.9)	39
	TF	15 (38.5)	24 (61.5)	39

PB: paucibacillary form; MB: multibacillary form; NM: nasal mucous; TF: tissue fluid.

multibacillary form, versus 26 patients who presented the paucibacillary form. Concerning microscopy, all the multibacillary patients were positive to Ziehl Neelsen coloration with a positive bacillary index ranging from 1+ to 5+. According to the type of sampling, it appeared easier to observe acid-fast bacilli from skin pulp fluid than from the nasal swab.[12] We also confirmed that it is more difficult to obtain a positive result with microscopy in an early infection. Microscopy suffers from low sensitivity because of the detection limit which is about 10^4 bacilli.

Of the 39 cases tested by PCR, 15 (38.5%) were positive (Table 2 and Figure 1). More than 90% of patients presenting with the multibacillary form were positive by PCR. This shows that it is simpler to diagnose the multibacillary form of leprosy, as the paucibacillary form could be confused with other skin diseases by inexperienced health workers.

Among the patients with the paucibacillary form, 3 out of 26 who presented a zero bacillary index were positive by PCR. This shows the value of PCR in confirming cases versus microscopy. Indeed, many studies have shown the value of PCR compared to microscopy or any other basic method used in microbiology, in the diagnosis of mycobacterial disease.[13,14]

The bioinformatic analysis of 6 of the amplified PCR products sequenced with the BLAST*n* program confirmed that those sequences obtained from PCR products were related to the *M. leprae* genome with 99–100% sequence identity.

Conclusion

PCR is now available for confirmation of leprosy cases in Côte d'Ivoire. It appears to be a useful method for detecting *M. leprae* for neglected skin disease control in Côte d'Ivoire, where we observe various skin diseases such as Buruli ulcer and yaws which sometimes present lesions similar to leprosy. Thus using PCR would help to reduce the number of Grade II disabilities by early and rapid confirmation of the diagnosis, and also lead progressively towards leprosy elimination.

Conflict of interest

The authors declare that they do not have any conflict of interests.

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