

Research Article

Genetic Diversity and Clonal Dissemination of ESBL-Producing *Klebsiella pneumoniae* from Clinical, Food, and Animal Sources in Bouaké, Côte d'Ivoire

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Abstract

Klebsiella pneumoniae is a major opportunistic pathogen responsible for multidrug-resistant infections in both hospital and community settings. The emergence and spread of extended-spectrum β -lactamase (ESBL)-producing strains have become a major public health concern due to limited treatment options and their potential spread among human, animal, and food reservoirs. This study aimed to assess the genetic relatedness of ESBL-producing *K. pneumoniae* isolates collected from human clinical samples, ready-to-eat foods, and animal sources using ERIC-PCR profiling. A total of 98 ESBL-producing *K. pneumoniae* isolates were included, comprising 96 clinical isolates from hospitalized ($n = 91$) and non-hospitalized ($n = 5$) patients at Bouaké University Hospital, one isolate from ready-to-eat food, and one isolate from raw cow's milk. Identification was performed using conventional bacteriological methods, and ESBL production was confirmed using CHROMagar ESBL medium and the double-disk synergy test. ERIC-PCR analysis revealed a high level of genetic diversity, with 25 distinct ERIC profiles identified, including nine common types and 16 unique types. Several dominant clonal lineages were observed among the clinical isolates, distributed across different hospital departments and outpatient settings. One foodborne isolate shared an ERIC profile identical to that of three clinical isolates, while the animal-derived isolate exhibited a unique genotype. Overall, most isolates showed genetic heterogeneity, although clonal dissemination was observed among hospital strains. These results indicate a predominance of ESBL-producing *K. pneumoniae* in the hospital setting and suggest limited genetic relatedness between human and non-human isolates. However, the interpretation of relationships involving food and animal isolates remains limited by their low representation in the study. These results underscore the importance of strengthening infection prevention and control measures and confirm the need for ongoing molecular surveillance of multidrug-resistant *K. pneumoniae*.

Keywords

Animal, Human, Ready-to-Eat Food, ESBL, *Klebsiella pneumoniae*, ERIC-PCR

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1. Introduction

Enterobacteriaceae are a large family of Gram-negative bacilli living in the digestive tract of humans and certain healthy animals [1]. They play an important role in human pathology and are among the most frequently isolated Gram-negative bacteria in clinical microbiology laboratories [2]. Infections caused by Enterobacteriaceae constitute a major public health problem because treatment options are increasingly compromised by resistance to several antibiotic classes, particularly β -lactam antibiotics, which remain among the most widely prescribed antibacterial agents worldwide [3]. The main mechanism of resistance of enterobacteria to β -lactams is the production of ESBL, an enzyme that inactivates almost all molecules in this family of antibiotics [4]. In addition, ESBL-producing strains are frequently accompanied by multidrug resistance to different therapeutic classes [5]. The presence of this type of resistance mechanism in pathogenic strains poses a major risk of therapeutic inadequacy and hence therapeutic failure, and is also a factor of diffusion [6].

Among these enterobacteria is *Klebsiella pneumoniae* (*K. pneumoniae*), a hospital pathogen responsible for about one-third of all Gram-negative infections [7]. It is associated with high patient morbidity and mortality [8]. *K. pneumoniae* is characterized by the presence of a thick polysaccharide capsule that complicates its detection by the host immune system [9].

For a long time associated only with hospitals, the epidemiology of ESBL-producing strains of *K. pneumoniae* has changed considerably since the years 2000 [10]. Since then, these ESBL strains have been spreading in the community, in food-producing animals and even in ready-to-eat foods, threatening food safety [10, 11]. This epidemic phenomenon is particularly worrying because of its rapid spread and geographical extent [10]. Food, being one of the main routes of introduction of antibiotic-resistant bacteria and their genes into the human digestive tract [12], consumption of contaminated food could therefore influence the diversity of antibiotic resistance genes in the gut [13]. In addition, these bacteria can transfer antibiotic resistance determinants to other pathogenic bacteria [14].

In this context, molecular epidemiological tools are essential to assess the genetic relatedness of isolates from different sources and to better understand transmission dynamics. Such approaches provide critical insights into whether ESBL-producing strains circulating in hospitals, animals, and food belong to shared or distinct clonal lineages.

