DTI
Drug Target Insights 2023; 17: 92-100
ISSN 1177-3928 | DOI: 10.33393/dti.2023.2613
ORIGINAL RESEARCH ARTICLE

Open Access

# ESBL and carbapenemase-producing Enterobacteriaceae in infectious pleural effusions: current epidemiology at Hôpital du Mali

**Aimé Césaire Kalambry[1], Tchamou Malraux Fleury Potindji[2], Ibrehima Guindo[3], Ambara Kassogue[1], Boubacar Sidiki Ibrahim Drame[1], Seydou Togo[4], Sadio Yena[4], Seydou Doumbia[5,6], Mahamadou Diakite[6,7]**

[1]Medical Biology Laboratory, "Hôpital du Mali" Teaching Hospital, Bamako - Mali
[2]Graduate School of Biological and Food Techniques, University of Lomé, Lomé - Togo
[3]National Institute of Public Health, Bamako - Mali
[4]Department of Thoracic Surgery, "Hôpital du Mali" Teaching Hospital, Bamako - Mali
[5]Faculty of Medicine and Odontostomatology of Bamako, Bamako - Mali
[6]University Clinical Research Center (UCRC), University of Science, Technique and Technologies of Bamako, Bamako - Mali
[7]Malaria Research and Training Center (MRTC), Bamako - Mali

**ABSTRACT**

**Background:** Antimicrobial resistance (AMR) is a global health concern, with extended-spectrum β-lactamases (ESBLs) and carbapenemases being major contributors. Pleural infection (PI) is a severe condition in West Africa, complicated by AMR. This study aimed to investigate the prevalence and molecular characteristics of ESBL and carbapenemase-producing enterobacteria in pleural effusions in Mali.

**Materials and methods:** Pleural fluid samples from 526 patients with pleuritis were analyzed. Enterobacterial species were isolated and identified, and the prevalence of resistance genes ($bla_{OXA-48}$, $bla_{NDM-1}$, $bla_{KPC}$, $bla_{TEM}$, $bla_{SHV}$) and virulence factors was determined.

**Results:** Among the patients, 110 were diagnosed with enterobacterial pleuritis. *Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis* were the main pathogens identified. Resistance to β-lactams and cephalosporins was high, while carbapenems showed good activity. ESBL production was detected in 33.6% of isolates, with $bla_{TEM}$ being the most common gene. Carbapenemase gene ($bla_{NDM-1}$) was found in three isolates.

**Conclusion:** The study highlights the high prevalence of multidrug-resistant bacteria and the need for appropriate antibiotic selection based on local resistance patterns. Understanding the molecular characteristics of resistance is crucial for optimizing patient care and developing effective therapeutic strategies. Further research is needed to monitor and control AMR in PIs in Mali.

**Keywords:** Carbapenem-resistant Enterobacteriaceae, ESBL, Mali, Pleural effusions

## Introduction

Infectious pleural effusion (IPE) is a severe clinical issue. Often secondary to a pre-evolving pulmonary infection, the condition presents with an increasing incidence worldwide (1,2). Care of IPE involves pleural drainage in most of the cases and antibiotic therapy; however, treatment proves inadequate in substantial cases, resulting in mortality rate ranging from 10.7% to 22% (3-5). Few studies have evaluated IPE management, status, and microbiology in Mali. A recent study by Tapia et al in 2021 reported 13.3% mortality rate (6). Among causal factors predictive of treatment failure is the alarming emergence of antimicrobial resistance (AMR).

AMR has emerged as a significant global health concern, undermining the effectiveness of antibiotics and exacerbating the burden of infectious diseases. Major contributors to AMR include extended-spectrum β-lactamases (ESBLs) and carbapenemases produced by Enterobacteriaceae (7,8). Both enzymes are β-lactamases with proven ability to degrade β-lactam antibiotics. The ESBLs exhibit hydrolytic activity against penicillins and all cephalosporins, yet are suppressed by β-lactam inhibitors (9). Mutation-wise, ESBLs encoding genes can be grouped into several variants: $bla_{TEM}$, $bla_{SHV}$, $bla_{IRT}$, etc (10). On the other hand, another important

