

Screening for Beta-Lactamase and Carbapenemase Phenotypes of *Klebsiella pneumoniae* Strains Isolated from Blood and Urine of Children Aged 0 to 59 Months at the Koutiala Reference Health Center, Mali

Abstract

Antimicrobial resistance, particularly in *Klebsiella pneumoniae*, poses a severe challenge to pediatric care in developing countries. The emergence of extended-spectrum beta-lactamase (ESBL)-producing and carbapenemase-producing strains dangerously restricts therapeutic options in children aged 0 to 59 months. To investigate the resistance phenotypes of beta-lactamases and carbapenemases in *Klebsiella pneumoniae* strains isolated from blood and urine samples of children aged 0 to 59 months at the Koutiala Reference Health Center (CSRéf). A prospective cross-sectional study was conducted over a period of 8 months at the microbiology laboratory of the Koutiala CSRéf. Blood (blood cultures) and urine samples (urocultures/ECBU) were cultured using selective and differential media. Biochemical identification of *K. pneumoniae* and antimicrobial susceptibility testing were performed following standard guidelines with the assistance of the Antibio application. Data were analyzed using WHONET, Excel, and SPSS. Among 2,771 collected samples, 623 yielded pathogenic growth (positivity rate of 22.5%). Out of these positive isolates, 25 strains of *K. pneumoniae* were recovered (12% of total pathogens), predominantly from blood (64%, n=16) and urine (36%, n=9). The male-to-female ratio was 1.27 (14 boys, 11 girls). Multi-drug resistant (MDR) phenotypes were detected in 80% of isolates (n=20). Phenotypic characterization revealed ESBL production in 60% of strains (n=15), high-level penicillinase in 20% (n=5), and suspected carbapenemase production in 20% (n=5). Confirmed resistance to carbapenems (imipenem/meropenem) was observed in 2 isolates (8%). Ciprofloxacin resistance reached 52%, whereas amikacin retained full susceptibility (100% sensitivity / 0% resistance). Overall mortality among infected patients was 12% (3/25). The high proportion of ESBL-producing *K. pneumoniae* and the emergence of suspected carbapenemase phenotypes in pediatric patients in Koutiala call for urgent enhancement of epidemiological surveillance and strict enforcement of antimicrobial stewardship.

Keywords: *Klebsiella pneumoniae*, Beta-lactamases, ESBL, Carbapenemases, Multi-drug resistance, Koutiala.

1. Introduction

In sub-Saharan Africa, and particularly in Mali, bacterial antimicrobial resistance (AMR) has become a major public health threat. In developing countries, several factors promote the emergence and dissemination of this phenomenon: irrational antibiotic use, availability of counterfeit or substandard drugs, lack of hygiene infrastructure, and inadequate infection control practices in hospital settings (Taitt et al., 2017). Furthermore, the lack of regular and reliable epidemiological data constitutes a major barrier to implementing effective policies for the rational use of anti-infective agents.

To address this challenge, the Koutiala health district was selected as one of five sentinel sites in Mali for antibiotic resistance surveillance under the GLASS project (Global Antimicrobial Resistance and Use Surveillance System), implemented by the WHO with financial support from KOICA.

Among high-concern pathogens, *Klebsiella pneumoniae*, a commensal enterobacterium of the digestive tract, is a formidable opportunistic pathogen responsible for severe healthcare-associated and community-acquired infections, such as bacteremia, pneumonia, and urinary tract infections. In children under 5 years of age (0 to 59 months), who are particularly vulnerable due to an immature immune system, *K. pneumoniae* infections are associated with high morbidity and mortality, reaching up to 50% in cases of severe pulmonary involvement or septic shock (Ran et al., 2025).

The management of these infections is greatly complicated by the ability of *K. pneumoniae* to acquire various enzymatic resistance mechanisms, including the production of extended-spectrum beta-lactamases (ESBLs), AmpC cephalosporinases, and more recently, carbapenemases (Bush and Bradford, 2016; Liu et al., 2016). Carbapenemase-producing strains (CP-KP) are rapidly spreading worldwide, conferring near-total resistance to the beta-lactam family and leading to dramatic therapeutic deadlocks in pediatric care.

