Fungi and Bacteria Isolated From Human Pulmonary Samples in Niger

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Abstract

The species name got by medical microbiology diagnostics can guide the treatment in human infections. This study aimed 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

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

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. aeruginos* were the most recorded in associations between bacteria species 28.60%.
Table-2: Distribution of association between bacterial species

<table>
<thead>
<tr>
<th>Bacterial association</th>
<th>Effective</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumonia + P. aeruginosa</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>K. pneumoniae + S. epidermidis</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>S. aureus + P. aeruginosa</td>
<td>2</td>
<td>28.60</td>
</tr>
<tr>
<td>S. pneumonia + S. maltophilia</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>S. aureus + S. pneumonia</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>S. aureus + S. epidermidis</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fungal association</th>
<th>Effective</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida sp + A. fumigatus</td>
<td>1</td>
<td>33.34</td>
</tr>
<tr>
<td>C. albicans A. fumigatus</td>
<td>1</td>
<td>33.33</td>
</tr>
<tr>
<td>Candida sp + A. flavus</td>
<td>1</td>
<td>33.33</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>

Association between fungal and bacterial species
Fungal and bacterial species Associations were reported 23 times (15.45%). P. aeruginosa, 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
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 Bruxel. 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. aeruginis (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 maltophila, 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.

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