**Received:** May 29, 2023
**Accepted:** August 2, 2023
**Published online:** August 29, 2023

**Corresponding author:**
Aimé Césaire Kalambry
Medical Biology Laboratory, Hopital du Mali
Bamako - Mali
kaimecesaire@gmail.com

Drug Target Insights - ISSN 1177-3928 - www.aboutscience.eu/dti
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enzyme is carbapenemase, which degrade carbapenem antibiotics and include gene variants like $bla_{KPC}$, $bla_{IMP}$, $bla_{OXA-1}$, etc (9). Thus, their inclusion in World Health Organization list as top priority pathogens underscores their potential to pose significant challenge in clinical settings (11). Data from recent studies found an association between extended-spectrum β-lactamases and carbapenemases producing Enterobacteriaceae (ESBL-E/CPE) infections and risk of mortality (12,13). Moreover, prior to antibiotherapy, comorbidities as well as persistent colonization were found to be strong predictors of infection and subsequent treatment failure (14,15). Yet to date, detailed reports on the magnitude of ESBL-E/CPE in West Africa are scarce (16). Although there are data on phenotypic and genotypic distribution of AMR in pathogens responsible for various clinical infections in Mali, there are still unanswered questions related to the genotypic distribution of these AMR genes in IPEs (17-19). Understanding the extent of resistance and identifying the underlying resistance mechanisms is crucial for designing effective therapeutic strategies and optimizing patient care in this region.

This study has mainly focused on the epidemiology of $bla_{OXA-48}$, $bla_{NDM-1}$, $bla_{KPC}$ genes responsible for the induction of carbapenem resistance; $bla_{TEM}$, $bla_{SHV}$ genes that are responsible for ESBL production, as well as genes associated with bacterial adhesins (*bfp, eae, ipah, eagg*) and toxin genes (*slt1, slt2, lt, sta*). The primary objective of this study was to isolate, identify, and analyze the diversity of enterobacterial species found in pleural fluid samples from patients with IPE. A better knowledge of IPE in Mali could facilitate the management of the condition and thus reduce the level of mortality.

## Material 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 CHU “Hôpital du Mali” in Bamako. The study included all 6,096 hospitalized patients with pleurisy in these two departments.

The inclusion criteria were: (1) clinical diagnosis of pleural infection (PI) with subsequent diagnostic thoracentesis and microbiological confirmation (pleural fluid culture positive to at least one microorganism). PI was defined as the presence of positive pleural fluid culture and/or purulent pleural fluid and clinically manifesting as complex parapneumonic effusion (CPPE) or empyema (20); (2) patients who provided written informed consent to participate in the study.

The exclusion criteria were: (1) patients who had non-purulent or no growth from pleural fluid; (2) patients with pleural effusion caused by noninfectious etiologies such as malignancy and congestive heart failure.

The data were collected using Microsoft Excel through questionnaires administered to consenting patients by physicians prior to sample collection. Subsequently, laboratory data were obtained and combined with the questionnaire responses.

### *Microbiological processing and identification*

All pleural fluid samples were processed within an hour after collection at the microbiology laboratory of CHU Hôpital du Mali. Samples were inoculated on brain-heart infusion (BHI) and an anaerobic blood culture flask and incubated for 18 to 24 hours at 35±2°C. From these broth cultures, fresh blood agar, enriched chocolate agar, and Sabouraud agar were plated. The colonies were characterized and identified using Gram stain, biochemical tests, and the Phoenix M50 automated system (panel 449044-NMIC/ID-435).

### *Susceptibility to antibiotics*

All isolates were subjected to susceptibility testing against 19 antibiotics (Tab. I). Table I provides an overview of the different antibiotic classes and specific antibiotic used for susceptibility tests. The antibiogram was conducted using the Phoenix M50 automated system (panel 449044-NMIC/ID-435) and was complemented by disk diffusion technique for antibiotics not covered by the automated panel. The results were expressed as susceptible or resistant in accordance with the guidelines previously described (21).

ESBL production was detected using the combination disk test method with the following combinations: ceftazidime-clavulanate, cefepime-clavulanate, and cefotaxime-clavulanate (21). *Klebsiella pneumoniae* ATCC 700603 was used as quality control strain.

