Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com/journal/sajb/home

OPEN ACCESS

Biosciences

Fungi and Bacteria Isolated From Human Pulmonary Samples in Niger

Oubayyou AM[1*], Zongo C[1], Mounkaila S[2], Gagara Issoufou MA[3], Traore AS[1], Moumouni H[4]

[1]Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles, Université de Ouagadougou, 3BP 7131, Ouagadougou 03, Burkina Faso
[2]Faculté des Sciences Techniques, Université d'Agadez
[3]Service de Pneumo-phtisiologie, Hôpital National Lamordé de Niamey, and BP: 11653 Niamey-Niger
[4]Faculté des Sciences de la Santé, Université Abdou Moumouni de Niamey, BP: 131/125 Niamey-Niger Sincère remerciement vont: A l'Agence Nigérienne des Bourses et des Allocations (ANAB) et au Service de Coopération et d'Action Culturelle (SCAC, Coopération Française au Niger et au Burkina Faso) pour avoir financé ce travail

DOI: 10.36347/sajb.2019.v07i11.001 | **Received:** 07.05.2019 | **Accepted:** 04.11.2019 | **Published:** 11.11.2019

***Corresponding author:** Oubayyou AM

Abstract **Original Research Article**

The species name got by medical microbiology diagnostics can guide the treatment in human infections. This study aim was to determine association of medical fungal and bacterial profile in pulmonary samples from patients. It's a cross-sectional prospective study, carried out in health centers in Niamey, from October 1st, 2013 to September 30th, 2016. Several microbial strains have been isolated in association. It concerned species of the same genus or from a group of germs to another. Age, tuberculosis, smoking, chronic disease, which are numerous in our area constitute risk factor. Also difficult access to health care, insufficient respect of recommendations to fight against infections. All of those are reasons favoring associated infections in lung diseases. The association between bacteria species were 7 types. It's predominated by that between *S. aureus* and *P. aeruginosa* with 28.60%. Three types of fungi association were reported with same frequency (33.33%). Associated infection between bacteria and fungi was observed in 23 times, or 15.45% of all isolates.

Keywords: Fungal, Bacteria, Lung infection, Niger.

INTRODUCTION

Microbial etiologies diversity reported in pulmonary diseases require lot of investigation. It is generally to bacteria, especially tubercle bacilli or common germs which have a central role in this evolution. Autopsy studies provide that the idea is probably carelessness of mycological diagnosis, particularly of acute invasive aspergillosis, in patients with lung diseases [1]. Medical microscopic fungi have significant share, about 30% of patients die before the diagnosis is made [2]. *Candida* and *Aspergillus* are the two main fungal pathogens. The mortality rate associated with invasive aspergillosis is very high 90% [2].

Nowadays microbial associations are known in etiological distribution by microbiological investigation techniques. According to literature, Paugam *et al*. [3] in comparative analysis with bacterial infections show that patients with positive *Aspergillus* have not only a higher contamination of mycobacterial and *Stenotrophomonas maltophilia* but also a more frequent association with *Pseudomonas aeruginosa*.

P. aeruginosa is also thought to be associated with *C. albicans* [4, 5]. Epidemiological and clinical microbiology criteria are poor and the choice of adequate therapy is difficult in countries where laboratory testing is not accessible. What's the association criteria share between those microorganisms? Also medical mycology diagnosis basic and their biotechnology highlight will interest this screaming in our country. Where hypothesis that bacteria does over infection with opportunists fungi pathogens in Niger.

MATERIAL AND METHODS

The Broncho alveolar fluid, pleural fluid and sputum samples were collected on sterile tubes in National Hospital of Niamey, National Hospital of Lamordé which host a large number of patients from the country added to this some health structures like: sanitary districts and some private clinic recognized by their capacity to take care of respiratory diseases. National Hospital of Lamordé has been choosing for confirmation of suspected microbial infection.

Over 2 years epidemiological data were collected from medical record or by questioning patients

suspected having fungal or bacterial infections and a mixing of both.

Classic medical micro biotechnology methods has been done to prove the infection by phenotypic germ detection. The identification of the species is based on the comparison of various phenotypic characters of the strain to be studied with respect to those of a reference strain.

