Research Article

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Multiplex PCR Detection of Bacteria and Risk Factors Associated with Acute Respiratory Infections (ARI) in Bouaké, Côte D'ivore

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ABSTRACT

Introduction: The advent of the polymerase chain reaction (PCR) technique has improved the etiological diagnosis of acute respiratory infections, with rapid and reliable results.

Objective: to identify by multiplex PCR the bacteria in the blood of patients with ARI and the associated risk factors.

Material and Methods: This was a cross-sectional study conducted from January 2018 to December 2020. Venous blood sampling on EDTA tube was performed in all patients with AKI, who were included at the Bouaké University Hospital and Air France CSU3. The collected samples were analyzed by multiplex RT-PCR using FTD pneumo Cap kit.

Results: Of 870 blood samples analyzed, 116 (13.33%) were positive for at least one bacterium. Bacteria detected were *Streptococcus pneumoniae* (7.59%), *Staphylococcus aureus* (4.14%), *Haemophillis influenzae* (2.41%), *Moraxella catarhalis* (2.18%), *Mycoplasma pneumoniae* (0.23%) and *Legionella pneumophilla* (0.11%). The highest positivity rates were observed in patients under 2 years of age (15.64%) and over 55 years of age (15.63%). *S. pneumoniae* and *M. catarhalis* were more detected in patients residing in the city of Bouaké ($p<0.05$). S. pneumoniae was detected in 46.97% of patients aged 0 to 2 years. The risk of developing S. aureus infection was higher in patients over 55 years of age (OR= 6.30; 95% CI 1.14-34.64; $p= 0.01$) and in persons exposed to pets (OR= 18.88: 95% CI 5.56-64.08; $p <0.01$). Patients residing in rural areas were less exposed to *M. catarhalis* infection. Smoking increased the risk of *M. catarhalis* infection 7-fold (OR= 6.94; 95% CI 2.51-19.23; $p<0.01$).

Conclusion: This study identified the bacteria associated with ARI in Bouaké and the associated factors by multiplex PCR. *S. pneumoniae* was the main bacterium identified in the patients. Because of the existence of a pneumococcal vaccine, it is necessary to identify the different serotypes of S. pneumoniae circulating in the Bouaké area.

KEYWORDS: Bacteria; Acute respiratory infection; Multiplex PCR; Risk factors; Côte d'Ivoire

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INTRODUCTION

Acute respiratory infections (ARIs) are caused by bacterial, viral, or fungal infections of the respiratory tract. ARIs are one of the major diseases that cause significant morbidity and mortality in children [1,2]. Each year, they contribute to approximately 4 million of the 15 million deaths worldwide among children under five years of age [3]. According to WHO ARIs are responsible for approximately 5.8 million deaths worldwide, 50% of which occur in developing countries [4]. Therefore, whether in developing or developed countries, ARIs represent an enormous human and economic burden for families and society as a whole [5,6].

Streptococcus pneumoniae, Haemophillis influenzae and *Moraxella catarhalis* are the most common bacteria in upper and lower respiratory tract infections [7,8]. The biological diagnosis of AKI relies on the detection of bacteria by conventional bacteriology techniques. However, the culture of some bacteria may be difficult or require longer periods of time, especially when the patient has been given antibiotics prior to collection [9]. The advent of the polymerase chain reaction (PCR) technique has improved the etiological diagnosis of ARI with rapid and reliable results [10]. This high sensitivity of PCR in the diagnosis of bacterial ARI is due to the fact that PCR is based on the detection of genetic material (DNA and RNA) and therefore does not require the survival of the microorganism. In addition, it is not affected by the previous use of antibiotics [11].

The etiological diagnosis of ARI remains a major health issue in Africa and in Côte d'Ivoire in particular. In 2020, ARI represented in Côte d'Ivoire the 2nd cause of consultation and hospitalization especially in children under 5 years of age with a national incidence of 167.44‰. The Gbêkè health region with 241.89‰ recorded one of the highest incidences of ARI in children under 5 years old [12]. In the study conducted by N'chott et al. [11] in Côte d'Ivoire to identify bacteria involved in lower respiratory tract infection in adults, test results isolated bacteria such as *S. pneumoniae* (15.1%) and *H. influenzae* (36.4%), *L. pneumophlia* (4.8%) and M. catarrhalis (4.8%). *S. pneumoniae* remains the leading cause of acute community-acquired pneumonia and more rarely H. influenzae, although vaccination and reduced smoking have decreased its frequency [8]. At the present state of knowledge, very few data are available on the etiology of bacteria associated with ARI in central Côte d'Ivoire. The aim of this study was to identify the bacteria and risk factors associated with ARI in patients suffering from acute respiratory infection in the Bouake region.