The present study aimed to investigate the genetic relatedness of ESBL-producing *Klebsiella pneumoniae* isolates recovered from human clinical samples, food, and animal sources using molecular typing methods, in order to assess their genetic diversity and explore potential links between these reservoirs.

2. Materials and Methods

2.1. Strains Collection

A total of 98 ESBL-producing *Klebsiella pneumoniae* isolates were recovered from different sources during January 2015 to June 2017. Of these, 91 clinical isolates were obtained from hospitalized patients ($n = 91$) distributed across ten hospital wards, whereas 5 isolates were obtained from non-hospitalized patients in the outpatient setting. One isolate was recovered from a ready-to-eat food sample purchased from street vendors, and one isolate was obtained from raw cow's milk collected from dairy farms. Bacterial strains were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Maldi Biotyper, Bruker Daltonics; Bremen, Germany). The screening of ESBL-producing *Klebsiella pneumoniae* strains was performed using CHROMagar™ ESBL medium and the double-disk synergy test (DDST) [15-17].

2.2. DNA Extraction

Genomic DNA was extracted by the simple boiling method as previously described [18]. Briefly, *K. pneumoniae* isolates were cultured on Mueller Hinton agar (Merck, Germany) and incubated at 37 °C. After 24 hours, one to five colonies were suspended in distilled water, and suspension was boiled for 30 min at 95 °C in water bath. The suspension was centrifuged at 14,000 rpm for five min and the supernatant transferred to filter columns. After a final centrifugation at 1200 rpm for five min, the supernatant was transferred to filter columns. After a final centrifugation at 1200 rpm for five min, the supernatant was transferred to a new microtube and stored at -20 °C.

2.3. ERIC-PCR

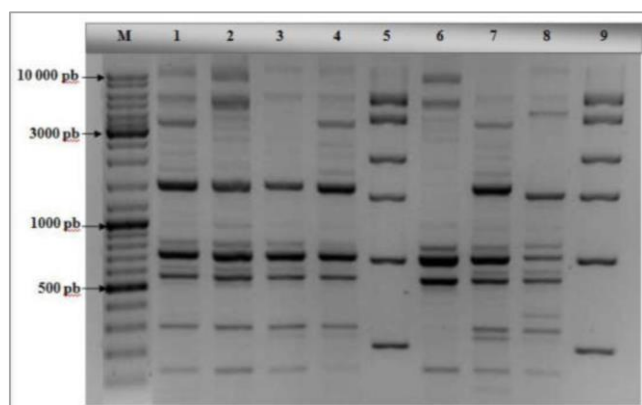
K. pneumoniae isolates were fingerprinted using the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). The primers used for the ERIC-PCR reaction were ERIC-1 (5' TGTAAGCTCCTGG GGATTCAC 3') and ERIC-2 (5' AAGTAAGTGACTGGGGTGAGCG 3') [19]. PCR amplification was performed in a reaction mixture of 25 μ L, and the cycling conditions were as follows: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 5 min and a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on 2% agarose gels and visualized using a gel documentation system (Vilber Lourmat, France) after staining with GelRed®. Products sizes were estimated using 100 bp to 10 000 pb DNA ladders as molecular size markers (Thermo Scientific).

2.4. ERIC-PCR Profiles Analysis

The ERIC patterns were analyzed by online data analysis service (insilico.ehu.es). ERIC profiles were compared using Dice method and clustered by UPGMA (unweighted pair group method with arithmetic mean) program and finally, the dendrogram was drawn [19].

3. Results

Analysis of the electrophoretic profiles of the DNA fragments obtained after PCR amplification showed a variation in the number of bands between 6 and 15 according to the strain, with sizes ranging from 100 bp to 10,000 bp. The gel image of the isolates is given in Figure 1.



M: molecular size marker; 1, 2, 3,...: bacterial strains

Figure 1. ERIC-PCR profiles from ESBL-producing-Klebsiella pneumoniae.

