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Original Article

Atypical Bacterial Agents in Pleural Effusions in Bamako: Bacteriology and Antibiotic Resistance Profile

Agents Bactériens Atypiques dans les Épanchements Pleuraux en Milieu Hospitalier À Bamako : Profils Bactériologiques et de Résistance aux Antibiotiques

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ABSTRACT

Background. The etiology of pleural infections differs significantly between community-acquired and hospital-acquired cases, and involves a range of bacterial pathogens, including atypical variants. In this study, we present the first report on the isolation of atypical pathogens in pleural infections in Mali, aiming to improve our understanding of their clinical implications. **Methods.** Pleural fluid samples were collected from patients with pleural infection. Conventional culture method for microbial identification was combined with antimicrobial susceptibility testing following EUCAST guidelines. **Results.** Among the 244 examined patients, atypical bacteria were found in 11 (4.5%) cases. The identified pathogens included: *Burkholderia cepacia* (n=1), *Chromobacterium violaceum* (n=1), *Aeromonas veronii* (n=2), *Aeromonas hydrophila* (n=1), *Achromobacter species* (n=1), *Cedecea davisae* (n=1), *Cedecea neteri* (n=1), *Cedecea lapagei* (n=1), *Moellerella wisconsensis* (n=1), *Kluyvera ascorbata* (n=1). The resistance rate to amoxicillin-clavulanate was 90.9%, to ciprofloxacin was 100%, and to gentamicin was 90.9%. Furthermore, 27.3% (n=3) of the isolates were carbapenemase producers. **Conclusion.** This study reveals that the atypical pathogens involved in pleural infections at the study center exhibit alarming resistance to empirical antibiotics. Therefore, further in-depth studies, coupled with on-the-field training on standardized diagnostic and management methods, are necessary to optimize detection, prevent therapeutic approach variability, and improve patient outcomes.

RÉSUMÉ

Introduction. L'étiologie des infections pleurales diffère entre les cas acquis en communauté et les cas acquis à l'hôpital, et implique une gamme de pathogènes bactériens, y compris des variantes atypiques. Nous présentons le premier rapport sur l'isolement de pathogènes atypiques dans les infections pleurales au Mali. **Méthodes**. Des échantillons de liquide pleural ont été collectés chez des patients atteints d'infection pleurale. La méthode de culture conventionnelle pour l'identification microbienne a été combinée à un test de sensibilité aux antimicrobiens selon les directives EUCAST. **Résultats.** Parmi les 244 patients examinés, des bactéries atypiques ont été trouvées dans 11 (4,5%) cas. Les agents pathogènes identifiés comprenaient : Burkholderia cepacia (n=1), Chromobacterium violaceum (n=1), Aeromonas veronii (n=2), Aeromonas hydrophila (n=1), Achromobacter species (n=1), Cedecea davisae (n=1), Cedecea neteri (n=1), Cedecea lapagei (n=1), Moellerella wisconsensis (n=1), Kluyvera ascorbata (n=1). Le taux de résistance à l'amoxicilline-clavulanate était de 90,9%, à la ciprofloxacine de 100% et à la gentamicine de 90,9%. En outre, 27,3% (n=3) des isolats étaient des producteurs de carbapénèmases.
Conclusion. Les pathogènes atypiques impliqués dans les infections pleurales au centre d'étude présentent une résistance alarmante aux antibiotiques empiriques. Par conséquent, des études plus approfondies, couplées à une formation sur le terrain aux méthodes de diagnostic et de prise en charge standardisées, sont nécessaires pour optimiser la détection, prévenir la variabilité de l'approche thérapeutique et améliorer les résultats des patients.

STUDY HIGHLIGHTS

What is already known
The aetiologies of infectious pleural infections (IPE) differ significantly between community-acquired and hospital-acquired cases.

What question this study addressed
Prevalence of atypical bacterial in patients with IPE in Mali, the extent of antimicrobial resistance

What this study adds to our knowledge
Atypical pathogens involved in pleural infections at the exhibit alarming resistance to empirical antibiotics. 27.3% (n=3) of the isolates were carbapenemase producers.

How this is relevant to practice, policy or further research
Monitoring these pathogens as possible threat to healthcare systems is of paramount importance.

INTRODUCTION

Pleural effusions are an important clinical entity with a wide variety of etiologies. The development of the condition depends largely on secondary complications of underlying pleural or pulmonary diseases, malignant tumours or tuberculosis (1,2). Here, the term infectious pleural effusion (IPE) is used to define all clinical entities resulting from the accumulation of fluid or pus in the pleural space, subsequent to bacterial penetration and replication (3,4).