While several studies have explored bacterial resistance in major university hospital centers in Bamako, very few works precisely document the phenotypic characterization of beta-lactamases and carbapenemases in the highly vulnerable 0-to-59-month age group in semi-urban areas such as Koutiala.

It is within this framework that the present study was conducted, the main objective of which was to determine the resistance phenotypes to beta-lactams specifically ESBL phenotypes, high-level penicillinases, and suspected carbapenemases among *Klebsiella pneumoniae* strains isolated from blood and urine in children aged 0 to 59 months admitted to the Koutiala Reference Health Center (CSRéf).

2. Materials and Methods

2.1. Study Setting and Site

The study took place at the microbiology laboratory of the Koutiala Reference Health Center (CSRéf) in Mali. Samples originated from various pediatric care units:

- Pediatric Intensive Care Unit (PICU)
- Neonatology Unit
- Inpatient Therapeutic Feeding Center (ITFC / URENI)
- General Pediatric Ward
- Burn Unit

2.2. Study Design and Population

This was a descriptive, prospective, cross-sectional study conducted over an 8-month period.

The study population consisted of all children aged 0 to 59 months hospitalized in the pediatric ward of the Koutiala Referral Health Center (CSRéf) who presented with clinical signs of bacterial infection (sepsis, neonatal infection, urinary tract infection, or severe acute malnutrition with complications) and for whom a biological sample (blood and/or urine) had been ordered.

2.3. Sampling and Specimen Collection

Blood (Blood Culture): 4 mL of venous blood was collected aseptically and inoculated into pediatric blood culture bottles using a closed system.

Urine (Uroculture / ECBU): Urine specimens were collected in young children using a sterile pediatric urine collection bag or via clean-catch midstream in older children, following rigorous antiseptic perineal cleansing.

2.4. Culture Media and Equipment

The following culture media were utilized:

- Blood culture bottles (BacT/ALERT® automated system)
- Chromagar Orientation agar
- MacConkey agar
- Columbia CNA agar (Nalidixic Acid + Colistin)
- Enriched Chocolate agar (PVX)
- Mueller-Hinton (MH) agar
- Kligler-Hajna medium

Laboratory equipment included the BacT/ALERT® detection system, 37°C incubators, optical microscopes, a water distiller, and steam sterilizer autoclaves.

2.5. Microbiological Procedures and Identification

2.5.1. Blood Cultures: Bottles were incubated in the BacT/ALERT® automated system. In case of a positive signal, a Gram stain was performed, followed by subculturing onto MacConkey and Chromagar plates.

2.5.2. Cytobacteriological Urine Examination (ECBU): Direct microscopic examination allowed leukocyte counting (significant threshold $\geq 10^4$ leukocytes/mL or 10 leukocytes/mm³). Quantitative inoculation was carried out on orientation agar.

2.5.3. Bacterial Identification: Identification of *K. pneumoniae* was based on colony morphology (mucoid, lactose-positive), Gram stain (Gram-negative bacilli), and a reduced biochemical panel (Kligler-Hajna, oxidase-negative, citrate-positive, urease-positive, indole-negative).

2.6. Antimicrobial Susceptibility Testing and Phenotypic Characterization

Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method (Kirby-Bauer method) on Mueller-Hinton agar, in accordance with the guidelines of the AntibioGram Committee of the French Society for Microbiology (CA-SFM / EUCAST). Reading and interpretation assistance for inhibition zone diameters were standardized using the AntibioGo mobile application.

Beta-lactam resistance phenotypes were defined as follows:

- **ESBL (Extended-Spectrum Beta-Lactamase):** Confirmed by the 'double-disk synergy' image between amoxicillin-clavulanic acid and 3rd/4th generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) or aztreonam.
- **High-Level Penicillinase:** Marked resistance to ampicillin and amoxicillin, associated with partial resistance to amoxicillin-clavulanic acid, without synergy with 3rd generation cephalosporins.

- **Suspected Carbapenemase:** Decreased susceptibility or overt resistance to at least one carbapenem (ertapenem, imipenem, meropenem) and/or ceftazidime resistance combined with complex synergy patterns.
- **Multi-Drug Resistance (MDR):** Acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

3. Data Analysis

Epidemiological and microbiological data were entered and structured using WHONET specialized software, and subsequently analyzed with IBM SPSS Statistics and Microsoft Excel. Qualitative variables are expressed as frequencies and percentages.

