Isolation of non-tuberculous mycobacteria at three rural settings in Zambia; a pilot study

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Abstract

To assess the role of non-tuberculous mycobacteria (NTM) as a cause of tuberculosis-like diseases in Zambia, 167 chronically ill patients, hospitalized in three rural hospitals in Katete, Sesheke and Chilonga, were examined by microscopy and liquid culture for the presence of NTM. The percentages of patients with a positive culture for *Mycobacterium tuberculosis* complex were similar in the three geographical locations (19–25%). In contrast, the percentage of NTM ranged from 78% in Katete and 65% in Sesheke to 21% in Chilonga. Furthermore, the distribution of NTM species was different at the three geographical sites. In seven patients, true NTM-associated disease was suspected: five with *Mycobacterium lentiflavum* and two with *Mycobacterium intracellulare*. Analysis of possible risk factors indicated that the OR for NTM culture-positive sputum was significantly higher for patients living in Katete and Sesheke. Female gender and chest X-ray appearances of tuberculosis were independently associated with NTM culture-positive sputum. NTM colonization and disease in hospitalized, chronically ill patients in rural Zambia appear to be common.

Keywords: Africa, colonization, non-tuberculous mycobacteria, tuberculosis, Zambia

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Introduction

Tuberculosis (TB) is nearly always caused by *Mycobacterium tuberculosis*. However, non-tuberculous mycobacteria (NTM) can also mimic this disease. Large geographical variation in the distribution of NTM has been reported worldwide. In the USA, NTM-associated lung disease is most commonly attributed to *Mycobacterium avium* complex (MAC), with *Mycobacterium kansasii* being the second most important causative agent [1]. In the UK, especially in England and Wales, *M. kansasii* is the pathogen most commonly associated with NTM-associated lung disease, whereas *Mycobacterium malmoense* is most commonly encountered in Scotland, and *Mycobacterium xenopi* predominates in southeastern England [2]. In Japan, the most common cause of NTM-associated lung disease is MAC, followed by *M. kansasii* [3]. However, in most parts of the world, especially in those areas with a high prevalence of TB, the distribution of NTM in clinical isolates is not known.

Reported rates of NTM colonization and disease are probably underestimates. Colonization rates are especially uncertain, as people without significant symptoms will not commonly undergo the intensive investigations needed to detect NTM. This is compounded by the lack of systematic reporting of NTM isolation in most countries.

In African countries, despite the fact that environmental exposure to NTM is very high, NTM colonization and disease are hardly reported, even among patients with AIDS [4]. However, it is not clear whether this is due to a truly low prevalence of NTM disease or whether the magnitude of this problem has not yet been revealed. A number of studies on the epidemiology of NTM have, however, been performed in South Africa. In two population-based studies, rates of NTM colonization were 1400 and 6700 per 100 000, respectively [5,6]. Two other studies on NTM in this country focused on a population of gold-miners [7,8]: the annual rate of NTM disease in this group was found to be 101 per 100 000, with the two most common organisms isolated being *M. kansasii* (66/100 000) and *Mycobacterium scrofulaceum* (12/100 000).

In recent years, the clinical importance of NTM has become increasingly recognized [9]. The set of criteria in a Statement of the American Thoracic Society (ATS) in 1997, revised in 2007, can be used to differentiate between true NTM disease, colonization, pseudo-infection, and contamination [2,10,11]. This differentiation is made on the basis of clinical, radiological and microbiological features.

The goal of this pilot study was to assess the role of NTM in TB-like diseases in various rural settings in Zambia.

Materials and Methods

One hundred and sixty-seven chronically ill adult patients, hospitalized in three rural hospitals in different provinces of Zambia from March to August 2001, were the subjects of this study. These hospitals were St Francis Hospital in Katete (Eastern Province), Yeta District Hospital in Sesheke (Western Province), and Our Lady's Hospital in Chilonga (Northern Province). All patients above the age of 15 years, with symptoms in any part of the body for more than 2 weeks, were included.