Clonal diversity was observed among 98 ESBL producing *K. pneumoniae* isolates by detecting 25 ERIC patterns (ERIC-types), nine of which were common type (A, B, C, D, E, J, L, W, X) and sixteen unique type. ERIC-type X was identified as the dominant type, with 18 isolates belonging to it originating from six inpatient wards and the outpatient ward. In addition, ERIC-type L had 17 strains isolated from patients also from six inpatient wards and the outpatient ward. Fourteen *K. pneumoniae* strains from five inpatient ward and the outpatient ward showed ERIC-type W. Genotype B was observed in 13 strains sharing the same origin as genotype W. ERIC-type C strains (five strains) came from 3 different inpatient wards, as did ERIC-type A strains (four strains). The strain isolated from ready-to-eat food and three strains from three inpatient wards had ERIC-type J. ERIC-type D and ERIC-type E each included 3 strains from different inpatient wards. As for the strain isolated from raw cow's milk, it had only one genotype of its own. The Dendrogram of *K. pneumoniae* isolates clustering based on ERIC patterns is given in Figure 2.

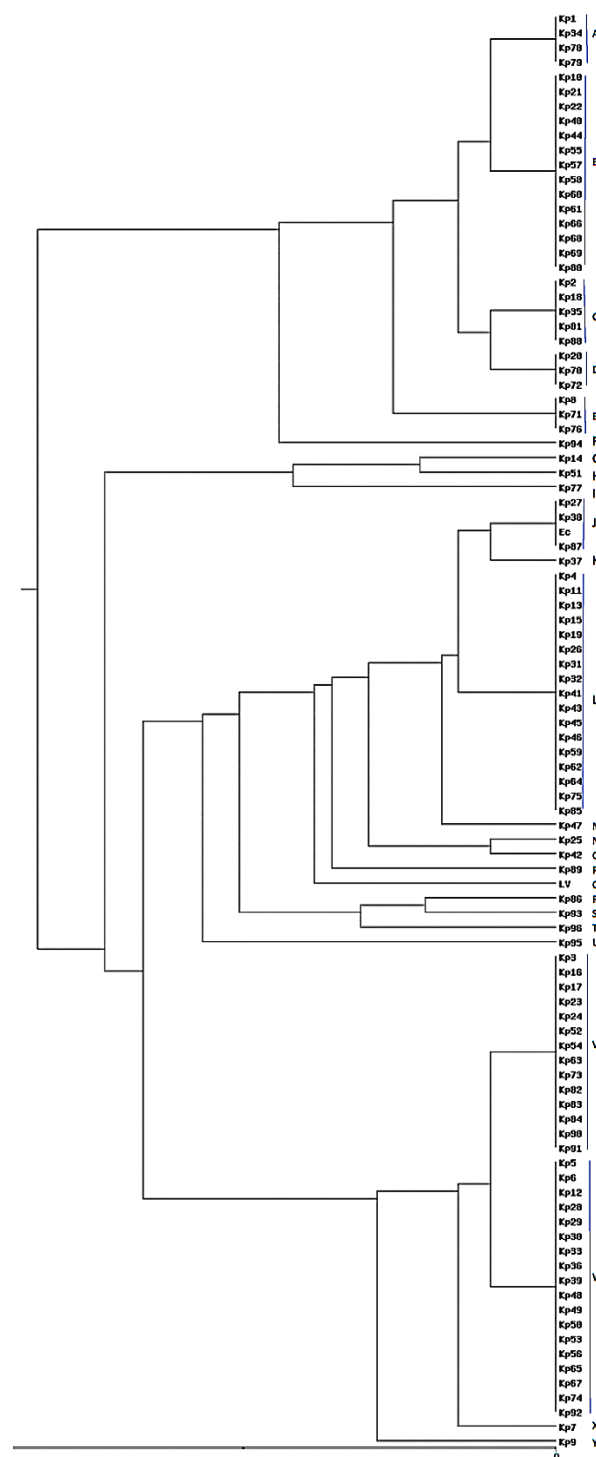


Figure 2. Dendrogram of 98 *K. pneumoniae* isolates clustering based on ERIC patterns.