**TABLE I -** Antibiotic classes and specific antibiotics used in this study

| Family | Antibiotics |
|---|---|
| Beta-lactam antibiotics | Amoxicillin (20 µg) |
| | Amoxicillin (20 µg) + clavulanic acid (10 µg) |
| | Piperacillin (30 µg) |
| | Piperacillin (30 µg) + tazobactam (6 µg) |
| | Ticarcillin (75 µg) |
| | Cefuroxime (30 µg) |
| | Cefoxitin (30 µg) |
| | Ceftazidime (10 µg) |
| | Ceftriaxone (30 µg) |
| | Cefepime (30 µg) |
| | Aztreonam (30 µg) |
| | Imipenem (10 µg) |
| | Meropenem (10 µg) |
| | Ertapenem (10 µg) |
| Aminosides | Amikacin (30 µg) |
| | Gentamicin (10 µg) |
| | Tobramycin (10 µg) |
| Quinolones | Ciprofloxacin (05 µg) |
| Other | Trimethoprim (1.25 µg) + sulfamethoxazole (23.75 µg) |

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## Molecular analysis

DNA was extracted according to the method described previously (22). Briefly, pure colonies were suspended in 200 µL of Tris-ethylenediamine tetraacetic acid (EDTA) solution, heated at 100°C for 10 minutes, and then immediately placed at −20°C for 5 to 10 minutes. After centrifugation at 12,000 rpm for 10 minutes, the obtained supernatant was used as DNA template. Quality control of the extraction was carried out using Thermo Scientific NanoDrop One/One[C] instrument at the molecular biology unit of the University Centre for Clinical Research (UCRC) in Bamako.

Using conventional polymerase chain reaction (PCR), eight virulence factor genes from *Escherichia coli*, namely *bfp, eae, eagg, ipah, slt1, slt2, lt* and *sta*, as well as ESBL coding genes, namely $bla_{TEM}$ and $bla_{SHV}$, were characterized. In addition, the following genes were screened for their role in conferring antibiotic resistance: the *catA1* gene responsible for encoding chloramphenicol acetyltransferase, mutations on genes encoding for topoisomerase IV, and DNA gyrase protective proteins targeted by quinolones (*qnrA, qnrB, qnrS*), as well as class 1 (*int1*), class 2 (*int2*), and class 3 (*int3*) integrons, which carry resistance genes for multiple antibiotics. We screened bacterial isolates for the presence of carbapenemase genes, namely: $bla_{KPC}$, $bla_{NDM-1}$, and $bla_{OXA-48}$.

PCR was performed using ABI 9700 thermocycler (Applied Biosystems, USA) with the following cycling parameters: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at variable temperature for each primer set for 39 seconds, and extension at 72°C for 60 seconds (Tab. II). Table II provides essential information about selected genes, including their nucleotide sequences, hybridization temperatures, amplicon sizes. The final extension step was carried out at 72°C for 5 minutes.

**TABLE II -** Primer sequences and characteristics of selected genes

| Gene names | Nucleotide sequences (5′ → 3′) | Hybridization temperature | Amplicon size | Reference |
|---|---|---|---|---|
| Adhesin genes | | | | 22 |
| *bfp* | GAC ACC TCA TTG CTG AAG TCG<br>CCA GAA CAC CTC CGT TAT GC | 57°C | 324 bp | |
| *eae* | TCA ATG CAG TTC CGT TAT CAG TT<br>GTA AAG TCC GTT ACC CCA ACC TG | 65°C | 494 bp | |
| *ipaH* | GAA AAC CTC CTG GTC CAT CAG G<br>GCC GGT CAG CCA CCC TCT GAG AGT AC | 53°C | 424 bp | |
| *Eagg* | ACG CAG AGT TGC CTG ATA AAG<br>AAT ACA GAA TCG TCA GCA TCA GC | 53°C | 630 bp | |
| Toxin genes | | | | |
| *slt1* | TTT ACG ATA GCA TTC TCG AC<br>CAC ATA TAA ATT ATT TCG CTC | 56°C | 130 bp | |
| *slt2* | CTT CAC GTC ACC ATA CAT AT<br>ACG ATG TGG TTT ATT CTG GA | 56°C | 346 bp | |
| *lt* | GGC GAC AGA TTA TAC CGT GC<br>CCG AAT TCT GTT ATA TAT GTC | 56°C | 707 bp | |
| *sta* | TTA ATA GCA CCC GGT ACA AGC AGG<br>CTT GAC TCT TCA AAA GAG AAA ATT AC | 43°C | 146 bp | |
| Antibiotic resistance genes | | | | |
| *int1* | ACATGTGATGGCGACGCA CGA<br>ATTTCTGTCCTGGCTGGC GA | 57°C | 580 bp | |
| *int2* | GTAGCAAACGACTGACGAAAT G<br>CACGGATATGCGACAAAA AGG T | 62°C | 806 bp | |
| *int3* | GCC CCG GCA GCG ACT TTC AG<br>ACG GCT CTG CCA AAC CTG ACT | 62°C | 1200 bp | |
| *SHV* | TTATCTCCCTGTTAGCCACC<br>GATTTGCTGATTTCGCTCGG | 55°C | 800 bp | |
| *Tem* | ATAAAATTCTTGAAGACGAAA<br>GACAGTTACCAATGCTTAATC | 55°C | 850 bp | |