Sputa of the patients were examined for presence of acid-fast bacteria and subjected to culture in Mycobacteria Growth Indicator Tubes (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Decontamination of sputum samples was performed using *N*-acetyl-L-cysteine–NaOH and sulphuric acid, as described previously [12]. In Katete and Sesheke, the sputum was collected twice on two consecutive days. In Chilonga, a single sputum sample was collected and cultured, for logistic reasons. Serological testing for human immunodeficiency virus (HIV) was performed using a qualitative immunoassay (Abbott Determine HIV-1/2) and the Vidas HIV DUO assay (BioMérieux, Marcy l'Etoile, France). Chest X-rays were evaluated in a blinded manner in The Netherlands. The Accuprobe culture confirmation test for *M. tuberculosis* complex (Accuprobe; BioMérieux) and/or 16S rRNA gene sequencing were used to identify *Mycobacterium* isolates [13].

Data analysis

Data were entered into SPSS 6 and analysed using STATA v. 8.0 (Stata Corporation, College Station, TX, USA). Student's *t*-test was used to compare means between groups, and the chi-square test to compare proportions. Univariate ORs with 95% CIs were calculated to assess associations of risk factors for NTM isolation. A stepwise backward regression approach was used for multivariate analysis.

Results

General characteristics

A total of 167 chronically ill patients were included in the study; 63 in Katete, 51 in Sesheke, and 53 in Chilonga. The patient characteristics are shown in Table 1. The proportion of HIV-positive patients varied between 69% and 79% in the three hospitals. The occupation of the majority of patients in Katete and Sesheke was farming: 71% and 61%, respectively. In Chilonga, more variation was observed. In Katete, most patients (52%) retrieved their water from a borehole. In Sesheke and Chilonga, the main source of water was a river in combination with tap water (40–45%). Patients who were

TABLE 1. Characteristics of included patients with chronic complaints for at least 2 weeks in Katete, Sesheke, and Chilonga

	Katete	Sesheke	Chilonga	p-value	All
Number of included patients, *n* (%)	63	51	53		167
Female, *n* (%)	37 (59)	26 (51)	19 (36)	0.05	82 (49)
Age (years), median (range)	36 (18–76)	37 (18–67)	39 (15–74)	0.9	38 (7–76)
HIV, *n* (%)	48 (79)	38 (76)	34 (69)	0.3	120 (75)
Farmer, *n* (%)	45 (71)	31 (61)	19 (36)	<0.001	95 (57)
Smoking, *n* (%)	1 (2)	11 (22)	–[a]	0.001	12 (11)
Using alcohol, *n* (%)	4 (7)	15 (31)	–[a]	0.01	19 (18)
Tuberculosis treatment in the past, *n* (%)	12 (19)	15 (29)	15 (28)	0.4	42 (25)
Water source, *n* (%)					
Well	16/60 (27)	7 (14)	5/39 (13)		29 (19)
Borehole	31/60 (52)	0	2/39 (5)		33 (22)
Tap	5/60 (8)	21 (41)	15/39 (40)		41 (27)
River	8/60 (13)	23 (45)	16/39 (42)	<0.001	47 (31)
Unboiled milk, *n* (%)	1 (2)	35 (69)	–[a]	<0.001	36 (33)
Died during study period, *n* (%)	9 (14)	1 (2)	5 (9)	0.07	15 (9)

HIV, human immunodeficiency virus.
[a]Data on smoking and using alcohol or (un)boiled milk were not available in Chilonga.

TABLE 2. **Patients with positive *Mycobacterium* cultures in Katete, Sesheke, and Chilonga**

	Katete, *n* (%)	Sesheke, *n* (%)	Chilonga, *n* (%)	p-value	All, *n* (%)
Number of included patients	63	51	53		167
Mycobacterium tuberculosis complex	12 (19)	13 (25)	13 (25)	0.7	38 (23)
NTM in any sputum specimen	49 (78)	33 (65)	11 (21)	<0.001	93 (56)
NTM in only first sputum specimen	42 (67)	25 (49)	11 (21)	<0.001	78 (45)
NTM in first and second sputum specimens	28 (44)	12 (24)	–[a]	0.02	40 (35)
Same NTM in both sputa	13 (21)	5 (10)	–[a]	0.1	18 (16)
NTM and *M. tuberculosis*	8 (13)	7 (14)	3 (6)		18 (10)

NTM, non-tuberculous mycobacteria.
[a]Only a single sputum specimen per patient was cultured in Chilonga.

milk consumers in Katete usually boiled their milk prior to use. The patients in Sesheke mainly used unboiled milk. Most of the 167 patients complained of regular coughing and/or chest pain (79%). Complaints of the digestive tract were found in 13% of the patients, and the other 8% of patients had either skin infections/abscesses, enlarged lymph nodes, or complaints of the urogenital tract or central nervous system.

