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Resistance profile of bacteria isolated from Broncho alveolar lavage fluid (BALF) of patients with severe acute lung disease at the University Hospital Center of Cocody in Abidjan, Côte d'Ivoire

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This study aimed to characterize the microorganisms responsible for severe acute respiratory infections in adult patients hospitalized at the University Hospital Center of Cocody in Abidjan. The biological material consisted of bronchoalveolar lavage fluid (BALF). Isolation and identification of bacterial strains, along with susceptibility testing, were performed using standard microbiological techniques. The bacteria identified included three strains of Klebsiella pneumoniae (75%) and one strain of Streptococcus pneumoniae (25%). Phenotypic analysis of the isolated strains revealed a β-lactam resistance phenotype, specifically the production of extended-spectrum β-lactamases (ESBL) in K. pneumoniae (66.7%), and a resistance phenotype to macrolides, lincosamides, and streptogramin B (MLSb). The resistance rate of K. pneumoniae strains to ceftriaxone, cefepime, cefotaxime, nalidixic acid, norfloxacin, levofloxacin, gentamicin, and tobramycin was 66.7%. The S. pneumoniae strain showed resistance to norfloxacin, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, clindamycin, and erythromycin. This study facilitated the isolation of two different bacterial strains responsible for pneumonia in adult patients.

Key words: Pneumopathy, BALF, bacteria, resistance, Abidjan.

INTRODUCTION

Acute respiratory infections (ARI) are common illnesses, leading to millions of emergency visits or hospitalizations each year. According to data published by the World Health Organization (WHO) in 2019, ARIs were one of

the leading causes of morbidity and mortality, representing a significant global public health concern (Hong et al., 2023). ARI can be disaggregated into lower respiratory infections and upper respiratory infections, depending on whether the infection's primary location is below the larynx (lower respiratory infection) or above it (upper respiratory infection) (Ross et al., 2023). Pneumopathy, also known as lower respiratory infections, is a disease of the lung parenchyma distal to the terminal bronchioles, caused by infections with bacteria, viruses, fungi, and, less frequently, parasites (Peto et al., 2014).

Although the diverse clinical and radiological presentations reflect a variety of responsible microorganisms for pneumonia, a clear etiology is essential for clinical diagnosis and treatment (Wang, 2023). Indeed, the identification of a causative pathogen not only aids in the diagnosis and classification of pneumonia but also guides antimicrobial therapy and infection control measures (Lim, 2022). In Côte d'Ivoire, the death rate associated with pneumonia, and particularly SARIs, has risen from 32.5 to 36% (Horo et al., 2012). Only data on tuberculosis are available as a cause of severe acute respiratory infections. Few data on the microbial etiologies of SARI have been addressed. The aim of this study was to characterize microorganisms responsible for severe acute respiratory infections in pneumonia patients in Abidjan, Côte d'Ivoire.

MATERIALS AND METHODS

Study framework

The samples were only taken in the pneumology department of University Hospital Center of Cocody from February 1, 2016, to October 31, 2017, because the pneumology department at Treichville University Hospital Center does not have a fiberscope for obtaining bronchoalveolar lavage fluid, and Yopougon University Hospital Center was closed for renovations. Laboratories of the Clinical Bacteriology Unit and the Antibiotics, Natural Substances, and Anti-Infectious Resistance Monitoring Unit at the Pasteur Institute of Côte d'Ivoire in Cocody were used for the various analyses.

Data collection and analysis

Data were collected on a pre-established survey form and processed using Microsoft Office Word 2010. Variables were entered and analyzed using STATA software version 15.0. The statistical test utilized was the Chi-square test. The significance threshold was set at the standard deviation test with an alpha threshold of 0.05.

Ethical considerations

Written informed consent was obtained from all patients prior to their inclusion in the study, ensuring anonymity through the use of anonymity numbers during data analysis. Data collection was conducted with the agreement of the Pneumophtisiology Department of University Hospital Center of Cocody. The study received approval from the National Ethics and Research Committee under reference No. 31/MSLS/CNER-dkn on June 23, 2015.

Biological material

The biological material consisted of bronchoalveolar lavage fluid.

Obtaining bronchoalveolar lavage fluid

When patients were free of tuberculosis and in good health, they underwent bronchial fibroscopy to obtain bronchoalveolar lavage fluid (BALF), an invasive sampling procedure performed under an endoscope. This process involves injecting and then aspirating 4 to 6 times a 50 ml volume of saline through a fiberscope placed in a sub-segmental bronchus. It offers the advantage of exploring a vast area of the lung, including distal bronchioles and up to 100 million alveoli. The BALF collected in a sterile single-use container was immediately sent to the Clinical Bacteriology Unit of the Pasteur Institute of Côte d'Ivoire at the Cocody site for microbiological analysis.