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| Gene names | Nucleotide sequences (5′ → 3′) | Hybridization temperature | Amplicon size | Reference |
|---|---|---|---|---|
| *catA1* | CCTGCCACTCATCGCAGTAC | 57°C | 450 bp | |
| | CTGCCTGGACAACATTGCTT | | | |
| *QnrA* | TCAGCACAAGAGGATTTCTC | 55°C | 657 bp | |
| | GGCAGCACTATTACTCCCA | | | |
| *QnrB* | GATCGTGAAAGCCAGAAAGG | 55°C | 469 bp | |
| | ACGATGCCTGGTAGTTGTCC | | | |
| *QnrS* | ACGACATTCGTCAACTGCAA | 55°C | 417 bp | |
| | TAAATTGGCACCCTGTAGGC | | | |
| Carbapenemase | | | | 27 |
| $bla_{KPC}$ | CATTCAAGGGCTTTCTTGCTGC | 55°C | 538 bp | |
| | ACGACGGCATAGTCATTTGC | | | |
| $bla_{OXA-48}$ | GCTTGATCGCCCTCGATT | 55°C | 281 bp | |
| | GATTTGCTCCGTGGCCGAAA | | | |
| $bla_{NDM-1}$ | ATGGAATTGCCCAATATTATGCAC | 55°C | 813 bp | |
| | TCAGCGCAGCTTGTCGGC | | | |

Each reaction was carried out in a 25 µL mixture prepared as described previously, with modifications (22). In all reactions, a negative control (water) was included alongside a positive control consisting of reference isolates (E2348-69, M90T, EDL 933, EDL 1493, R3, R4, R5, R6, and R7).

The sequence of primers used for amplification and the expected amplicon size are detailed in Table II. PCR products were visualized by transillumination after migration in 1.5% Tris base, acetic acid and EDTA (TAE) buffer.

### *Statistical analysis*

The data were analyzed using IBM SPSS Statistics for Windows, Version 23.0. Student's t-test was used to compare mean values of continuous variables, while chi-square test was employed to analyze categorical variables. A p-value of less than 0.05 was considered to indicate statistical significance. Statistical analysis of AMR data was performed using the software R (version 4.3.0) and the integrated development environment R Studio (version 2023.03.1+446). The package "AMR" was employed for AMR data processing (23).

### *Ethical approval*

Written consent was obtained from all included participants, and the study protocol was subjected to review and approval by the Ethics Committee of the University of Sciences, Techniques, and Technologies of Bamako. The approval was granted under reference number 2021/228/USTTB on June 9, 2021.