Culture results

The percentages of patients with a positive culture for *M. tuberculosis* complex were almost equal in the three locations, and varied between 19% and 25% (Table 2). The number of patients with positive NTM cultures was significantly higher in Katete (78%) and Sesheke (65%) than in Chilonga (21%). However, in Chilonga, only one sputum specimen was cultured per patient, unlike in Katete and Sesheke. Nevertheless, taking only the first sputum specimen in Katete and Sesheke into account, the number of patients with positive NTM cultures was still significantly higher in Katete and Sesheke than in Chilonga. The sputum smear was positive in 19 of 38 patients (50%) with sputum culture positive for *M. tuberculosis* complex and in only three of 76 (4%) patients with sputum culture positive for only NTM.

In all three hospitals, the sputum specimens were split into two equal parts before decontamination and culturing. In Katete, 217 (split) sputum specimens were cultured from 63 patients (Table 3). Therefore, 109 (217/2) sputum specimens were collected in Katete, in 92 (184/2) in Sesheke, and 52 (103/2) in Chilonga. Mycobacteria were isolated from 140 of the 217 (65%) sputum specimens collected in Katete; *M. tuberculosis* was found in 16 of the 217 (7%) cultured sputum specimens, and NTM in 124 (57%). The most commonly isolated NTM were *Mycobacterium lentiflavum* (42) and *Mycobacterium intracellulare* (19). The number of mycobacteria isolated in Sesheke was similar to the number isolated in Katete. In contrast, no *M. lentiflavum* was found in Sesheke. In Chilonga, usually only one sputum specimen was collected per patient, and this was also divided and pretreated in two ways

TABLE 3. **Mycobacteria isolated from sputum specimens in Katete, Sesheke, and Chilonga**

Culture result	Isolates in Katete, *n* (%)	Isolates in Sesheke, *n* (%)	Isolates in Chilonga, *n* (%)
Negative	77 (35)	55 (30)	67 (65)
Mycobacterium tuberculosis	16 (7)	25 (14)	18 (17)
Mycobacterium avium complex			
Mycobacterium intracellulare	19 (9)	19 (10)	0
M. avium	0	0	1 (1)
Mycobacterium chelonae	8 (4)	0	0
Mycobacterium lentiflavum	42 (19)	0	0
Various unknown *Mycobacterium* species	18 (8)	29 (16)	5 (5)
Various other *Mycobacterium* species[a]	7 (3)	14 (8)	6 (6)
AFB without identification	30 (14)	42 (23)	6 (6)
Total number of split sputum specimens	217	184	103

AFB acid-fast bacilli.
[a]Various other *Mycobacterium* species in Katete included *Mycobacterium mucogenicum*, *Mycobacterium elephantis*, *Mycobacterium gordonae*, *Mycobacterium gilvum* and *Mycobacterium rhodesia*. Various other *Mycobacterium* species in Sesheke included *Mycobacterium fortuitum*, *M. mucogenicum*, *Mycobacterium asiaticum*, *Mycobacterium terrae* and *Mycobacterium triplex*. Various other *Mycobacterium* species in Chilonga included *M. mucogenicum*, *M. triplex*, *Mycobacterium obuense* and *M. gordonae*.

before culturing. There were 103 sputum specimens cultured from 53 patients but, in contrast to Katete and Sesheke, only 36 (35%) cultures were positive for mycobacteria. *M. tuberculosis* was found in 18 of the 103 (17%) cultured sputum specimens and NTM in the other 18 (17%). *M. intracellulare* and *M. lentiflavum* were not isolated in Chilonga.