While conventional bacterial pathogens have been well-documented in IPEs, emerging evidence highlights the presence of atypical pathogens as a significant contributor to this clinical entity (5). Those pathogens encompass a diverse group of bacteria, including both rare members of the Enterobacteriaceae family, such as *Cedecea davisae*, *Cedecea neteri*, etc., as well as non-Enterobacteriaceae species (5,6). Infrequent yet recently increasing, infections caused by *Cedecea* spp have been reported, especially in critically ill and immunocompromised individuals (7). These ubiquitous and opportunistic pathogens may become the next growing public health concern worldwide.

Though IPEs is treatable, antimicrobial resistance (AMR) exacerbates the risks of clinical failure and mortality. In a robust systematic review, *Wang et al*. estimated *Mycoplasma*, *Legionella* and *Chlamydia species* to be responsible of 8.1% of cases of severe pneumonia, with mortality as high as 33% in some instances (8). Moreover, recent studies have documented the production of extended-spectrum β-lactamase (ESBL) enzyme, as well as several other resistance to broad spectrum antibiotics (9–11).

However, relatively little research has been carried out on those pathogens in our region, and even less on the specific pathogens discussed in this article despite recent epidemiologic data suggestive of an increasing incidence of pleural infections (3,12). Understanding the role and clinical implications of atypical pathogens in IPEs is crucial for optimizing diagnostic strategies, tailoring antimicrobial therapy, and improving patient outcomes.

The aim of this study was therefore to present the first report documenting the isolation and prevalence of atypical bacterial in patients (mostly immunocompetent) with IPE in Mali, and to determine the extent of antimicrobial resistance.

MATERIALS AND METHODS

Study Design and Population

The present research is a prospective cross-sectional study conducted between October 2021 and December 2022 in the Thoracic Surgery and Pediatrics departments of the "Hôpital du Mali" University Hospital in Bamako.

The inclusion criteria were as follows: 1) clinical diagnosis of pleural infection with subsequent diagnostic thoracentesis and microbiological confirmation (positive pleural fluid culture for at least one microorganism). Pleural infection was defined as the presence of a positive pleural fluid culture and/or purulent pleural fluid, clinically manifested by a complex parapneumonic effusion (CPPE) or empyema (13) ; 2) patients who provided written and informed consent to participate in the study.

The non-inclusion criteria were as follow: 1) patients whose pleural fluid was non-purulent or showed no growth; 2) patients with non-infectious causes of pleural effusions such as malignant tumor or congestive heart failure.

Microbiological treatment and pathogen identification

All pleural fluid samples were processed within one hour of collection in the microbiology laboratory of the "Hôpital du Mali" University Hospital. The samples were inoculated into brain-heart infusion (BHI) broth and anaerobic blood culture bottles and incubated for 18 to 24 hours at 35°C±2. From these broth cultures, fresh blood agar, enriched chocolate agar, and Sabouraud agar were streaked. Colonies were characterized and identified using Gram staining, biochemical tests, and the automated Phoenix M50 system (panel 449044-NMIC/ID-435).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the automated Phoenix M50 system (panel 449044-NMIC/ID-435) and supplemented with disk diffusion technique for antibiotics not covered by the automated panel. The results were interpreted as susceptible or resistant according to previously described guidelines (15).

ESBL production was detected using the combination disk test method with the following combinations: Ceftazidime-Clavulanate, Cefepime-Clavulanate, and Cefotaxime-Clavulanate (15). *K. pneumoniae* ATCC 700603 strain was used as a quality control strain.

Ethical Approval

Written consent was obtained from all participating individuals, and the study protocol was submitted for review and approval by the Ethics Committee of the University of Sciences, Techniques and Technologies of Bamako. Approval was granted under reference number 2021/228/USTTB on June 9, 2021.

RESULTS

Sociodemographic Characteristics of Patients

During the study period, a total of 6,096 patients were hospitalized. The study specifically analyzed pleural fluid samples obtained from 526 patients with pleural effusion. Bacterial infection was detected in 244 patients, including 11 cases (4.5%) caused by atypical bacteria (Table 1). Among these cases, seven (63.6%) patients were male, and four (36.4%) were female, resulting in a male-to-female sex ratio of 1.8.

