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High Prevalence of Bacterial Pathogens in Sputum of Tuberculosis Suspected Patients in Buea

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Authors' contributions

This work was carried out in collaboration between all authors. Author SN drafted the protocol, carried out most of the bench work, analyzed data and wrote the first manuscript. Author BTP contributed in the identification of bacteria, supervised and made input to the manuscript. Author HDM is the scientist at the TB laboratory where sputum microscopy and culture were made. He ensured the standard procedure and closely supervised the bench work. Author ALN approved proposal, read the manuscript and made inputs. Author JCNA read the manuscript and made inputs. Author IAA is the senior scientist in charge of the TB lab in the Buea Regional Hospital. She designed the study, approved the protocol supervised and contributed in writing the manuscript.

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ABSTRACT

Aim: To investigate the prevalence of non AFB (acid fast bacilli) bacterial pathogens among HIV positive and HIV negative TB suspected patients.
Study Design: A cross sectional laboratory based study was used.
Place and Duration of Study: Tuberculosis Unit, Buea Regional Hospital, between February and May 2015.
Methodology: We included 145 TB suspected patients referred to do a sputum test (82 women, 63

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men, 44 HIV positive, 101 HIV negative, age range 21-70 years). Socio-demographic factors and clinical history were abstracted using structured questionnaires. One early morning sputum sample was examined microscopically and cultured on blood, chocolate and MacConkey's agars

Results: Non AFB bacterial infections were identified in 89 (61.4%) out of the 145 study participants. The prevalence of non AFB bacterial infection in the HIV positive group (33 0ut 0f 44) was significantly higher than in the HIV negative group (56 out of 101), (*P*= .032). Bacteria isolated included 42 *S. pneumoniae*, 19 *H. influenzae*, 15 *K. pneumoniae*, 14 other enterobacteriaceae, 11 *P. aeruginosa* and 7 *S. aureus*. Although the prevalence of bacterial infection was 67.1% in females and 54% in males, the difference was not statistically significant (*P* =.149).

Conclusion: There is a high prevalence of non AFB bacterial pathogens among TB suspected patients. HIV positivity significantly increased the risk of developing LRTIs.

Keywords: Lower respiratory tract infection; non-AFB bacterial infection; TB suspected; Streptococcus pneumoniae; AFB; non AFB bacteria.

DEFINITIONS AND ABBREVIATIONS

Non AFB: Bacteria other than those that are acid fast bacilli; LRTI: Lower respiratory tract infection.

1. INTRODUCTION

LRTIs (lower respiratory tract infections) are some of the most prevalent diseases of humans worldwide [1]. These diseases directly result in about 7 million deaths annually in persons of all ages [2]. According to WHO, tuberculosis and acute lower respiratory tract infections are two among the 6 leading causes of death [3]. Of all the deaths caused by acute respiratory tract infections, LRTIs account for 20 – 24% [4].

Tuberculosis is one of the most debilitating bacterial pulmonary infections and it presents with similar signs and symptoms as LRTIs of other etiologies. A third of the world's population is said to be infected with *Mycobacterium tuberculosis* [5] with about 95% of cases coming from developing countries [6].

There has however been such a great emphasis on tuberculosis almost suppressing the amount of attention given to other lower respiratory tract infections which are also major health challenges both among communities and in hospitalized patients [4].

As populations evolve and immunocompromising conditions become more prevalent many more people become at risk of LRTIs [1]. The HIV pandemic has even worsened morbidity and mortality due to LRTIs. The lungs are said to still be the number one infection site for people living with HIV [7]. LRTIs are reported to cause about 70% of illnesses in AIDS patients [8].

The outcome of other non-mycobacterial bacterial infections especially in HIV immune-compromised patients may be fatal if not properly diagnosed and treated. During the time taken to rule out a TB diagnosis, the patient invariably deteriorates with an infection which may have been adequately treated if bacterial culture and sensitivity was done.

Different studies have shown different bacterial pathogens to be implicated to diverse extents but generally the major pathogens encountered are *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Moraxella catarrhalis, Streptococcus pyogenes* and some other enteric gram negative rods. These pathogens however differ in prevalence between geographical regions.