Identification of bacteria

A microscopic examination was performed on the bronchoalveolar lavage fluid, followed by Gram staining to guide the choice of culture media for seeding. The identification of bacteria was carried out by analyzing their morphological and biochemical characteristics.

Antibiotic susceptibility testing

The production of extended-spectrum β-lactamases was confirmed by the synergy test between amoxcillin-clavulanic acid, cefotaxime, cefepime, ceftriaxone and aztreonam (Jarlier et al., 1988). Susceptibility testing was performed using Mueller Hinton (MH) agar for non-demanding bacteria and MH agar with defibrinated fresh horse blood and added β-NAD (MH-F) (BioMérieux, Marcy l'Etoile, France) for demanding bacteria according to the disc diffusion method described by "Antibiogram Committee of the French Society of Microbiology (EUCAST/CA-SFM, 2016). A panel of antibiotic-impregnated discs (SirScan Discs, France) from different families were used for bacterial resistance determination in this study. The list of antibiotic discs used for *K. pneumoniae* and *S. pneumoniae* and the critical diameters (break points) were recorded in Tables 1 and 2. Reference strains *Escherichia coli* ATCC 25922 and *S. pneumoniae* ATCC 49619 were used for quality control.

RESULTS

Seasons of occurrence of pneumonia

The incidences of pneumonia were grouped according to the climatic seasons. Indeed, 33.4% of cases of

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Table 1. Antibiotic list for *Klebsiella pneumoniae* strains (CA-SFM, 2016).

Family	Antibiotics	Code	Charge of disc (µg)	Critical diameters	
				R<	S≥
Penicillin	Piperacillin	PIP	30	17	20
	Ticarcillin	TIC	75	23	23
	Ampicillin	AMP	10	14	14
	Mecillinam	MEC	10	15	15
Penicillins+ beta-lactamase inhibitor	Amoxicillin+ clavulanic acid	AMC	20	19	19
	Ticarcillin + clavulanic acid	TCC	75-10	23	23
Cephalosporins	Cefixime	CFM	5	17	17
	Cefoxitin	FOX	30	15	19
	Ceftriaxon	CRO	30	22	25
	Cefotaxim	CTX	5	17	20
	Cefepim	FEP	30	21	27
	Cefuroxim	CXM	30	19	19
Monobactam	Aztreonam	ATM	30	21	26
Carbapenem	Imipenem	IPM	10	16	22
Fluoroquinolones/Quinolones	Norfloxacin	NOR	10	19	22
	Nalidixic acid	NAL	30	14	19
Aminoglycosides	Gentamicin	GMN	10	14	17
	Tobramycin	TMN	10	14	17
Sulphonamides	Trimethoprim/Sulfamethoxazole	SXT	1.25-23.75	11	14
Phenicol	Chloramphenicol	CHL	30	17	17

Table 2. Antibiotic list for *Streptococcus pneumoniae* (CASFM, 2016).

Family	Antibiotics	Code	Charge of disc (µg)	Critical diameters	
				R<	S≥
Fluoroquinolones/Quinolones	Norfloxacin	NOR	10	12	12
	Ciprofloxacin	CIP	5	16	50
	Nalidixic acid	NAL	30	14	19
Sulphonamides	Trimethoprim/Sulfamethoxazole	SXT	1.25-23.75	15	18
Phenicol	Chloramphenicol	CHL	30	19	19
Glycopeptides	Vancomycin	VAN	5	13	13
Aminoglycosides	Gentamicin	GEN	500	17	17
Macrolides	Erythromycin	ERY	15	19	22
Lyncosamides/Synergistin	Clindamycin	CMN	2	19	19
	Pristinamycin	PTN	15	19	19

pneumonia occurred between the months of April to July, followed by 12.1% from August to September, then 24.2% from October to November, and finally 30.3% of cases from December to March.

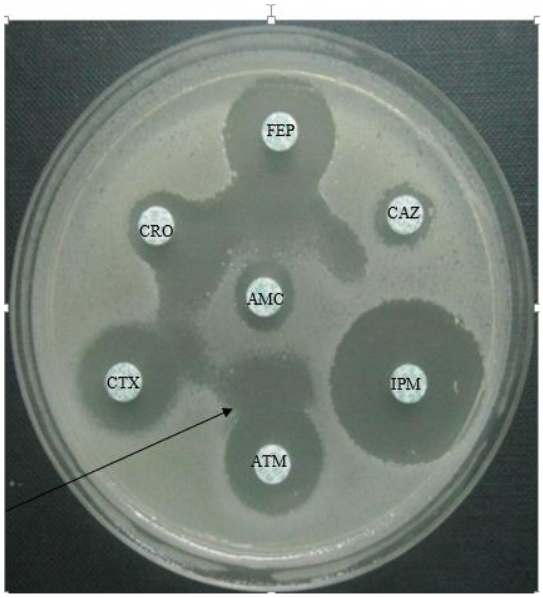

Figure 1. Detection of ESBL by champagne cork.