MATERIAL AND METHODS

This was a cross-sectional study conducted from January 2018 to December 2020 in two health centers in the city of Bouaké: The University Hospital Center (CHU) of Bouaké and the Urban Health Center (CSU) of Air France 3. Bouaké is the second largest city in central Côte d'Ivoire at latitude 7°69 N and longitude 5°03 W. The city covers an area of 72km2 with a population of approximately 1,542,000. The study was part of the ANDEMEIA project (African Network for Improved Diagnosis, Epidemiology and Management of Common Infectious Agents), a multi-center research project that aimed to strengthen collaboration between German and African research institutions to address current health-related research questions.

Study Population

The study population consisted of outpatients or inpatients with an acute respiratory infection evolving for less than 10 days with fever (T ≥38°C) or history of fever, in one of the a forementioned health facilities. The duration of the patient's hospitalization should not exceed 24 hours.

Sampling

Venous blood samples were collected from each patient included in the study using EDTA tubes. Samples collected at the Air France 3 CSU were transported to the Bouaké University Hospital laboratory in coolers containing cold accumulators, where they were stored in freezers at -80°C until analysis.

Data Collection

Adult patients should give their consent to be included in the study. For other patients (children and minors), the agreement of the parent or guardian was required as well as the assent of the patient. A unique identification code was used to link the sample and laboratory result to the participant. A standardized questionnaire was used to collect clinical and sociodemographic data as well as the following factors associated with ARI: place of residence, sex, age, smoking, and exposure to pets.

Laboratory Analysis

Extraction of bacterial genomes: Total nucleic acid extraction was performed from 200µL of venous blood samples using the Indispin Pathogen Mini kit (Reference: Sp 54106, Germany) according to the manufacturer's protocol. To validate the quality of the extraction an internal extraction control was included. Elution was performed with 60µL of AVE elution buffer. The obtained nucleic acid extracts were directly used for molecular analysis.

Gene amplification: The bacterial genomes were amplified by multiplex qRT-PCR using the FTD PneumoCap kit (Reference: FTD-29.1-34) according to the manufacturer's recommendations. This kit targets specific regions of the genomes of bacteria responsible for acute respiratory infections, including *Streptococcus pneumoniae, Staphylococcus aureus, Haemophillis influenzae, Moraxella catarhalis, Mycoplasma pneumoniae, Legionella pneumophilla*. Amplification was performed in an ABI® 7500 FAST thermal cycler according to the amplification program used by Safiatou et al. [13].

Statistical Analysis of Data

Clinical, demographic and microbiological data of the patients were entered into a database (Voozanoo). The data were then exported into an Excel spreadsheet and analyzed using STATA version 15 software. A descriptive analysis was performed to describe the sociodemographic and clinical characteristics of the patients. The Chi-square test was used to investigate the association between sociodemographic data and the identified pathogens. Univariate and multivariate analyses were used to identify risk factors associated with ARI. The threshold of significance was set for a value of $P<0.05$.

RESULTS

Sociodemographic and Clinical Characteristics of Included Patients

A total of 870 patients were included in this study. Male patients were in the majority (54.94%) with a M/F ratio =1.22. The mean age of the patients was 2 years with extremes of 0 months and 99 years. Thirty-five percent (35%) of the participants were less than 2 years old and 15% were over 55 years old. More than 83% of the patients resided in the city of Bouaké. Fever and cough were the main clinical signs in the patients, accompanied or not by dyspnea, sputum, and chest pain (Table 1).

Table 1: Socio-demographic and clinical data of patients.

Variables	Participants (N=870)	Percentage (%)
Gender		
Male	478	54.94
Female	392	45.06
Total	870	100
Residence		
Village	147	16.9
City	723	83.1
Age (year)		
0-2	307	35.29
3-5	76	8.74
6-15	80	9.2
16-25	84	9.66
26-35	87	10
36-45	53	6.09
46-55	55	6.32
> 55	128	14.71
Toux (T >38°C)	349	40.11
Cough	808	92.87
Cough +Expectoration	544	62.52
Cough + Dyspnea	436	50.11
Cough +Expectoration + Dyspnea	263	30.22
Cough +Expectoration + Douleur Thoraxique	270	31.03

Bacteria detected by multiplex PCR

Of a total of 870 blood samples tested, 116 were positive for at least one bacterium, a detection rate of 13.33%. Among the 7 bacteria tested with the FTD pneumoCap kit, 6 were detected: *Streptococcus pneumoniae* (7.58%), *Staphylococcus aureus* (4.13%), *Haemophillis influenzae* (2.41%), *Moraxella catarhalis* (2.18%), *Mycoplasma pneumoniae* (0.02%), *Legionella pneumophilla* (0.01%). Only one case of *L. pneumophilla* was detected (Table 2).