Small number of characters considered important were determined such as morphology, and cultural characteristics or sometime biochemical and immunological tests.

Table-1: List of commonly characters is detailed in the following table

Morphology: it's based on macroscopic and microscopic observation before or after coloration of Gram, methylene blue, Chinese ink	appearance of colonies: - Mold - Creamy germs form: - oval shape - bacillary form - filamentous form
Cultural character	• Aerobic / anaerobic growth • Fungal medium: pure Sabouraud, Sabouraud + chloramphenicol and Sabouraud + chloramphenicol + actidione as well as PCB medium • Bacterial medium: regular agar, fresh blood agar, Polyvitex ® enriched chocolate agar, Mac Conkey agar, eosin bleu de methylene agar and Chapman agar • Temperature: it was 37 ^{0}c for bacteria and 25 à 37 ^{0}c for fungal
Biochemical character	• Gram-negative bacilli, have been identified through API 10 S or 20 E gallery
	• Gram-positive cocci such as Staphylococcus and Streptococcus from catalase test result were identified with SLIDEX® and confirmed at API Strepto or API Staph

Epi-Info 6.04 and XLstat. 7.1 software were used to calculate the descriptive and comparative various analyzed parameters. Any P value < 0.05 was considered as statistically significant.

RESULTS

A total of 247 samples were obtained from 218 patients, of which 101 samples were positive after microbiological examination. Prevalence of bacterial, parasitic and fungal lung diseases in the population was 44.95%. Pleural fluid samples were the most obtained (75.51%). But the sputum had a very high sensitivity to isolate a germ 69.23%.

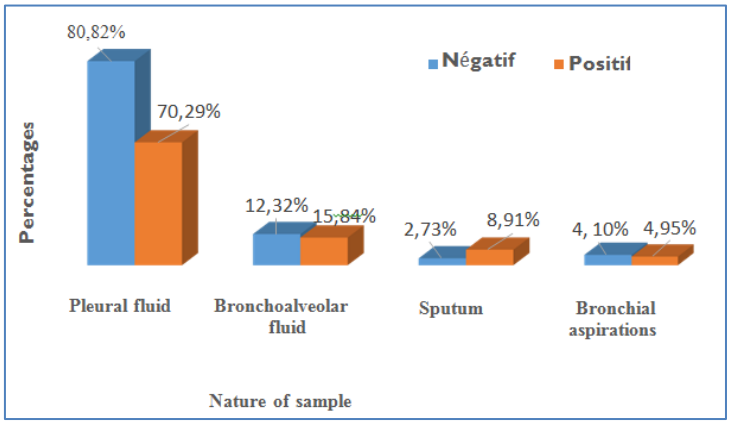

Fig-1: Frequency of samples nature

Associations between bacterial species

S. aureus and *P. aeruginosa* were the most recorded in associations between bacteria species 28.60%.

Table-2: Distribution of association between bacterial species

Bacterial association	Effective	Percentage
K. pneumonia + P. aeruginosa	1	14,28
K. penumoniae + S. epidermidis	1	14,28
S. aureus + P. aeruginosa	2	28,60
S. pneumoniea + S. maltophilia	1	14,28
S. aureus + S. pneumoniea	1	14,28
S. aureus + S. epidermidis	1	14,28
Total	**7**	**100**

Association between fungal species

Infection between fungi was 3 types with the same frequency. It showed only between *Candida* and *Aspergillus* genus.

Table-3: Distribution of association between fungal species

Fungal association	Effective	Percentage
Candida sp + A. fumigatus	1	33,34
C. albicans A. fumigatus	1	33,33
Candida sp + A. flavus	1	33,33
Total	**3**	**100**