Comparison of patients with and without NTM in their sputum

The demographic and clinical characteristics of patients with positive and negative NTM cultures were analysed. The medical history, physical examination and chest X-ray pathology did not show significant differences (Table 4). Table 5 shows the analysis of possible risk factors for patients with NTM in their sputum. The OR for NTM culture-positive sputum was significantly higher for patients living in Katete and Sesheke. Furthermore, female gender and suspicion of TB on chest X-ray were independently associated with NTM culture-positive sputum. A higher OR (although not significant in the multivariate analysis) was associated with farming and using

TABLE 4. Medical history, physical examination and chest X-ray of non-tuberculous mycobacteria (NTM) culture-positive and culture-negative patients

	NTM-positive	NTM-negative	p-value	All
Number of patients	93	74	–	167
Female sex, *n* (%)	55 (59)	27 (36)	0.004	82 (49)
Age (years), median (range)	37 (17–76)	38 (15–74)	0.9	38 (15–76)
HIV-positive, *n* (%)	71 (79)	49 (70)	0.2	120 (75)
BMI, mean	17.3	17.7	0.6	17.5
Weakness, *n* (%)	75 (82)	63 (88)	0.3	138 (84)
Vomiting, *n* (%)	19 (21)	18 (25)	0.5	37 (23)
Diarrhoea, *n* (%)	20 (22)	15 (21)	0.9	35 (22)
Dysuria, *n* (%)	10 (11)	13 (18)	0.2	23 (14)
Hepatomegaly, *n* (%)	19 (22)	13 (22)	0.9	32 (220)
Splenomegaly, *n* (%)	21 (24)	10 (17)	0.3	31 (21)
Lymph nodes, *n* (%)	52 (56)	44 (60)	0.6	96 (58)
Chest X-ray compatible with TB in the absence of *Mycobacterium tuberculosis* in culture, *n* (%)	25 (47)	15 (32)	0.1	40 (40)
Previous TB treatment, *n* (%)	23 (25)	19 (26)	0.9	42 (25)
Died, *n* (%)	8 (9)	7 (9)	0.8	15 (9)

BMI, body mass index; HIV, human immunodeficiency virus; TB, tuberculosis.

TABLE 5. Risk factors for positive non-tuberculous mycobacteria cultures from sputum

	Univariate analysis, OR (95% CI)	Multivariate analysis, OR (95% CI)
Location		
Chilonga	1	1
Sesheke	7.0 (2.9–16.8)	4.7 (1.6–13.7)
Katete	13.4 (5.5–32.6)	11.8 (4.5–30.8)
Sex (female)	2.5 (1.5–4.3)	2.0 (1.0–4.5)
Chest X-ray suggesting TB	1.9 (1.0–3.9)	2.5 (1.1–5.9)
Age ≥25 years	0.8 (0.3–2.1)	NS
Farmer	2.0 (1.1–3.8)	NS
Water (in comparison with tap water = 1)		
Borehole	3.1 (1.2–8.2)	NS
Well	2.6 (0.9–7.0)	
River	1.2 (0.5–2.8)	
Unboiled milk	1.0 (0.4–2.4)	NS
Smoking	0.4 (0.1–1.3)	NS
Using alcohol	0.7 (0.2–1.9)	NS
Mycobacterium tuberculosis isolated from sputum	0.6 (0.3–1.2)	NS
Tuberculosis treatment in the past	1.0 (0.5–1.9)	NS
HIV-positive	1.6 (0.8–3.3)	NS
Underweight (BMI < 18)	1.0 (0.5–2.1)	NS

BMI, body mass index; HIV, human immunodeficiency virus; NS, not (statistically) significant; TB, tuberculosis.

water from the borehole. HIV status and the influence of unboiled milk were not statistically significant.

Repeated isolation of NTM from sputum

The diagnostic criteria for NTM-associated lung disease according to the ATS include repeated isolation of the same species *Mycobacterium* from sputum samples in symptomatic patients together with imaging findings consistent with lung disease. In this study, two sputum specimens were collected and cultured from a total of 101 patients. *M. tuberculosis* was isolated from at least one sputum specimen in 21 (21%) of these patients, and NTM in 66 (65%). In ten patients, both *M. tuberculosis* and NTM were isolated.

In 56 patients (55%), NTM alone were found in both sputum specimens. In 15 of these 56 patients (15%) the same NTM species was found in both specimens (Table 6); in 29, NTM could not be identified in at least one sputum specimen, and in the remaining 12, different NTM species were isolated. In three more patients, the same species of NTM was found in both sputum samples, together with *M. tuberculosis* (Table 6).