Table 2: Prevalence of bacteria isolated from patients' blood.

Type of Bacteria	Number of Bacteria (N)	Frequency (%)
Streptococcus pneumoniae	66	7.59
Staphylococcus aureus	36	4.14
Haemophillis influenzae	21	2.41
Moraxella catarhalis	19	2.18
Mycoplasma pneumoniae	2	0.23
Legionella pneumophilla	1	0.11
Chlamydia pneumoniae	0	0

Distribution of Detected Bacteria According to Patient Characteristics

In general, infection was more predominant in male patients (14.64%) and in those residing in the city of Bouaké. The highest positivity rates were observed in patients under 2 years of age with 15.64% (48/307) followed by those over 55 years of age with 15.63% (20/128). The distribution of the different bacteria identified according to sex showed a predominance of *S. pneumoniae* (60.61%) and *H. influanzae* (66.67%) in male patients. While *M. catarhalis* was more identified in female patients. These differences were not statistically significant. *S. pneumoniae* and *M. catarhalis* were more detected in patients residing in the city of Bouaké, with statistically significant differences ($p<0.05$). S. pneumoniae was detected in 46.97% of patients aged 0-2 years with a statistically significant difference ($p=0.04$). For the other bacteria, positivity was also higher in patients aged 0-2 years, but these differences were not significant ($p>0.05$) (Table 3).

Table 3: Distribution of bacteria detected according to patient characteristics.

Variables	S. pn (n=66)	P	S. aureus (n=36)	P	H. inf. (n=21)	P	Morax. cat. (n=19)	P	Myco. Pn (n=2)	P	L. pn (n=1)	P
Gender												
Female	26 (39.39%)		18 (50.00%)		7 (33.33%)		12 (63.16%)		1 (50.00%)		0	
Male	40 (60.61%)	0.33	18 (50.00%)	0.54	14 (66.67%)	0.27	7 (36.84%)	0.46	1 (50.00%)	0.88	1 (100%)	0.36
Residence												
Village	18 (27.27%)		7 (19.44%)		5 (23.81%)		7 (36.84%)		0		0	
City	48 (72.73%)	0.01	29 (80.56%)	0.67	16 (76.19%)	0.39	12 (63.16%)	0.01	2 (100%)	0.52	1(100%)	0.65
Age (year)												
0 - 2	31 (46.97%)		19 (52.78%)		9 (42.86%)		9 (47.37%)		0		1 (100.00%)	
5-Mar	3 (4.55%)		2 (5.56%)		2 (9.52%)		0		0		0	
15-Jun	4 (6.06%)		1 (2.78%)		2 (9.52%)		1 (5.26%)		1 (50.00%)		0	
16 - 25	4 (6.06%)	0.04	1 (2.78%)	0.07	3 (14.29%)	0.87	4 (21.05%)	0.13	0	0.67	0	0.96
26 - 35	4 (6.06%)		4 (11.11%)		0		1 (5.26 %)		1 (50.00%)		0	
36 - 45	0		2 (5.56%)		1 (4.76%)		0		0		0	
46 - 55	5 (7.58 %)		5 (13.89 %)		1 (4.76%)		3 (15.79 %)		0		0	
> 55	15 (22.73%)		2 (5.56 %)		3 (14.29 %)		1 (5.26%)		0		0	

Types of Infection in Patients

The mixed detection rate was 4.25% (37/870), with 31 cases of double infection and 6 cases of triple infection. These co-infections were dominated by the association *M. catarhalis + H. influenzae* (21.62%), *S. pneumoniae + H. influenzae* (16.21%) and *S. pneumoniae + M. catarhalis* (16.21%). Triple infections were dominated by the combination of *S. pneumoniae + M. catarhalis + H. influenzae* (8.10%). Co-infections were more frequently detected in patients residing in Bouaké (58.13%) and in patients aged 0 to 2 years (81.39%) (Table 4).

Table 4: Distribution of mixed infections.