4. Ethical Considerations

The study received ethical approval from the local health authorities of CSRéf Koutiala. Informed consent was obtained from parents or legal guardians of all participating children prior to sampling. Anonymity and data confidentiality were strictly maintained throughout.

5. Results

5.1. Overall Sample Distribution and Isolation Frequency

Over the 8-month study period, a total of 2,771 samples (blood and urine) were received at the microbiology laboratory. Blood accounted for the majority of specimens at 82.7% (n=2,292), compared to 17.3% for urine (n=479).

A positive culture with a pathogenic organism was obtained in 623 cases, representing an overall positivity rate of 22.5%.

Among the 623 pathogenic isolates, 25 strains of *Klebsiella pneumoniae* were identified, corresponding to a prevalence of 12% among all isolated pathogenic bacteria, (Table 1).

Table 1: Overall distribution of samples according to specimen type and bacteriological results

Collection Type	Blood	Urine	Total	Percentage (%)
Number of samples	2,292	479	2,771	100.0%
Pathogens	499	124	623	22.5%
Contaminations	263	14	277	10.0%
Normal flora	-	94	94	3.4%
Negative	1,530	247	1,777	64.1%

5.2. Biological Origin of *Klebsiella pneumoniae* Strains

The 25 *Klebsiella pneumoniae* strains were predominantly isolated from blood cultures (64.0%, n=16), compared to urine samples (36.0%, n=9), Table 2.

Table 2: Distribution of 25 *Klebsiella pneumoniae* isolates by biological sample type

Specimen Type	Number of Isolates (n)	Percentage (%)
Blood (Blood culture)	16	64.0%
Urine (ECBU)	9	36.0%
Total	25	100.0%

5.3. Socio-Demographic Characteristics of Children

Sex Distribution: Among the 25 infected children, 14 were male (56%) and 11 were female (44%), corresponding to a male-to-female sex ratio of 1.27. No statistically significant difference was observed according to sex ($p > 0.05$).

Age Distribution: Infants (1 to 23 months) were the most affected group (64%, n=16), followed by children aged 24 to 59 months (32%, n=8) and neonates aged 0 to 28 days (4%, n=1).

5.4. Resistance Phenotypes to Beta-Lactams and Other Antibiotics

In-depth phenotypic analysis of the 25 *Klebsiella pneumoniae* strains revealed a very high frequency of enzymatic resistance mechanisms, (Table 3):

- **Extended-Spectrum Beta-Lactamases (ESBL):** Nearly two-thirds of the strains (60%, n=15) exhibited a typical ESBL phenotype.
- **Suspected Carbapenemase:** A phenotype suggestive of carbapenemase production was detected in 20% of strains (n=5). Among these, 2 strains (8% of total) exhibited confirmed in vitro resistance to imipenem and meropenem.
- **High-Level Penicillinase:** 20% of isolates (n=5) presented high expression of native penicillinase.
- **Multi-Drug Resistance (MDR):** In total, 80% of *K. pneumoniae* strains (n=20) were multi-drug-resistant bacteria.

Table 3: Resistance phenotypes to beta-lactams, aminoglycosides, and quinolones in *K. pneumoniae* (N = 25)

Antibiotic Families / Phenotypes	Number of Strains (n)	Resistance Rate (%)
ESBL Phenotype	15	60.0%
Suspected Carbapenemase	5	20.0%
High-Level Penicillinase	5	20.0%
Overall Multi-Drug Resistance (MDR)	20	80.0%
Gentamicin	10	40.0%
Tobramycin	10	40.0%
Amikacin	0	0.0%
Ciprofloxacin	13	52.0%

Regarding other antibiotic classes:

For aminoglycosides, gentamicin and tobramycin each displayed a resistance rate of 40% (n=10). In contrast, amikacin maintained full activity (0% resistance / 100% susceptibility) across all 25 strains.

For fluoroquinolones, ciprofloxacin resistance reached 52.0% (n=13).

5.5. Patient Clinical Outcomes

Among the 25 children treated for documented *K. pneumoniae* infection:

- 22 children recovered (88.0%).
- 3 deaths were recorded (12.0%). All 3 fatalities occurred in infants harboring multi-drug-resistant strains accumulating both the ESBL phenotype and fluoroquinolone resistance.