A chest X-ray was performed in ten of the 15 cases with identical NTM in both sputum specimens, and in four of these the pathology on the chest X-ray suggested TB. *M. lentiflavum* was isolated in both sputum samples from all four patients. The sputum smears were positive in two patients. In six patients, the chest X-ray showed no signs of TB. Unfortunately, no chest X-ray was performed in five patients. In one patient, *M. lentiflavum* was cultured from a lymph node biopsy and from two sputum samples. In three of these five patients, *M. intracellulare* was identified in both sputum specimens. In the last patient without an X-ray, mycobacteria were cultured from both sputum specimens but could not be identified to species level.

Discussion

A high percentage (56%) of patients presenting with mainly coughing and/or chest pain at three African hospitals yielded positive NTM sputum cultures. This finding may be due to the extremely high rate of HIV-positivity (between 69% and 79%) among the patients examined. However, because the group of HIV-negative patients was relatively small in this study, correlations between NTM isolation and HIV status could not be studied adequately. The proportion of patients with positive NTM cultures was significantly higher in Katete (78%) and Sesheke (65%) than in Chilonga (21%). In Katete and Sesheke, more patients were working as farmers than in Chilonga. Farming has not been mentioned in the literature as a risk factor for NTM infections, but this is probable, as farmers have intensive exposure to NTM in soil, water, and other environmental sources. Food and water are also sources of human exposure to NTM [14]. There were differences between the villages in their water supply and consumption of unboiled milk. This suggests that direct contact with the environment may be a risk factor for NTM colonization in Zambia. Other studies have also described a patient group of elderly women with

TABLE 6. Cases with the same non-tuberculous mycobacteria species isolated from two sputum samples

Case	Isolate from sputum	ZN	Male/female	Age (years)	BMI[a]	HIV	Temperature[b]	Complaints[c]	Duration (weeks)[d]	Chest X-ray	Died	Remarks
1	*Mycobacterium lentiflavum*	Positive	Female	50	20	Indiff	?	Resp	21	TB	Yes	*M. lentiflavum* also in urine, started with anti-TB treatment
2	*M. lentiflavum*	Positive	Male	35	24	Positive	39	Resp	34	TB	No	
3	*M. lentiflavum*	Negative	Female	30	18	Positive	38.5	Resp	5	TB	No	TB in past, improvement on anti-TB treatment
4	*M. lentiflavum*	Negative	Female	25	?	Positive	38.5	Resp	4	TB	Yes	TB in past
5	*M. lentiflavum*	Negative	Male	29	14	Positive	38.5	Resp	3	No pathology	No	Started with anti-TB treatment
6	*M. lentiflavum*	Negative	Female	40	17	Positive	38.5	Resp	13	No pathology	No	TB in past
7	*Mycobacterium intracellulare*	Negative	Male	45	16	Positive	36	Dig	43	No pathology	No	
8	*Mycobacterium chelonae*	Negative	Female	41	14	Positive	38	Resp	32	No pathology	No	
9	*M. intracellulare*	Negative	Male	57	14	Negative	36.5	Resp	17	Not suspicion of TB	No	
10	*M. intracellulare*	Negative	Female	40	16	Positive	?	Resp	57	No pathology	No	Started with anti-TB treatment
11	*M. lentiflavum*	Negative	Female	67	?	Negative	35.5	Dig	8	No chest X-ray	No	*M. lentiflavum* cultured from lymph node
12	*M. intracellulare*	Negative	Female	43	?	?	?	Resp	4	No chest X-ray	No	Started with anti-TB treatment
13	*M. intracellulare*	Negative	Female	49	17	Positive	36.6	Resp	78	No chest X-ray	No	
14	*M. intracellulare*	Negative	Male	59	21	Negative	?	Resp	46	No chest X-ray	No	Started with anti-TB treatment
15	UMS	Negative	Male	36	17	Positive	39	Resp	8	No chest X-ray	No	TB in past
16	*M. lentiflavum*	Negative	Female	63	20	Negative	36.5	Resp	3	TB	No	Also *Mycobacterium tuberculosis* in sputum, started with anti-TB treatment
17	*M. lentiflavum*	Positive	Male	34	13	Positive	38	Resp	6	TB	No	Also *Mycobacterium tuberculosis* in sputum, started with anti-TB treatment
18	*M. chelonae*	Positive	Male	24	?	Positive	37	Resp	20	TB	No	Also *Mycobacterium tuberculosis* in sputum, started with anti-TB treatment