## Results

### *Sociodemographic characteristics of patients*

The study specifically analyzed pleural fluid samples obtained from 526 patients with pleurisy, out of which 110 were diagnosed with enterobacterial pleuritis (Tab. III). Table III provides an overview of patient characteristics and their distribution within the thoracic surgery and pediatrics' ward. It allows for a comparison between the two groups and helps identify any statistically significant differences in

**TABLE III** - Patient characteristics and distribution by thoracic surgery and pediatric wards

| | Thoracic surgery | Pediatrics | Total | P |
|---|---|---|---|---|
| Characteristics | n = 92 (%) | n = 18 (%) | n = 110 (%) | 0.000 |
| *Gender* | | | | 1.000 |
| Male gender | 59 (64.1) | 12 (66.7) | 71 (64.5) | |
| Female gender | 33 (35.9) | 6 (33.3) | 39 (35.5) | |
| Median age | 42 | 8.5 | 37.5 | |
| *Age group* | | | | |
| 0–4 | – | 13 (72.2) | | 0.000 |
| 5–9 | – | 5 (27.8) | | |
| 10–14 | – | | | |
| 15–19 | 3 (3.3) | – | | |
| 20–24 | 3 (3.3) | – | | |
| 25–29 | 16 (17.4) | – | | 0.000 |
| 30–34 | 12 (13.0) | – | | |
| 35–39 | 6 (6.6) | – | | |
| 40–44 | 10 (10.9) | – | | |
| 45–49 | 6 (6.5) | – | | |
| 50–54 | 10 (10.9) | – | | |
| 55–59 | 9 (9.8) | – | | |
| 60–64 | 8 (8.7) | – | | |
| 65–69 | 4 (4.3) | – | | |
| 70–90 | 5 (5.4) | – | | |

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gender, age, and age group distribution. A total of 71 (64.5%) patients were men and 39 (35.5%) were women with a male-to-female sex ratio of 1.8. The majority of the patients (76/110, 69.1%) resided in urban areas. Pediatric patients aged 0-4 years, and young adults (25-29 years) constituted 72.2% of the cases, highlighting the vulnerability of these age groups to the condition. The observed distribution within these age groups was found to be statistically significant (p = 0.000).

### *Bacterial diversity and antibiotics resistance profile*

The three main pathogens isolated in this study were *Escherichia coli* (44.5%; n = 49), *Klebsiella pneumoniae* (11.8%; n = 13), and *Proteus mirabilis* (13.6%; n = 15). Antibiotic resistance profile of *E. coli*, *P. mirabilis*, and *K. pneumoniae* was assessed against several antibiotics (Tab. I). Results showed marked differences in antibiotic susceptibility between β-lactams, cephalosporins, and carbapenems. β-Lactams showed no activity against the three Enterobacteriaceae; second-generation cephalosporins had shown moderate activity against *E. coli* and *K. pneumoniae* (65.3% and 53.8% respectively) but had no activity against *P. mirabilis*, while third-generation cephalosporins showed moderate activity against all three types of isolates, with susceptibility rates ranging from 8.2% to 91.8%. In contrast, all carbapenems had high activity against all three types of isolates. In terms of efficacy, *E. coli* and *P. mirabilis* isolates exhibited higher susceptibility to the combination of penicillins and β-lactamase inhibitors (piperacillin/tazobactam [TZP]); however, they were moderately to highly resistant to trimethoprim/sulfamethoxazole (Tab. IV). Table IV provides valuable information on the susceptibility patterns of *E. coli, P. mirabilis,* and *K. pneumoniae* to various antibiotics. It assists in understanding the effectiveness of different antibiotics against these bacterial species, aiding in the selection of appropriate treatment options.

The combination disk test method revealed that 33.6% (n = 37) of all isolates were ESBL producing. *E. coli* had the highest prevalence at 24.5% (n = 27), followed by *K. pneumoniae*, *Enterobacter cloacae*, and others.

It is noteworthy that a substantial proportion of the patients, precisely 77.3% (85/110), had previously undergone at least one course of antibiotic treatment before the sampling procedure. Moreover, an alarming 94.5% of the isolates displayed multidrug-resistant (MDR) profiles, as per the guidelines set forth by Magiorakos et al (24).

### *Molecular characterization of genes*

Table V lists the frequencies of the virulence genes identified in *E. coli* isolates. Table V provides valuable information on the presence and distribution of genes related to adhesins, enteroxins, antibiotic resistance, and carbapenemases in *E. coli* and related species. It helps in understanding the genetic characteristics and potential resistance patterns of these bacterial strains.