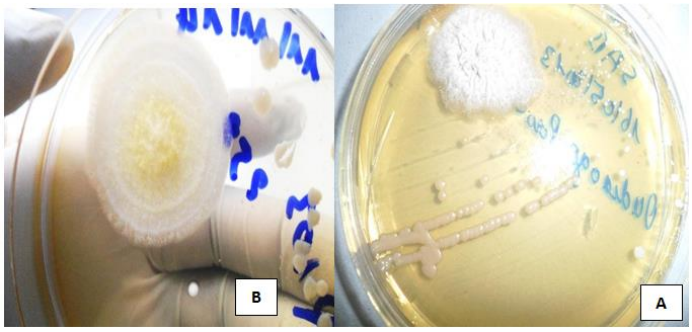

Fig-2: Culture of *Candida* and *Aspergillus* in association: Image: A and B

Association between fungal and bacterial species Fungal and bacterial species Associations were reported 23 times (15.45%). *P. aeruginisa*, *S. odorofera* and *Y. pseudotuberculosis* Strains were associated only with yeasts. *Candida* sp. was most incriminated fungal species (9/23) in associations with bacteria. According to analysis results the association began at 23 years of age. Men were most represented at 66.66%. The disease had chronic course in 93.33%. It also had 100% community look. At least one risk factor had been reported in the patients. These are corticosteroids therapy at 41.17%, antibiotic therapy 58.82%, decoctions 52.94% and HIV-AIDS 11.76%. Radiological examination of the lungs revealed bilateral pneumopathy in 66.66% of cases. Association with TB bacillus was 27.65%. Smoking has important role in occurrence of lung infection (23.4%).

Table-4: Distribution of association between bacterial and fungal species

Bacterial species	Fungal species							
	A. fumigatus	*Candida spp.*	*C. albicans*	*C. neoformans*	*A. flavus*	*P. Jurovecii*	*A. nidulans*	**Total**
A. baumannii	1	-	-	-	-	-	-	1
K. pneumoniae	1	1	-	-	-	-	-	2
P. aeruginosa	-	2	1	1	-	-	-	4
S. odorifera	-	2	-	-	-	-	-	2
S. aureus	2	1	1	-	2	-	-	6
S. epidermidis	-	1	-	-	1	-	-	2
S. matophilia	-	-	-	-	-	1	-	1
S. pneumoniae	-	-	-	1	1	-	1	3
Y. pseudotuberculosis	-	2	-	-	-	-	-	2
Total	**4**	**9**	**2**	**2**	**4**	**1**	**1**	**23**

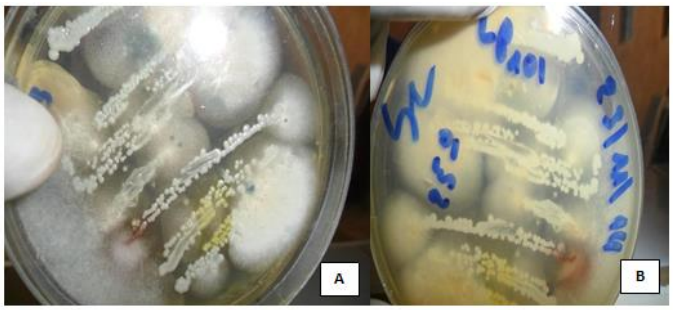

Fig-3: Colonies of yeast and mold in a kneaded box: Image: A front, Image: B back

DISCUSSION

The technical platform of mycology diagnosis was very poor in our laboratories; this confirms the idea that it is probably an underestimation of fungal infections, particularly in acute invasive aspergillosis, in patients with lung diseases [1]. Hence the very high rate of chronicity is 93.33% of the mixed pathogen isolates in this study. The serious consequences weigh about 30% of deaths before the diagnosis is made [2].

The motivations to collect pulmonary secretions have moved from the therapeutic field to complementary examination in the diagnosis and monitoring of humans pulmonary pathologies [6, 7]. Several natures were obtained according to level of attack, diagnosis interest and means implemented. Among these are pleural fluid (LP), broncho-alveolar lavage (BAL), bronchial aspirations, sputum, and lung biopsies.

Bacterial are also subject to underlying infection. However our results show 6 types of associations. *S. aureus* and *P. aeruginosa* was most recorded infection between bacteria at 28.60%. Afane *et al*. [8] found a similar value of tuberculosis in infection with common germs 31.85%. *Streptococcus* was represented 75% of associated infections in their study [8]. It was around 17% with Joris *et al*. Study in 2016 [20]on broncho-pulmonary infections with atypical germs in children in Bruxcel. The association between bacteria has been observed by other literature reviews [9, 10].