Table 1: Characteristics and distribution of patients in thoracic surgery and pediatrics departments.

	Thoracic surgery	Paediatrics	Total
Characteristics	n= 9 (%)	n= 2 (%)	n= 11 (%)
Gender			
Male gender	6 (66,7)	1 (50)	7 (63,6)
Female gender	3 (33,3)	1 (50)	4 (36,4)
Age group			
(1-10)	/	2 (18,2)	
(20-30)	1 (9,1)	/	
(35-50)	3 (27,3)	/	
(51-60)	3 (27,3)	/	
(61-70)	2 (18,2)	/	

Many patients (7/11, 63.6%) resided in urban areas. The children aged 1 to 10 years accounted for 2 out of 11 cases (18.2%), while adults (aged 35 to 50 years) represented 3 out of 11 cases (27.3%), highlighting the vulnerability of these age groups to the disease.

Bacterial diversity and antibiotic resistance profile

The atypical bacteria isolated in this study were *Burkhorderia cepacea* (n=1), *Chromobacterium violaceum* (n=1), *Aeromonas veronii* (n=2), *Aeromonas hydrophila* (n=1), *Achromobacter species* (n=1), *Cedecea davisae* (n=1), *Cedecea neteri* (n=1), *Cedecea lapagei* (n=1), *Moellerella wisconsensis* (n=1), and *Kluyvera ascorbata* (n=1). The antibiotic resistance profile of the enterobacterial isolates *Cedecea davisae*, *Cedecea neteri*, *Cedecea lapagei*, *Moellerella wisconsensis*, and *Kluyvera ascorbata* was evaluated against multiple antibiotics (Table 2) for enterobacteria and Table 3 for non-enterobacteria. The results showed that β-lactams, with the exception of carbapenems, had no activity against the three species of Enterobacteriaceae. Amikacin and carbapenems showed moderate activity against all three types of isolate, with sensitivity rates ranging from 33.3% to 100%.

Table 2: Antibiotic resistance profile of *Cedecea spp*, *Moellerella wisconsensis*, and *Kluyvera ascorbata*.

Antibiotics	***Cedecea spp***		***Moellerella wisconsensis***		***Kluyvera ascorbata***	
	S%	R%	S%	R%	S%	R%
Amoxicillin	0	3 (100)	0	1 (100)	0	1 (100)
Amoxi+ clavulanic acid	0	3 (100)	0	1 (100)	0	1 (100)
Piperacillin	0	3 (100)	0	1 (100)	0	1 (100)
Piperacillin+tazobactam	2 (66,7)	1 (33,3)	0	1 (100)	0	1 (100)
Ticarcillin	0	3 (100)	0	1 (100)	0	1 (100)
Aztreonam	1 (33,3)	2 (66,7)	0	1 (100)	0	1 (100)
C2G	0	3 (100)	0	1 (100)	0	1 (100)
C3G	0	3 (100)	0	1 (100)	0	1 (100)
Cefoxitin	0	3 (100)	0	1 (100)	0	1 (100)
Ertapenem	2 (66,7)	1 (33,3)	0	1 (100)	0	1 (100)
Imipenem	2 (66,7)	1 (33,3)	0	1 (100)	0	1 (100)
Amikacin	1 (33,3)	2 (66,7)	1 (100)	0	1 (100)	0
Gentamicin	0	3 (100)	0	1 (100)	0	1 (100)
Tobramycin	0	3 (100)	0	1 (100)	0	1 (100)
Ciprofloxacin	0	3 (100)	0	1 (100)	0	1 (100)
Triméthoprim + sulfaméthoxazol	1 (33,3)	2 (66,7)	0	1 (100)	0	1 (100)

Table 3: Antibiotic resistance profile of *Aeromonas spp*, *Chromobacterium violaceum*, and *Burkholderia cepacia*.