Type of infection	Bacteria	Number (N=37)
Double Infection		
	S. aureus + S. pneumoniae	2 (5.40%)
	S. aureus + H. influenzae	5 (13.51%)
	S. pneumonia + Myco. Pneumoniae	1 (2.70%)
	S. pneumoniae + H. influenzae	6 (16.21%)
	H. inflenzae + Myco. Pneumoniae	1 (2.70%)
	M. catarhalis + H. influenzae	8 (21.62%)
	S. pneumoniae + M. catarhalis	6 (16.21%)
	S. aureus + M. catarhalis	2 (5.40%)
Triple Infection		
	S. aureus + S. pneumoniae + H. influenzae	1 (2.70%)
	S. pneumoniae + H. influenzae + M. pneumoniae	1 (2.70%)
	S. aureus + S. pneumoniae + M. catarhalis	1 (2.70%)
	S. pneumoniae + M. catarhalis + H. influenzae	3 (8.10%)

Factors Associated With The Detection of Bacteria

The results of this study showed that patients over 55 years of age were at greater risk of *S. aureus* infection (OR= 6.30; 95% CI 1.14-34.64). Exposure to pets also increased the risk of developing S. aureus infection (OR= 18.88; 95% CI 5.56-64.08) p <0.01). Patients who resided in villages had a lower risk of developing *M. catarhalis* infection, whereas smoking increased the risk of *M. catarhalis* infection 7-fold (OR= 6.94; 95% CI 2.51-19.23; p<0.01) (Table 5).

Table 5: Risk factors associated with the detection of bacteria.

Variable	*S. aureus* OR (95% IC)	Pv	*Morax. Catarrhalis* OR (95% IC)	Pv	*S. pneumoniae* OR (95% IC)	Pv
Gender						
Male	1		1		1	
Female	1.22(0.63 -2.39)	0.54	0.70 (0.27-1.81)	0.46	0.77 (0.46-1.29)	0.33
Residence						
Village	1		1		1	
City	0.83 (0.35-1.94)	0.67	0.33 (0.13- 0.87)	0.01	0.50 (0.28-0.90)	0.01
Age (year)						
0-2	4.15 (0.94-18.27)	0.04	3.83 (0.477-30.80)	0.17	0.84 (0.43-1.62)	0.61
5-Mar	1.70 (0.23-12.42)	0.59	0	0.44	0.30 (0.08-1.12)	0.05
15-Jun	0.79 (0.07-8.99)	0.85	1.60 (0.098-26.26)	0.73	0.39 (0.12-1.25)	0.1
16-25	0.75 (0.06-8.55)	0.82	6.35 (0.68-59.21)	0.06	0.37 (0.11-1.19)	0.08
26-35	3.03 (0.53-17.14)	0.18	1.47 (0.09-24.09)	0.78	0.36 (0.11-1.14)	0.07
36-45	2.47 (0.33-18.20)	0.35	_	0.51	_	0
46-55	6.30 (1.14-34.64)	0.01	7.32 (0.72-74.35)	0.04	0.75 (0.25-2.19)	0.6
> 55	1		1		1	
Smoking						
No	1		1		1	
Oui	1.26 (0.37- 4.24)	0.7	6.94 (2.51-19.23)	0	0.19 (0.02-1.45)	0.07
Domestics Animals						
No	1		1		1	
Yes	18.88 (5.56-64.08)	0	6.06 (1.97-18.61)	0	–	0

DISCUSSION

This study, which used multiplex PCR to detect bacteria and risk factors associated with ARI from venous blood samples of patients, is one of the first studies conducted in the Bouake region. The overall identification rate of bacteria was 13.33% (116/870). This rate is lower than that obtained by N'chott et al. [11] in a study conducted in Abidjan to identify bacteria involved in lower respiratory tract infection in adults. These authors obtained an overall bacterial positivity rate of 63.6% after analysis of 33 bronchoalveolar lavage fluid samples. Serin et al. [14] also reported a 62% bacterial positivity rate in the United States after multiplex PCR analysis of deep tracheal aspirate samples from 50 patients hospitalized with pneumonia. These differences in positivity results could be explained by differences in sample size and types of samples tested. Higher bacterial positivity rates of 99% were obtained in hospitalized children with severe respiratory infection in Lusaka, Zambia [15].

In the present study, the positivity rates of the bacteria identified in the patients' blood (*S. pneumoniae* (7.59%), *S. aureus* (4.14%), *H. influenzae* (2.41%), *M. catarhalis* (2.18%), *Myco pneumoniae* (0.23%), *L. pneumophilla* (0.11%)) were lower than those obtained in the study of N'chott et al. [11] which were: *S. pneumoniae* (14.3%); *H. influenzae* (42.8%); *M. catarrhalis* (4.8%). In another study conducted in Senegal in children under 5 years of age, Assane et al. [16] reported the following detection rates: *S. pneumoniae* (17%), *M. catarrhalis* (15.43%), *H. influenzae* (8.02%). These differences with the results of this study could be explained by differences in study methodology, study population, type of specimens tested and method of specimen analysis. However, the high positivity rates of *S. pneumoniae* and *H. influenzae* in this study are in agreement with the results of Lodre et al. [17].