Association between fungi species was 3 orders with same frequency (Table 3). It concerned only *Candida* and *Aspergillus* genus. Adou-Bryn *et al*. [1] already reported infection between *Aspergillus* and *Candida* in Ivory Coast, as well as in the study of Fujisfuta *et al*. [11]. In a recently published study in 2016 Oubayyou *et al*. observed one case of association between *A. fumigatus* and *C. albicans* in Burkina Faso. In addition to this case, other mixed species of *aspergillus* genus has been isolated [12].

The consequences of association between fungi and bacteria are not fully known, but studies show that they correlate with degradation of lung function [5].

Several bacteria were associated with mushrooms, ie 15.45%. *Staphylococcus aureus* (26.08%) was most incremented followed by *P. aeruginisa* (17.39%). *P. aeruginisa*, *S. odorofera* and *Y. pseudotuberculosis* Strains were associated only with yeasts. *Candida* sp. was the most incriminated fungal species (39.13%) in associations with bacteria. In the literature, *Pseudomonas aeruginosa* is also thought to be associated with *C. albicans* [4, 5]. Our results confirm data found by Paugam *et al*. In 2010. They reported in comparative analysis with prevalence of bacterial infections that patients with positive culture to *Aspergillus* have a higher prevalence of mycobacterial contamination and *Stenotrophomonas maltophilia*, as well as a more frequent association with *Pseudomonas aeruginosa*. We must also note frequency of fungal pathogen association with pulmonary tuberculosis in this study was 6.9%. Tuberculosis infection cover opportunistic germ up: *Aspergillus fumigatus* was 4/7 in front of *Candida* sp. 2/7 and *Aspergillus flavus* 1/7. This association was reported at 4.2% by Ader *et al*. in 2006. Otherwise association between *A. fumigatus* and *Mycobacteria* has been reported [3]. Marine, 2012 had noticed that non-tuberculosis mycobacterial positive patients accounted for 13.1% of patients with *A. fumigatus* versus 4.6% of patients without *A. fumigatus*. This part of the work shows interest of simultaneous realization of BK and other germs research to take care lung infection. If only BK is searching by bacilloscopy, common germ will be unknown. Indeed, there is some cellular immune depression related to TB infection. The antimicrobial function of lung based on phagocytosis by alveolar macrophage is deteriorated by tuberculosis infection [13]. According to our results association began at 23 years of age. There is a large disparity between ages of series published by Gleason in 2002[19]. He reported that two bacteria associated *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are responsible for more than 40% of pediatric pulmonary disease. Explanation could be difference between: the diagnostic methods, population studied as well as patient's field. Men were the most represented (66.66%), as the same case for most authors in West Africa [14, 15]. Interpretation emerged from our results showing 100% of smokers were men. The urban population was the most affected at 66.66%. At least one

contributing factor has been reported in patients. These same factors are those conventionally described in the literature [16-18]. Radiological examination of lungs revealed bilateral pneumopathy in 66.66% of cases of bacterial and fungal association. Bilateral involvement could be explained by the fact that the majority of patients consulted late, so lesions initially unilateral (left or right) extend contralateral side by bronchogenic dissemination.

CONCLUSION

The management of infections is very difficult because of the many risk factors and the lack of correlation between clinical symptomatology and the causative organism. Mixed isolates of germs in the pulmonary samples are interest of this study. The study has shown that elderly, tuberculosis, HIV, steroids, antibiotics, smoking, are numerous and leads groups of people with decreased immunity. They are vulnerable to these infections. To this is added the insufficient respect of the recommendations concerning the fight against the infections, are as many reasons for microbial association. It is therefore legitimate to introduce surveillance methods aimed at reducing the risk of exogenous or endogenous contamination and to continue the search for sensitive and efficient diagnostic tests.