This study was carried out among TB suspected patients to note the prevalence of non-mycobacterial bacterial pathogens mimicking tuberculosis both in HIV positive and HIV negative patients. We also investigated the major risk factors for developing lower respiratory tract infections among patients.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling

This study was carried out at the Buea Regional Hospital TB Centre. Buea is the South West Regional capital of Cameroon and a major gateway city into the region. The TB Centre receives suspected cases from all over the region. Patients presenting with lower respiratory

tract infections that were sent to do a sputum test for AFB and who gave their informed consent by signing our forms were included in the study. The patients were all screened for HIV antibodies according to hospital protocol for TB suspected patients. Forty-four HIV reactive (Alere Determine® HIV ½ (Abbot Laboratories, Tokyo, Japan) and HIV Bioline (Standard Diagnostics, Inc.) confirmed] and 101 HIV nonreactive patients suspected of TB formed our study groups.

2.2 Study Design

This study was a cross sectional laboratory based study involving 145 TB suspected patients at the Buea Regional Hospital.

Ethical clearance was sought from the Faculty of Health Science Institutional Review Board (FHS-IRB) of the University of Buea. Administrative clearance for specimen collection was obtained from the South West regional delegation for public health and from the director of the Buea Regional Hospital. The researcher only commenced study after obtaining due authorization from necessary authorities. Patients were recruited into the study only if they gave their informed consent. To ensure confidentiality, no name was required from research participants. Unique identification codes were used for participant identification. Furthermore, the researcher assured patients that there would be no unauthorized disclosure of test results, no other analysis would be performed on participant's samples except those stated on the test protocol included in the research proposal and consent form, and that no part of participant's personal information or laboratory findings would be used for purposes other than strictly for educational and scientific advancement. Participants benefited by receiving their test results and the necessary advice on how to proceed.

2.3 Sample Collection and Processing

An early morning expectorated sputum sample was collected in sterile containers from all patients included in the study. Patients presented before a physician with signs and symptoms such as cough lasting more than two weeks after commencing antibiotics, fever, night sweats, chest pain and other symptoms suggestive of lower respiratory tract infections and were thus sent for AFB testing. Samples were collected from morning to mid-day when they were processed and inoculated into media. Patients were asked not to eat 1 hour before expectoration, to rinse their mouth with sterile water and then to cough deeply to expectorate into a provided sterile container.

The quality of sputum samples were assessed both microscopically and macroscopically. Watery and non-purulent sputa were considered unsuitable for further processing. All sputa were examined microscopically under the low power objective and the number of epithelial cells and/or polymorphonuclear leukocytes were counted to establish to establish the level of contamination. Sputa that had a majority of epithelial cells with few to no leukocytes were rejected as poorly collected sample. All unsuitable specimens were discarded and new specimens collected.

2.4 Laboratory Procedure

Gram staining was done to investigate bacterial pathogens while Ziehl Neelson staining was done to check for acid fast bacilli.

2.5 Culture of Sputum

Sputum specimens were inoculated into blood, chocolate and MacConkey's agars. The inoculated plates were incubated at 37℃ for 24-48 hours aerobically, except for chocolate agar, in which the plates were incubated for 24-48 hours at 37℃ in an atmosphere of 5-10% CO_2 [9]. Cultures with significant growth were presumptively identified based on their cultural and morphological characteristics on selective and differential media [9]. Standard microbiological techniques and biochemical tests were used to confirm bacterial species.

Patients that tested positive to HIV were referred to the Buea regional hospital HIV unit for follow up. All positive cultures were reported back to the requesting physicians.

2.6 Statistical Analysis

Data acquired were analyzed using the Epi Info software (version 7) (Centers for Disease Control and Prevention, USA). Categorical variables were compared by chi-square. P values of <0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

In all at least one pathogen was identified in 99 of the 145 study participants. 79 had exclusively

non-AFB bacterial infection, 10 had exclusively AFB and 10 had co-infections of both. Overall non AFB bacterial infections were identified in 89 (61.4%) out of the 145 study participants. The prevalence of non AFB bacterial infection in the HIV positive group was significantly higher than in the HIV negative group (*P*= .032). Table 1 shows the number of positive sputum smears and bacterial isolates from both study groups.