*Eagg* was found in 6.1% (n = 3) of the *E. coli* isolates while *bfp* and *eae* were not detected; the gene *ipah* was found in a higher percentage (n = 27; 55.1%). Among isolates, $bla_{TEM}$ was the most common ESBL, being present in 29.7% of *E. coli.* In contrast, $bla_{SHV}$, *int2, int3, qnrS, qnrA,* and *qnrB* genes were not detected. In terms of carbapenemase genes, $bla_{NDM-1}$ was detected (Tab. V).

**TABLE IV -** Antibiotic susceptibility profiles of *Escherichia coli, Proteus mirabilis*, and *Klebsiella pneumoniae*

| **Antibiotics** | ***E. coli*** | | ***P. mirabilis*** | | ***K. pneumoniae*** | |
|---|---|---|---|---|---|---|
| | **S%** | **R%** | **S%** | **R%** | **S%** | **R%** |
| Amoxicillin | 0 | 49 (100) | 0 | 15 (100) | 0 | 13 (100) |
| Amoxi + clavulanic acid | 4 (8.2) | 45 (91.8) | 0 | 15 (100) | 2 (15.4) | 11 (84.6) |
| Piperacillin | 0 | 49 (100) | 0 | 15 (100) | 0 | 13 (100) |
| Piperacillin + tazobactam | 46 (93.9) | 3 (6.1) | 12 (80) | 3 (20) | 10 (76.9) | 3 (23.1) |
| Ticarcillin | 0 | 49 (100) | 2 (13.3) | 13 (86.7) | 0 | 13 (100) |
| Aztreonam | 46 (93.9) | 3 (6.1) | 11 (73.3) | 4 (26.7) | 12 (92.3) | 1 (7.7) |
| C2G | 4 (8.2) | 45 (91.8) | 0 | 15 (100) | 3 (23.1) | 10 (76.9) |
| C3G | 4 (8.2) | 45 (91.8) | 2 (13.3) | 13 (86.7) | 5 (38.5) | 8 (61.5) |
| Cefoxitin | 32 (65.3) | 17 (34.7) | 0 | 15 (100) | 7 (53.8) | 6 (46.2) |
| Ertapenem | 48 (98.0) | 1 (2.0) | 14 (88.2) | 1 (6.7) | 11 (84.6) | 2 (15.3) |
| Imipenem | 46 (93.9) | 3 (6.1) | 11 (73.3) | 4 (26.7) | 11 (84.6) | 2 (15.3) |
| Amikacin | 39 (79.6) | 10 (20.4) | 10 (66.7) | 5 (33.3) | 9 (69.2) | 4 (30.8) |
| Gentamicin | 12 (24.5) | 37 (75.5) | 2 (13.3) | 13 (86.7) | 5 (38.5) | 8 (61.5) |
| Tobramycin | 13 (26.5) | 36 (73.5) | 2 (13.3) | 13 (86.7) | 5 (38.5) | 8 (61.5) |
| Ciprofloxacin | 5 (10.2) | 44 (89.8) | 6 (40) | 9 (60) | 4 (30.7) | 9 (69.2) |
| Trimethoprim + sulfamethoxazole | 2 (4.1) | 47 (95.9) | 6 (40) | 9 (60) | 3 (23.1) | 10 (76.9) |

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**TABLE V** - Distribution of genes of adhesins, enteroxins, antibiotic resistance, and carbapenemases in *Escherichia coli* and related species