Antibiotics	***Aeromonas sp***		***Chromobacterium violaceum***		***Burkholderia cepacea***	
	S%	R%	S%	R%	S%	R%
Amoxicillin	0	3 (100)	0	1 (100)	0	1 (100)
Amoxi+ clavulanic acid	0	3 (100)	0	1 (100)	0	1 (100)
Piperacillin	0	3 (100)	0	1 (100)	0	1 (100)
Piperacillin+tazobactam	2 (66,7)	1 (33,3)	0	1 (100)	0	1 (100)
Ticarcillin	0	3 (100)	0	1 (100)	0	1 (100)
Aztreonam	1 (33,3)	2 (66,7)	0	1 (100)	1 (100)	0
Fosfomycine	2 (66,7)	1 (33,3)	0	1 (100)	1 (100)	0
Tigecyline	1 (33,3)	2 (66,7)	0	1 (100)	1 (100)	0
Cefoxitin	0	3 (100)	0	1 (100)	1 (100)	0
Ertapenem	3 (100)	0	0	1 (100)	1 (100)	0
Imipenem	3 (100))	0	0	1 (100)	1 (100)	0
Amikacin	1 (33,3)	2 (66,7)	1 (100)	0	1 (100)	0
Gentamicin	0	3 (100)	0	1 (100)	0	1 (100)
Tobramycin	0	3 (100)	0	1 (100)	1 (100)	0
Ciprofloxacin	1 (33,3)	2 (66,7)	0	1 (100)	0	1 (100)
Triméthoprim + sulfaméthoxazol	1 (33,3)	2 (66,7)	0	1 (100)	0	1 (100)

On the other hand, all carbapenems showed high activity against the isolates except for 3 (27.3%). In terms of effectiveness, the combination of penicillin with beta-lactamase inhibitors (pipercillin/tazobactam) showed higher sensitivity rates for *Aeromonas* and *Cedecea species*, while trimethoprim/sulfamethoxazole had low activity against all three types of isolates. Two isolates among the enterobacteria (*Cedecea neteri* and *Kluyvera ascorbata*) were carbapenemase producers (Table II), while one isolate (*Chromobacterium violaceum*) among the non-enterobacteria showed this characteristic. Fosfomycin was active in 66.7% of *Aeromonas* isolates and 100% of *Burkholderia cepacea* isolates (Table III). None of the isolates were found to be ESBL producers.

It is worth noting that a significant proportion of patients, 100% to be exact, had undergone at least one antibiotic treatment prior to the sampling procedure. Furthermore, 100% (11/11) of the isolates exhibited multidrug resistance (MDR) profiles, in accordance with the guidelines established by *Magiorakos et al.* (14).

Study limitations

We did not investigate virulence factors, nor the molecular resistance of isolates. We also don't know whether the bacteria isolated are from healthcare-associated infections.

DISCUSSION

From *Cedecea spp*, our investigation on pleural fluid samples yielded three species acknowledged for theri clinical relevance. In 2013, the first reported case of pneumonia caused by *C.lapagei* in America was documented by Lopez et al. (15). Many recent studies have also described *Cedecea spp* as cause of various in factions in blood stream, urinary tract as well as lung (9,10,16–18). Moreover, prior studies have noted the important occurrence of *Cedecea spp* in chronically ill and immunocompromised patients (19). The current study found none of this although the median age of our study cohort was 34.4. This finding, somehow unanticipated provide some support for the conceptual premise of a surveillance program aimed at these pathogens, especially since a genomic study underscored common antimicrobial resistance features with other enterobacteria and the possible emergence of acquired resistance (19).

In 2022, a case report from Cyprus, followed by a systematic review reported the uncommon features of *M.wisconensis* in human infection. The report found the bacterium to be susceptible to piperacillin/tazobactam combination, aztreonam and carbapenems (20). This did not appear to be the case in our study as the isolate showed total resistance to the above mentioned antibiotics.

Among our isolates, only *C.violaceum* was specifically reported in a previous study as causative agent in a pleural space infection (21). Compared to that report, our study participant was a 38 year old male. The isolate was resistant to all antibiotics tested, except Amikacin. No follow-up could be performed on whether tere was clinical failure or not, but the infrequent nature of the infection as well as the unexpected antibiotic resistance profile posed a significant challenge to clinicians.

In the light of the above discussed findings and of the previous literature, it may be inferred that there is a notable paucity of scientific literature on the current prevalence of the above discussed pathogens in Africa region. Therefore, monitoring these pathogens as possible threat to healthcare systems is of paramount importance.

CONCLUSION

Many of the pathogens investigated in this study are either inhabitants of aquatic ecosystems or commensal microorganisms. Infections caused by these bacteria could become a health concern in the near future, given the population mobility and the inadequacy of hospital hygiene monitoring mechanisms in our facilities. While we have data on the studied isolates, various questions arise, including those about potential new species, unusual resistance mechanisms, and mixed infections with varying disease progressions. Therefore, it's important to continue studies in order to provide more precise answers to these questions.

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Conflict of interest

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