A total of 108 bacterial pathogens were isolated from 89 patients. Bacteria isolated included 42 *S. pneumoniae*, 19 *H. influenzae*, 15 *K. pneumoniae*, 14 other enterobacteriaceae, 11 *P. aeruginosa* and 7 *S. aureus*. 8 of the 11 isolates of *P. aeruginosa* came from the HIV positive group while only three were seen in HIV non-reactive patients. Table 2 shows the number of each bacterial pathogen isolated in both study groups of a total of 20 co-infections of two non AFB bacteria, 8 were seen among the HIV reactive group. One HIV patient was seen with a co-infection of three different bacteria.

The prevalence of bacterial infection was 67.1% in females and 54% in males There was however no significant difference in the prevalence of LRTI between men and women (*P*=.149). Women had more risk than men of developing bacterial lower LRTI (Odds ratio = 1.7), (Table 3).

Although prevalence of bacterial LRTIs was not significantly different by age (*P*=.072), the risk steadily increased with increase in age group (Table 3).

The lower economic class defined as income less than XAF 100,000 had the highest prevalence of bacterial infections 65%, the middle economic class, XAF 100,000 < income < XAF 500,000 had prevalence of 46.1% and lastly the upper class of income > XAF 500,000 which constituted only two participants in the study had a 50% prevalence. However economic class was not significantly associated to infection (*P*=.204) (Table 3).

Smoking, alcoholism and the presence of comorbidities were not found to be significantly associated to infection (Table 3).

Table 1. HIV status and bacterial lower respiratory tract infection

	n=145	Acid fast bacilli (%) 95% CI of %	Other bacteria (%) 95% CI of %
HIV reactive	44	9 (20.4)	33 (75)
		7.7- 33.2	64.2- 85.8
HIV non-reactive	101	11 (10.9)	56 (55.4)
		0.2- 22.0	53.9- 57.0
P-value		.119	.032

Table 2. Etiologic agents in bacterial lower respiratory tract infections

Bacterial pathogen	Prevalence n (%) 95%CI of %	HIV reactive n (%) 95%CI of %	HIV non-reactive n (%) 95%CI of %
Streptococcus pneumoniae	42 (38.9)	17 (40.5)	25 (59.5)
	35.1- 42.7	31.3- 49.6	34.4- 44.1
Haemophilus influenzae	19 (17.6)	8 (42.1)	11(57.9)
	13.8- 21.14	9.9- 28.2	10.2- 23.1
Klebsiella pneumoniae	15 (13.9)	4 (26.7)	11(73.3)
	10.1- 17.7	0.4- 18.7	10.2- 23.1
Other enterobacteriaceae	14 (12.9)	3 (21.4)	11(78.6)
	9.1- 16.8	2.0- 16.3	10.2- 23.1
Pseudomonas aeruginosa	11 (10.2)	8 (42.7)	3 (27.3)
	6.4- 14.0	9.9- 28.2	1.9- 11.0
Staphylococcus aureus	7 (6.5)	2 (28.6)	5 (71.4)
	2.7- 10.3	4.4- 13.9	1.1- 14.0
Total	108 (100)	42 (38.9)	66 (61.1)