BMI, body mass index; Dig, digestive tract complaints/symptoms; HIV, human immunodeficiency virus; TB, tuberculosis; ZN, Ziehl–Neelsen; Resp, respiratory tract complaints/symptoms; UMS, unknown *Mycobacterium* species.
[a]?, BMI not known.
[b]Temperature at time of inclusion; ?, temperature at time of inclusion not known.
[c]Complaints, reason for visiting the hospital.
[d]Duration, duration of complaints at the time of hospitalization and inclusion in study.

no pre-existing lung disease at increased risk of NTM colonization, especially with MAC and *M. kansasii* [1,15]. In the present study, female gender was also a risk factor for NTM culture-positive sputum.

Little is known about the prevalence of different NTM in Africa. In this study, clear geographical differences between isolated NTM species were observed; however, the study group was small, and the NTM isolation was restricted to one season. Interestingly, unidentifiable mycobacteria were found in all three villages (between 5% and 16% of the cultured sputum specimens). These data may indicate that there is a different distribution of mycobacteria in Africa than in the USA and Europe, but this needs to be confirmed in larger studies.

Chronic respiratory symptoms not responding to antibiotics, radiological features suggesting TB and also two positive sputum cultures fulfil the ATS diagnostic criteria. In this study, 15 patients had the same NTM species in both sputum specimens, suggesting that NTM isolation may be clinically relevant in this group. Chest X-rays were performed in ten of these 15 patients, and had the appearance of TB in four patients with repeated *M. lentiflavum* sputum isolates. In at least three of the five patients without a chest X-ray, there was a suspicion of NTM lung disease. In one patient, *M. lentiflavum* was isolated from two sputum specimens and from a (normally sterile) lymph node. In two other patients with chronic respiratory complaints and no response to antibiotics, anti-TB treatment was started. *M. intracellulare* was isolated from consecutive sputum specimens in both patients. The combination of symptoms, together with the positive cultures, is highly suggestive of NTM lung disease. However, the sputa of these patients were not tested by molecular methods for the possible presence of *M. tuberculosis*. Ziehl–Neelsen-positive sputa should ideally have been subjected to such a test. Furthermore, culture of sputum was performed in Mycobacteria Growth Indicator Tubes and not on solid medium, and quantitation of CFUs and visualization of mixed cultures were therefore not possible. A low number of CFUs could have been an indication of colonization by NTM. Moreover, NTM are found ubiquitously in the environment, and colonization by NTM may result from drinking contaminated water or showering with it.

Recently, *M. lentiflavum* was isolated from public water distribution systems [16]. Isolation of the same NTM species from two different sputum specimens collected on consecutive days could therefore be a result of colonization with NTM because of exposure to drinking water contaminated with NTM. Disease caused by *M. lentiflavum* is difficult to treat with standard anti-TB drugs; in most cases, clarithromycin is added to the treatment, but the management of this NTM disease remains difficult [17]. The improvement of a patient (no. 3) in our study on anti-TB treatment without clarithromycin therefore suggests disease caused by *M. tuberculosis* rather than by *M. lentiflavum*.

Overall, for almost half (7/15) of the patients with the same NTM species in both sputum specimens, there was a basis for suspicion of NTM disease according to the criteria of the ATS. *M. lentiflavum* was isolated twice in five patients, and *M. intracellulare* was isolated twice in two patients. In this study, the estimated rate of colonization was 58% (59/101), with a striking rate of disease of about 7% (7/101). The fact that all patients presented at the hospitals with chronic complaints could partially explain such a high rate.

Laboratory cross-contamination is considered to be highly unlikely in this study, for the following reasons. The same methods were used at all three villages; the mycobacterial isolates represented several evolutionary lineages and strain variations [18]; and, in a significant proportion of the cases, the same NTM were isolated from consecutive sputa, indicating that the quality of NTM isolation was adequate.

We conclude, therefore, that NTM colonization and infection are common in rural Zambia and deserve further study.

Transparency Declaration

None of the authors has any conflict of interest.

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