| **Genes of adhesins** | ***Escherichia coli* n = 49 (%)** | | | |
|---|---|---|---|---|
| *Eagg* | 3 (6.1) | | | |
| *Bfp* | 0 | | | |
| *eae* | 0 | | | |
| *ipaH* | 27 (55.1) | | | |
| **Enteroxin genes** | ***E. coli* n = 49 (%)** | | | |
| *lt* | 1 (2.0) | | | |
| *slt1* | 1 (2.0) | | | |
| *slt2* | 0 | | | |
| *Sta* | 1 (2.0) | | | |
| **Antibiotic resistance genes N = 37** | ***E. coli* n = 27** | ***Klebsiella pneumoniae* n = 4** | ***Enterobacter cloacae* n = 2** | ***Others** n = 4*** |
| *Tem* | 11 (29.7) | | 1 (2.7) | 0 |
| *SHV* | 0 | – | – | 0 |
| *Int1* | 10 (27.0) | 4 (10.8) | 1 (2.7) | 0 |
| *Int2* | 0 | 0 | 0 | 0 |
| *Int3* | 0 | 0 | 0 | 0 |
| *QnrS* | 0 | 0 | 0 | 0 |
| *QnrA* | 0 | 0 | 0 | 0 |
| *QnrB* | 0 | 0 | 0 | 0 |
| *CatA1* | 12 (32.4) | 0 | 0 | 0 |
| **Carbapenemase N = 13** | ***Proteus penneri* n = 1 (%)** | ***K. pneumoniae* n = 2 (%)** | ***Providencia rettgeri* n = 2 (%)** | ***Others*** n = 8** |
| *KPC* | 0 | 0 | 0 | 0 |
| *OXA-48* | 0 | 0 | 0 | 0 |
| *NDM-1* | 1 (7.7) | 1 (7.7) | 1 (7.7) | 0 |

Based on molecular screening, only one carbapenemase gene, $bla_{NDM-1}$, was detected in three different isolates only with single occurrence, namely *K. pneumonia, Providencia rettgeri,* and *Providencia penneri.*

## Discussion

The results reported in this study provide valuable insights into the bacteriology of PIs in Mali. By addressing resistance rates toward various antibiotics, and the frequency of ESBL, carbapenemases, and MDR bacteria, this study is the first to comprehensively analyze these factors and lay the groundwork for future clinical studies to determine whether improved bacterial diagnosis and antibiotic selection can positively impact the outcomes of PIs. According to the British Thoracic Society (BTS) Guidelines for Pleural Disease, PI was clinically addressed in this discussion as a case of CPPE or empyema and literature was searched accordingly (20).

Out of the 526 samples analyzed, 244 were positive to culture; of which 110 cultures tested positive for Enterobacteriaceae, indicating a significant number of negative results. These results could potentially be attributed to alternative etiologies or could be a result of previous antibiotic utilization (as 77.3% of our patient's population have admittedly taken at least one antibiotic prior to sampling).

In the current study, *E. coli, K. pneumoniae*, and *P. mirabilis* were the main bacteria identified, indicating their significant role as causative agents of PI. These findings align with previous studies that have also identified the above-mentioned pathogens as the primary contributors to PI (25,26). Furthermore, the study revealed that those pathogens exhibited higher rate of resistance to third-generation cephalosporins, indicating their classification as ESBL phenotypes. The prevalence rates of ESBL reported in this study were significantly lower than a study from Burkina Faso, which reported 70% of ESBL-producing isolates in hospitalized patients (27). Genotypically, the most common ESBL gene was $bla_{TEM}$. This finding was also reported by Sonda et al (28). We did not detect $bla_{SHV}$ gene.

The observed high resistance among the most common isolates to penicillin, quinolones, cephalosporins, and others is likely attributed to the higher utilization of those antibiotic classes in hospital settings. The existence of such association between antibiotic prescriptions and susceptibility pattern was previously highlighted in another hospital setting in Eritrea (29). Clinicians' choice of broad-spectrum antibiotics or combination therapy may be suggestive of the infection being acquired in a hospital setting. It could be inferred that despite the treatment recommendations as per European Respiratory Society (ERS) and American Association for Thoracic Surgery (AATS), the selection of the treatment regimen must typically depend on infection setting, the local prevalence of microorganisms, and antibiotic resistance patterns (30,31).

Additionally, our study reported a high prevalence of MDR bacteria. This MDR rate aligns with previous study in Turkey reporting the widespread occurrence of MDR bacteria (26). The study findings revealed a higher prevalence of carbapenem resistance (7.2%) compared to the previous report by Dwomoh et al (32), which documented a resistance rate of 5.6%. Hackman et al (33) reported a similar rate of carbapenem resistance. However, our study observed significant disparities in the resistance rates of individual carbapenems (ertapenem, imipenem, meropenem) as well as the TZP combination compared to their study. These variances can be attributed to our study's focus on the three predominant pathogens isolated (*E. coli, K. pneumoniae, P. mirabilis*). On the basis of available data, TZP and carbapenems appear to maintain significant effectiveness against the tested pathogens; the results were in accordance with those of a Malaysian observational cohort study (34). The recorded prevalence aligns with other studies conducted in Africa, such as those conducted in Nigeria (35) and South Africa (36). However, Egypt has recently reported a notably higher prevalence of carbapenem resistance (37).