6. Discussion

Surveillance of antibiotic resistance in *Klebsiella pneumoniae* in pediatrics is crucial in the West African context. Our study provides valuable data on enzymatic inactivation phenotypes (beta-lactamases and carbapenemases) circulating in the Koutiala health district.

During the study period, 2,771 samples were examined, yielding a pathogenic positivity rate of 22.5%, associated with a contamination rate of 10%. These proportions are consistent with previous data reported by Diallo (2010) at the Gabriel Touré University Hospital in Bamako (21% positive cultures and 5% contamination).

K. pneumoniae isolates predominated in blood (64%) compared to urine (36%). Epidemiologically, this result is explained by the preferential admission of severe sepsis and suspected bacteremia in intensive care and neonatology at CSRéf Koutiala, generating a demand for blood cultures that far exceeds that for urocultures. This predominant blood culture profile contrasts with other series, such as that of SebaisandBoughachiche (2016) in Algeria, where *K. pneumoniae* was primarily of urinary origin (40% urine vs. 10% blood), reflecting differences in patient recruitment (adults vs. pediatrics).

Infants aged 1–23 months were the most impacted group (64%). This finding aligns with observations by Tigoi (2024) in Kenya, demonstrating that *K. pneumoniae* and *E. coli* represent

major enterobacterial threats in young African children, with carriage or invasive infection rates reaching 63%. Immunological vulnerability associated with young age and the high frequency of nutritional disorders (ITFC/URENI) in Koutiala account for this heightened susceptibility.

The frequency of the ESBL phenotype in our study reached 60%. This elevated rate is alarming but accords with recent sub-regional data. Cissé (2025) reported an ESBL production rate of 73% among clinical *K. pneumoniae* isolates in Bamako. Similarly, Sebais and Boughachiche (2016) noted a 50% ESBL prevalence in hospital settings in Constantine.

The rapid dissemination of genes encoding ESBLs (particularly CTX-M types) in hospital and community environments is exacerbated by the frequent and often empirical first-line pediatric use of 3rd generation cephalosporins (ceftriaxone) Villegas et al., (2004); Bush and Bradford, (2016).

One of the salient findings of this study is the detection of suspected carbapenemase production in 20% of strains (n=5), with 2 strains expressing confirmed in vitro resistance to meropenem and imipenem (8%).

Although carbapenem resistance long remained marginal in West Africa, the study by Ran et al. (2025) highlights that carbapenem-resistant *K. pneumoniae* (CRKP) strains are currently undergoing dramatic global pediatric expansion. The emergence of carbapenemases (such as KPC, NDM, or OXA-48-like) in children under 5 years represents a threat of complete therapeutic impasse in a rural reference health center lacking access to newer antibiotic combinations such as ceftazidime-avibactam Kang et al., (2024).

Co-resistance to other antibiotic classes was common, reaching 52% for ciprofloxacin and 40% for gentamicin and tobramycin. This high level of multi-drug resistance (80% MDR strains) aligns with trends reported by Sidibé (2020) in Mali and Diarra et al., (2022) in Sikasso.

Remarkably, amikacin maintained 100% susceptibility (0% resistance). This finding confirms that amikacin remains the aminoglycoside of choice and a strategic reserve therapeutic alternative in combination regimens for treating severe neonatal and pediatric bacteremia caused by ESBL-producing Enterobacteriaceae in Koutiala, as corroborated by Amraoui (2021).

The 12% mortality rate (3 deaths out of 25) associated with *K. pneumoniae* infections reflects the severity of septic conditions in young children. Although substantially lower than the mortality reported by Diallo (2010) (32.5% mortality), it underscores the critical prognostic impact of bacterial multidrug resistance, where delays in initiating effective initial antibiotic therapy compromise infant survival.

7. Conclusion

The study of *Klebsiella pneumoniae* resistance phenotypes in children aged 0 to 59 months at CSRéf Koutiala reveals a concerning epidemiological situation characterized by:

- A major prevalence of multi-drug resistant (MDR) strains (80%).
- A very high frequency of the ESBL phenotype (60%).
- The emergence of suspected carbapenemase phenotypes (20%), with confirmed carbapenem-resistant strains (8%).
- Fortuitously, complete retention of amikacin susceptibility (100% sensitivity).

8. Acknowledgements

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