Table 3. Effect of clinical and socio-demographic factors on bacterial LRTIs

Factor	Divisions	N°	Prevalence (%) 95% CI of %		odds ratio (95% CI)	*P* value
			No growth	Growth		
HIV status	Negative	101	45 (44.6) 43.8- 45.3	56 (55.4) 54.7-56.2	1	.032
	Positive	44	11(25) 19.6- 30.4	33 (75) 69.6-80.4	2.4 (1.1-5.3)	
comorbidities	Absent	121	49 (40.5) 38.0- 42.9	72 (59.5) 57.1-62.0	1	.321
	Present	24	7 (29.2) 16.9- 41.5	17 (70.8) 58.5-83.1	1.4 (0.8-3.9)	
Smoking	Yes	18	7 (38.9) 31.3-46.5	11 (61.1) 53.5-68.7	1	.817
	No	127	49 (38.6) 35.7- 41.5	78 (61.4) 58.5-64.3	1.0 (0.4-2.8)	
Alcohol consumption	Hazardous	13	6 (46.1) 43.0- 49.3	7 (53.9) 50.7-57.0	1	.774
	Non-hazardous	132	50 (37.9) 34.9- 40.9	82 (62.1) 59.1-65.1	1.4 (0.4-4.2)	
Gender	Male	63	29 (46.0) 44.6- 47.5	34 (54.0) 52.5-55.4	1	.149
	Female	82	27 (32.9) 27.6- 38.3	55 (67.1) 61.7-72.4	1.7 (0.9-6.8)	
Age	21-30	57	28 (49.1) 48.8- 49.5	29 (50.9) 50.5-51.2	1	.072
	31-40	29	12 (41.4) 36.8- 46.0	17 (58.6) 54.0-63.2	1.4 (0.6-3.4)	
	41-50	19	7 (36.8) 28.1-45.6	12 (63.2) 54.4-71.9	1.7 (0.6-4.8)	
	>50	40	9 (22.5) 10.0- 35.0	31 (77.5) 65.0-90.0	3.0 (1.2-7.2)	
Economic status	Upper class	2	1 (50.0)	1 (50.0)	1	.204
	Middle class	26	14 (53.9) 51.7- 56.0	12 (46.1) 44.0-48.3	1.2 (0.1-20.7)	
	Lower class	117	41(35.0) 31.1- 39.0	76 (65.0) 61.0-68.9	1.9 (0.4-22.3)	

Income (I) < 100,000XAF = lower class, 100,000XAF < I < 500,000XAF = middle class, I > 500, 000XAF= upper class

3.1 Discussion

This study focused on identifying non-acid-fast bacteria involved in lower respiratory tract infections among patients suspected to be suffering from tuberculosis. Tuberculosis is a leading public health problem globally with one third of the world's population said to be infected with the tubercle bacilli [10]. However other lower respiratory tract infections caused by bacteria other than *M tuberculosis* are major causes of mortality and morbidity in both HIV negative and positive persons [1]. Our study recorded 13.7% smear positivity for AFB but other respiratory tract bacterial pathogens were isolated in 61.4% of participants. This highlights the importance of these bacterial infections which are often not specifically targeted once TB is suspected after one failed antibiotic empirical trial. In Cameroon the present algorithm for the diagnosis of TB does not include gram stain and culture for these other bacterial pathogens which could be the cause of disease. Our study therefore shows that majority of patients sent to be tested for AFB having presented with lower respiratory symptoms may rather be suffering from other bacterial infections and not tuberculosis. The prevalence of these bacterial infections was significantly higher in HIV reactive patients (P=0.03) than in HIV non-reactive patients. This can be explained by the diminished immunity of HIV patients [11] which gives route for opportunistic and normal pathogens to attack.

The pathogens isolated in our study were *S. pneumoniae* (26.8%), *H. influenzae* (17.6%), *K. pneumoniae* (13.9%), other enterobacteriaceae (13.0%), *P. aeruginosa* (10.2%), and lastly *S. aureus* (6.5%). *S. pneumoniae* was the most frequently isolated pathogen in both study groups. This pathogen has been reported as the most significant pathogen in lower respiratory tract infections both in studies involving the general population and those among HIV patients [12,13,14]. In his study involving HIV infected African adults with lung diseases due to common bacteria; Tchamran recorded 81% of infections due to *S. pneumoniae* and stated it to be the most disturbing pathogen in HIV reactive patients [14].

Our study recorded *H. influenzae* as the second most prevalent pathogen. This is a well-known agent of pneumonia and other lower respiratory tract infections as reported by different studies [15,16]. Ndip in his study of upper respiratory tract bacteria among boarding school students in Buea isolated *H. influenzae* in 20% of patients being the most prevalent pathogen in his study [15]. Our study carried out in the same area further shows the important role played by *H. influenzae* in patients with bacterial lower respiratory infections even though we never found it to be the most prevalent among TB patients.