For the majority of the bacteria identified in this study, overall detention rates were higher in male patients. These results are consistent with data in the literature showing a predominance of pneumonia cases in males [18]. The results of this study showed a difference in bacterial detection by age group. Children aged 0 to 2 years were more likely to develop ARI. *S. pneumoniae, S. aureus, H. influenzae, M. catarhalis* were more identified in patients aged 0 to 2 years with respective rates of 46.97%, 52.78%, 42.86% and 47.37%. The differences observed were statistically significant for *S. pneumoniae* (p=0.04). These results could be explained, on the one hand, by the fact that the study population consisted mostly of children aged 0-2 years and, on the other hand, by the immaturity of the immune defenses, and the relatively high permeability of the mucosa of the gastrointestinal tract in children [19].

These main pathogens detected are responsible for bacterial meningitis and represent a major public health problem in developing countries where vaccination coverage is still low [20]. The last 20 years have been marked by the emergence and spread of antibiotic resistance and often multidrug resistance in many

countries. Two main pathogenic species are involved in community-acquired respiratory tract infections: Streptococcus pneumoniae and Haemophilus influenzae [21]. The study conducted by Touré et al. in central Côte d'Ivoire on the epidemiology of pediatric meningitis reported that *S. pneumoniae* was the main bacterium causing meningitis in children with a detection rate of 48.4%. Bacteria such as *H. influenzae* and *S. aureus* were detected at low rates of 3.2% each [22]. In another study conducted in Abidjan from 2010 to 2016 to determine the etiology of bacterial meningitis, Boni-Cisse et al. [23] reported that 69.5% of bacterial meningitis cases were caused by *S. pneumoniae* with 71.4% of meningitis cases in children 0-11 months of age, 70.6% in children 12-23 months of age and 66.7% in children 24-59 months of age. The national pneumococcal PCV-13 vaccine coverage rate was 89%, 84%, and 82% for pneumo 1, pneumo 2, and pneumo 3, respectively [24], However, despite this national immunization coverage, cases of *S. pneumoniae* bacterial meningitis remain high, as reported in other studies. This raises questions about the *S. pneumoniae* serotypes circulating in the community and the susceptibility of bacterial strains to antibiotics. In this study, serotyping was not carried out, nor was the sensitivity of the strains to antibiotics, notably related to the analysis method based on the multiplex PCR technique.

Co-infections were dominated by the association *M. catarhalis + H. influenzae* (21.62%), *S. pneumoniae + H. influenzae* (16.21%) and *S. pneumoniae + M. catarhalis* (16.21%). These co-infections were more detected in patients aged 0 to 2 years (81.39%). This could be explained by the immaturity of children's immune defenses [25]. The study of factors associated with ARI revealed that the age group 46-55 years (OR=6.30; 95% CI: 1.14-34.64; p=0.01) and exposure to pets (OR=18.88: 95% CI: 5.56-64.08; p<0.05) are risk factors for *S. aureus* infection. Smoking was a risk factor for *M. catarhalis* infection (OR=6.94 95% CI: 2.51-19.23; p=0.01). In a similar study, Coronel et al. [26] reported that exposure to tobacco smoke, age less than 1 year, and presence of pets were the factors associated with ARI in children aged 0-5 years. Other authors have found that the main risk factors associated with AKI in children were maternal literacy, smoking and nutritional status of children, male gender [27-29]. These differences with our results could be explained by differences in the study methodology.

CONCLUSION

The present study allowed the identification of bacteria and factors associated with acute respiratory infections in central Côte d'Ivoire using molecular biology techniques. *S. pneumoniae* was the main bacterium identified in the patients followed by *S. aureus* and *H. influenzae*. Children aged 0 to 2 years were more likely to develop bacterial ARI. Risk factors associated with bacterial detection were smoking, age range 0-2 years, and exposure to pets. It would be useful to continue this study by characterizing *H. influenzae* strains, as well as serotyping *S. pneumoniae* in order to identify the strains circulating in Bouaké and to take them into account when choosing the type of pneumococcal vaccine.

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ETHICAL CONSIDERATIONS

The National Life Sciences and Health Ethics Committee, No. 105/MSHP/CNER-dk dated 10/21/2016, approved the study protocol. The participant or accompanying parent signed a written consent after reading the information note.

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