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The relatively low abundance of carbapenem resistance genes further supports this notion, indicating that the isolates in this study may not possess robust mechanisms of resistance to carbapenems. We did not detect cases of $bla_{KPC}$ and $bla_{OXA-48}$ type of carbapenemase but 7.7% of $bla_{NDM-1}$. In 2022, a study investigating the global epidemiology of *OXA-48*-like β-lactamases, treatment option and pipeline development were conducted by Sara E. Boyd et al (38). The study found that the enzymes of $bla_{OXA-48}$ type are the most common carbapenemases among Enterobacteriaceae in much of western Europe. In Africa, the same study reported circulation of these types of β-lactamase in Tunisia, Algeria, Egypt, and South Africa. In West Africa, cases have been reported in Senegal and Nigeria. Mali did not provide data probably due to lack of adequate health care infrastructure and limited molecular diagnostic capabilities (38). Nabi Jomehzadeh et al found higher levels of $bla_{NDM-1}$ (31%) (39). In 2020, Muggeo et al reported the first description of $bla_{NDM-5}$ in Mali (18). Overall, β-lactam/β-lactam inhibitor combinations and carbapenems are suitable choices, as presented previously in observational studies and guidelines (40-42). However, it is crucial to interpret these results within the context of the provided data. The analysis is limited to the specific isolates and may not be representative of the broader population or other geographical regions.

According to this study, the presence of virulence factor genes in *E. coli* isolates was limited to a specific set. The occurrence of these factors varied, ranging from 2% for genes *sta, slt*, and *lt*, to 55.1% for the *ipah* gene. The *ipah* gene encodes Invasion Plasmid Antigen and is closely linked to immune system modulation in the host and bacterial survival. It is commonly detected in the majority of EIEC isolates (43).

The study has certain limitations. Firstly, it was conducted in a single center, which might restrict the applicability of the results to other populations or settings. Additionally, the sample size was relatively small, warranting larger multicenter studies to validate the findings. The study also lacked detailed information on patients' antibiotic exposure history and prior hospitalizations, which could have influenced the prevalence of antibiotic resistance. Despite these limitations, the study emphasizes the significant prevalence of antibiotic resistance in the examined setting and underscores the ongoing need for surveillance and efforts in antibiotic stewardship to address this critical public health concern. Further research involving larger sample sizes, multicenter designs, and comprehensive patient data would greatly enhance our understanding and approach toward combating antibiotic resistance in our region.

In countries with limited resources such as those in West Africa, more specific socioeconomic and behavioral factors contribute to exacerbating this threat, among others: (i) certain common societal practices such as self-medication; (ii) a failing medical sector with insufficiently trained prescribers and inefficient diagnostic tools; or (iii) an uncontrolled drug chain with over-the-counter, improperly stored, counterfeit, and/or expired antibiotics favor the emergence of resistance. This study on the prevalence and characteristics of ESBL and carbapenemase-producing Enterobacteriaceae in pleural effusion in Mali holds great significance for low- and middle-income countries. By providing critical insights into the extent of resistance and molecular epidemiology, this research will facilitate the development of effective strategies to combat AMR, improve patient outcomes, and safeguard public health in Mali.

## Acknowledgments

The authors would like to thank the managers of the molecular biology laboratories of the National Institute of Public Health and the University Clinical Research Center. The authors would also like to express their sincere gratitude to the Fogarty International Center for funding the registration of PhD student Aimé Césaire Kalambry through grant D43TW008652. Their support has been instrumental in the advancement of our understanding in this field and has contributed significantly to the successful completion of this manuscript.

## Disclosures

Conflict of interest: The authors declare no conflict of interest.
Financial support: This research is part of a PhD study whose PhD program received financial support from the Fogarty International Center to cover the student's registration fees only. No specific funds were allocated to this particular research project. The funding agency played no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

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