Bacteria identified

The bacteria identified were *K. pneumoniae* and *S. pneumoniae*. The most frequently isolated germ was *K. pneumoniae* (3 strains, a rate of 75%), followed by *S. pneumoniae* (one strain, a rate of 25%). Of the 3 *K. pneumoniae* strains isolated, 2 were from women (66.67%) and one from a man (33.33%). And *S. pneumoniae* strain was isolated from a woman. The M/F sex ratio was 0.5. The physiological criterion of sex does not appear to influence infections.

Antibiotic resistance of *Klebsiella pneumoniae* strains

All *K. pneumoniae* strains were tested with antibiotics belonging to the β-lactam, aminoglycoside, quinolone, phenol and sulfonamide families. Phenotypic analysis of the 3 *K. pneumoniae* strains isolated showed two β-lactam resistance phenotypes with production of extended-spectrum β-lactamases (66.7%) and a wild-type phenotype (low-level penicillinase). The production of extended-spectrum β-lactamases (ESBLs) in *K. pneumoniae* was demonstrated by the synergy (champagne cork) between amoxicillin/clavulanic acid, aztreonam (ATM), ceftriaxone (CRO), cefepime (FEP) and cefotaxime (CTX) disks (Figure 1).

Strain resistance to β-lactams was 66.7% to amoxicillin and clavulanic acid, piperacillin, ceftriaxone, cefepime, cefotaxime, cefuroxime and cefixime respectively. Only one strain was resistant to imipenem, a rate of 33.3%. Similarly, resistance to quinolones was also marked by a 66.7% resistance rate to nalidixic acid, norfloxacin and levofloxacin respectively. With regard to resistance to aminoglycosides, 66.7% of strains were resistant to gentamicin and tobramycin, and only one strain was resistant to amikacin, with a rate of 33.3%. Antibiogram results are shown in Table 3**.**

Resistance of *Streptococcus pneumoniae* to antibiotics

S. pneumoniae was resistant to the fluoroquinolone and quinolone antibiotics: norfloxacin, ciprofloxacin, and nalidixic acid. It is also resistant to trimethoprim-sulfamethoxazole, clindamycin, and erythromycin. It is a strain with Macrolide-lincosamide-streptogramin b (MLSb-type) resistance, that is, resistance to all Macrolides, Lincosamides, and Streptogramin b.

DISCUSSION

The aim of this study was to characterize the microorganisms responsible for severe acute respiratory infections in adult patients hospitalized at University Hospital Center of Cocody, Abidjan, Côte d'Ivoire. Identification of microorganisms seeded on culture media yielded a positivity rate of 12.1%, compared with a negativity rate of 87.9%. Of the four positive samples, three strains of *K. pneumoniae*, which are Gram-negative Bacillus enterobacteriaceae, and one strain of *S. pneumoniae*, Gram-positive Cocci, were isolated. Ahui's study of 302 respiratory samples taken showed a positivity rate of 42%. P*seudomonas aeruginosa* and *K. pneumoniae* were encountered in 26.4% of cases each, followed by *Echerichia coli* in 10.4% of cases (Ahui et al.,

Table 3. Susceptibility profile of *Klebsiella pneumoniae* to antibiotics.

Family	Antibiotics	Code	Resistance rate (%)
Penicillin	Ticarcillin	TIC	100
	Piperacillin	PIP	66.7
	Ampicillin	AMP	100
	Mecillinam	MEC	33.3
Penicillins+Beta lactamase inhibitor	Amoxicillin/Clavulanic acid	AMC	66.7
	Ticarcillin/Clavulanic acid	TCC	66.7
	Cefixime	CFM	66.7
Cephalosporins	Cefoxitin	FOX	33.3
	Cefriaxon	CRO	66.7
	Cefotaxim	CTX	66.7
	Cefepim	FEP	66.7
	Cefuroxim	CXM	66.7
Carbapenem	Imipenem	IPM	33.3
Monobactam	Aztreonam	ATM	66.7
Fluoroquinolones/Quinolones	Norfloxacin	NOR	66.7
	Levofloxacine	LVX	66.7
	Nalidixic acid	NAL	66.7
Aminoglycosides	Tobramycin	TBN	66.7
	Gentamicin	GMN	66.7
	Amikacin	AMK	33.3
Phenicol	Choramphenicol	CHL	66.7
Sulphonamides	Trimethoprim/Sulfamethoxazole	SXT	100

2024). Similarly, *K. pneumoniae* strains were the most frequently isolated in Coulibaly (2020) work. The negativity of the culture results in our study could be explained by the use of inadequate antibiotic therapy by patients before going to hospital or either a viral origin of the pneumopathy. Variability in the frequency of uropathogenic bacteria may be linked to regional environmental factors (climate) and the usual activities of local populations (hygiene) in the various studies carried out.