REFERENCES

1. Adou-Bryn KD, Ouhon J, Assoumou A, Kassi EA, Kone M, Therizol-Ferly M. 1999.
2. Sylvie C, Pharm B. Les antifongiques dans le traitement des infections invasives.*Pharmactuel*, 2003; 36 (1): 25-41.
3. Paugam A, Baixench MT, Demazes-Dufeu N, Burgel PR, Sauter E, Kanaan R, Dusser D,Dupouy-Camet J, Hubert D. 2010. Characteristic and consequences of airways colonization by filamentous fungi in 201 adult patients with cystic fibrisis in France. *Medical Mycology*, 48: 32-36.
4. Chotirmall SH, Greene CM, McElvaney NG. *Candida* species in cystic fibrosis: A road less travelled. *Medical Mycology*, 2010; 48 (1): S114-S124.
5. Whittaker L, Hogan D. Mixed bacterial-fungal infection in the cystic fibrosis respiratory tract. *Medical Mycology*, 2010; 48 (1): 125-132.
6. Balquet MH, Hatron PY, Wallaert B, Janin A, Gosset D, Devulder B. Signification des données du lavage broncho-alvéolaire au cours de la sclérodermie systémique: l'alvéolite lymphocytaire est en relation avec un syndrome de Sjögren associé. La Revue de Médecine Interne. 1990 May 1;11(3):S39.
7. Basset P, Bernaudin JF, SOLER P, Chollet S. Lavage bronchoalvéolaire d'exploration. *EMC*, 1977; 6-000-M-50.
8. Ze EA, Tafeukeng ND, Vouking M. Étiologies des Pneumopathies Bactériennes non Tuberculeuses en Milieu Pneumologique à Yaoundé. HEALTH SCIENCES AND DISEASES. 2013 Sep 3;14(3).
9. Hammerschlag M. 2003. Pneumonia due to *Chlamydia* pneumoniae in children: Epidemiology, diagnosis, and treatment. *Pediatr Pulmonol*, 36: 384-90.
10. Benitez AJ, Thurman KA, Diaz MH, Conklin L, Kendig NE, Winchell JM. Comparison of real-time PCR and a microimmunofluorescence serological assay for detection of Chlamydophila pneumoniae infection in an outbreak investigation. Journal of clinical microbiology. 2012 Jan 1;50(1):151-3.
11. Fujishita M, Kataoka R, Kobayashi M, Miyoshi I. Clinical features of 32 cases of fungal pneumonia. The Japanese journal of thoracic diseases. 1991 Apr 25;29(4):420-8.
12. Oubayyou AM, Zida A, Moumouni H, Savadogo A, Traore AS, Ouedraogo M. Aspects épidémiologiques et étiologiques des affections pulmonaires d'origine parasitaire et fongique en milieu hospitalier à Ouagadougou (Burkina Faso). International Journal of Biological and Chemical Sciences. 2016;10(3):1286-94.
13. Chaparas SD. L'immunité dans la tuberculose. Bulletin of the World Health Organization. 1982;60(6):827.
14. Kayantao D, Kone A, Pouabe Tchameni R, M'Baye O. Aspects épidémiologiques, cliniques et évolutifs des pneumopathies bactériennes à l'hôpital du Point G à Bamako. Médecine d'Afrique Noire. 2001;48(10).
15. Zoubga AZ, Ouedraogo M, Boncoungou K, Ki C, Ouedraogo SM, Ouedraogo G, Bambara M, Birba E, Millogo GR, Some L, DRABO Y. Aspects epidemiologiques, cliniques et therapeutiques des pneumopathies aiguës bacteriennes. Médecine d'Afrique Noire. 2000;47(11).
16. Chidiac C. Révision de la IVe Conférence de consensus en thérapeutique anti-infectieuse de la Société de pathologie infectieuse de langue française (SPILF). Médecine et maladies infectieuses. 2001;5(31):268-301.
17. Dellamonica P. Épidémiologie bactérienne des infections ORL et bronchopulmonaires en 1998. Presse Médicale. 1992;28(1):3-4.
18. Mouton Y, Bignolas G, Chidiac C, Decazes JM, Gehanno P. Recommandations sur la prise en charge de la pathologie infectieuse respiratoire. Médecine et Maladies Infectieuses. 1995 Oct 1;25(10):1021-8.
19. Gleason PP. The emerging role of atypical pathogens in community-acquired pneumonia. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2002 Jan;22(1P2):2S-11S.
20. Joris C, Kourani E, Vermeulen F. Les infections broncho-pulmonaires à germes atypiques chez l'enfant: Mythe ou réalité?. Revue médicale de Bruxelles. 2016;37(4):331-7.