Klebsiella pneumoniae was the third most isolated bacterial pathogen in our study. Ndip in his study of upper respiratory tract bacteria among boarding school students in Buea also recorded *K. pneumoniae* as third most implicated pathogen [15]. This pathogen has however been reported as the number one bacterial pathogen among patients with LRTIs both by Shailaja in India [17] and Akingbade in Nigeria [18]. Prince et al in New York reported a case study of a patient who was initially thought to have pulmonary tuberculosis but turned out to be suffering from klebsiellosis by *K. pneumoniae* [19]. Therapy was adjusted and convalescence occurred. Infections with *K. pneumoniae* are usually associated with alcoholism and diabetes mellitus [20]. However this was not investigated in our study.

P. aeruginosa infection among patients positive for HIV is often associated with considerable mortality. Studying the clinical manifestations of *P. aeruginosa* infection among patients with AIDS, Dropulic recorded 13 pneumonia cases among 73 cases of *P. aeruginosa* infections [21,22]. In our study *P. aeruginosa* contributed 10.2% of all bacterial infections and 72.7% of the isolates were among the HIV reactive group. *P. aeruginosa* has been said to be the most common bacterial pathogen in nosocomial infections among HIV patients with mortality rate up to 23% [23].

Earlier studies have identified *S. aureus* as mostly common with patients in intensive care units where it is implicated in nosocomial pneumonia [24,25] *S. aureus* was identified in 6.5% of cases in this study and had dominance in the age group greater than fifty years. A majority of our patients in the age group greater than fifty were hospitalized and critically ill.

The effect of gender on the prevalence of lower respiratory tract infections has been varied according to different studies. While some report higher prevalence in women [26,27] others report higher prevalence in men due to lifestyle factors like alcohol consumption and smoking [18,28]. Our study however recorded no significant difference in prevalence between genders although the risk in women was 1.7 times higher than in men. Egbe in Nigeria also recorded no significant difference in prevalence of LRTIs between males and females. The higher risk in women from our study may be due to the dominance in the daily burning of wood for cooking in our setting by the women.

Although our study recorded no significant difference in bacterial infections with respect to age, the risk increased with age with the age group 31 to 40 having 1.4 times more risk than the age group 21 to 30, the age group 41 to 50 having 1.7 times more risk than the age group 21 to 30 and the age group >50 having 3 times more risk than the age group 21 to 30. In his study of the microbiology of lower respiratory tract pathogens in Benin City Nigeria, Egbe recorded an increase in prevalence with age [29]. Millet working with older adults in the United Kingdom also recorded an increase in prevalence of lower respiratory tract infections with increase in age [30]. A diminishing immunity due to age as well as other health complications are probable reasons for this trend [29].

Other factors such as smoking, alcoholism, income status and the presence of comorbidities were found not to be significantly associated to bacterial infection. Smoking and alcoholism usually increase risk to lower respiratory tract infections by diminishing mucosal immunity [18],

but the risk from these factors may have been eclipsed in our study population by the overwhelming dominance of other factors such as the cold weather in Buea and the burning of wood for cooking which all favor LRTIs.

4. CONCLUSION

The overall prevalence of non AFB bacterial LRTIs among TB suspected patients was 61.4%. HIV positivity significantly increased the risk of developing LRTIs. Age and gender did not significantly affect the prevalence of LRTIs although the risk increased with age and was higher in female. Other risk factors like smoking, alcoholism and presence of comorbidities were found not to be significantly associated to LRTI among TB suspected patients. *S. pneumoniae* was the most prevalent pathogen both among HIV positive and HIV negative individuals. The findings of this study will help in redesigning diagnostic algorithm for TB suspected cases to include investigation of non-mycobacterial pathogens.

CONSENT

Patients were enrolled into the study only if they gave their written informed consent for both participation in the study and for HIV testing.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Faculty of Health Sciences Institutional Review Board, University of Buea and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. The Faculty of Health Sciences Institutional Review Board Project number is 2015/345.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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