The distribution of *K. pneumoniae* according to sex showed that female subjects were more affected (n=2) by *K. pneumoniae* infections than male subjects (n=1). The M/F sex ratio was in favour of females at 0.5. These results differ from those of Gebre et al., 2021 according to their study which found that the bacterial pathogens (*K. pneumonia*, Pseudomonas spp, *E. coli*) were more predominant in male (64.0%) than female.-Regarding age, the age group most represented in our study was 30 to 39 years old, or 48.5% of cases. This result may be explained by the fact that acute pneumonia is particularly frequent and serious in elderly people, who are also often aggravated by other diseases (Coulibaly, 2020). Concerning the influence of environmental factors, a rate of 33.4% of hospitalized patients was hospitalized between April and July. Côte d'Ivoire has a four-season equatorial climate, with two rainy seasons alternating with two dry seasons. The major rainy season runs from April to July, the minor dry season from August to September, the minor rainy season from October to November and the major dry season from December to March (Fadika et al., 2019). This high rate of pneumonia cases at this time of year may be due to the fact that respiratory infections are sensitive to meteorological changes (cold and rainy seasons in southern countries, atmospheric water content) (Dembélé, 2020).

According to the analysis of antibiogram results, 66.7% of strains were resistant to the amoxicillin-clavulanic acid combination, and 3rd generation cephalosporins. This result is similar to that obtained in the study by (Ahui et al., 2024) where 51% were resistant to the amoxicillin-clavulanic acid combination and 66% were resistant to ceftriaxone.

Furthermore, the results showed that of the three *K. pneumoniae* strains isolated, two were extended-spectrum β-lactamase (ESBL) producers at 66.7%, followed by the wild-type at 33.3%. This result is similar to that obtained in the study by Bandić-Pavlović et al. (2020) where Four *K. pneumoniae* were ESBL positive. Indeed, the intensive and abusive use of antimicrobials in the treatment of infections caused by enterobacteria has led to the production of expanded spectrum beta-lactamase (Kot et al., 2023). For aminoglycosides, a resistance rate of 66.7% was observed for gentamicin, but much lower for amikacin at 33.3%. This rate of resistance to amikacin is worrying because this aminoglycoside is the first choice in the treatment of nosocomial infections with *K. pneumonia*e and especially *K. pneumoniae* producing extended-spectrum β-lactamases (Logre et al., 2020). On the other hand, only one strain of *S. pneumoniae* was isolated, with a detection rate of 25%.

The low detection rate of *S. pneumoniae* could be explained by the fact that *S. pneumoniae* is a fragile bacterium, and culture methods are time-consuming with relatively low sensitivity, particularly when antibiotics have been administered to the patient prior to sampling. Also, early and accurate diagnosis of pneumococcal pneumonia in healthcare settings remains challenging due to the limitations of conventional diagnostic methods (Yang et al., 2015). In this study, *S. pneumoniae* was sensitive to penicillin. Indeed, *S. pneumoniae* has remained uniformly susceptible to penicillin for over 25 years of use of this antibiotic. Increased use of antibiotics in various health sectors has caused the emergence of antibiotic resistant microorganisms worldwide (Pulingam et al., 2022). The *S. pneumoniae* strain was resistant to erythromycin. This result is in line with that reported by Sharew and colleagues in their work in Ethiopia (Sharew et al., 2021).

Conclusion

This study enabled the isolation of two bacteria responsible for pneumonia in adults, namely *K. pneumoniae* and *S. pneumoniae*. The predominant germ was *K. pneumoniae*, isolated from 2 females and 1 male. These strains were resistant to third-generation cephalosporins, fluoroquinolones and aminoglycosides. The *S. pneumoniae* strain had MLSB-type resistance, i.e. resistance to all Macrolides, Lincosamides and Streptogramine B. The limitations of this work lie in the study framework. The study was carried out in a single university hospital, as the pneumology department at Treichville university hospital center does not have a fiberscope for obtaining bronchoalveolar lavage fluid, and Yopougon university hospital center was closed for renovations. This situation implies that results cannot be generalized to the entire population of Abidjan, as patients may have turned to clinics or community centers, resulting in a low sample rate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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