Ec. ready-to-eat food isolate, LV. raw cow's milk isolate, Kp. Clinical isolate

4. Discussion

Investigation of the genetic relatedness of 98 ESBL-producing strains of *K. pneumoniae*, including 96 clinical strains, one foodborne strain and one strain of animal origin, showed a genetic diversity of these strains characterized by 25 distinct

genotypes. Similar results have been obtained in previous studies by several researchers, including Abe *et al.* in Côte d'Ivoire [20], Moosavian and Emam in Iran [21] and Firmo *et al.* in Brazil [22].

Of these 98 strains, 82 showed common profiles differentiated into nine genotypes. This could suggest a similar origin of dissemination. Each of these nine genotypes comprised between 4 and 18 strains from the same and different departments. Similar results have been reported by several researchers in their studies of enterobacterial strains of nosocomial origin [23, 24]. These findings may be explained by clonal dissemination within hospital wards, potentially associated with inadequate hygiene practices and the predominant mode of transmission of *K. pneumoniae*, particularly via hand-mediated spread.

In addition, a common genotype was identified in the food BLSE strain isolated from cooked cow's milk and three human clinical strains from the Pediatrics, Neurology and Surgical Emergency wards. These three clinical strains were isolated from urine and pus samples. This result indicates the genetic homogeneity of these strains. Several researchers, including Giri *et al.* [11], have demonstrated the good discriminatory power and typing capacity of ERIC-PCR for bacterial isolates of human and food origin. The same applies for Tang *et al.* [25], whose study showed genetic homogeneity between isolates of human and food origin. According to Bray et Zafar [26], the acquisition and gastrointestinal carriage of *K. pneumoniae* ESBL is an important intermediate step in the process of developing *K. pneumoniae* ESBL infections in patients, and may also serve to perpetuate a large reservoir of organisms within the hospital. These findings are consistent with those reported by Tsitsos *et al.* [27], who investigated ESBL- and carbapenemase-producing *K. pneumoniae*, *Escherichia coli*, and *Acinetobacter* spp. in patients and kitchen environments of two Greek hospitals. Their study identified antimicrobial-resistant *K. pneumoniae* in kitchen-associated samples and demonstrated epidemiological relatedness between some isolates recovered from the kitchen environment and hospitalized patients. These findings suggest that hospital kitchens may act as potential reservoirs and sites of circulation of antimicrobial-resistant bacteria, thereby contributing to their dissemination within healthcare facilities [27]. In addition, Parmar *et al.* [28] demonstrated a clonal relationship between *K. pneumoniae* isolates recovered from broilers and their handlers, suggesting possible transmission through direct contact and highlighting the role of poultry production environments in the dissemination of ESBL-producing *K. pneumoniae*. Modeling studies have also identified cross-contamination in a kitchen as a primary source of exposure to foodborne bacteria [29]. Therefore, good hand and kitchen hygiene are key control points for reducing the risk of exposure to foodborne bacterial pathogens such as *K. pneumoniae*.

In the present study, distinct genotypes were observed among the 15 human clinical strains and the single animal-origin strain, indicating genetic heterogeneity among these

isolates. Similar genetic diversity has been reported among *Klebsiella* isolates recovered from human, animal, and environmental sources in a genomic One Health study conducted in Ghana [30]. Conversely, close genetic relatedness between human- and animal-origin *K. pneumoniae* has also been reported. Menezes *et al.* [31], using whole-genome sequencing, identified transmission of ESBL-producing *K. pneumoniae* strains between companion animals and their cohabiting humans. Similarly, comparative genomic analysis of *K. pneumoniae* from the poultry production chain identified closely related multidrug-resistant lineages shared between poultry and human clinical isolates [32].

A major limitation of this study lies in the very limited number of isolates of animal and food origin included. Consequently, the observed genetic similarities should be interpreted with caution, as a larger and more representative sampling from non-clinical sources would be necessary to better assess the potential role of the food chain and animal reservoirs in the dissemination of ESBL-producing *K. pneumoniae*.

5. Conclusions

This study highlights significant genetic diversity among ESBL-producing *Klebsiella pneumoniae* isolates, as well as the presence of several dominant clonal lineages circulating among patients in various wards at Bouaké University Hospital in Côte d'Ivoire. These findings indicate significant clonal dissemination within the hospital environment, suggesting ongoing nosocomial transmission of multidrug-resistant strains. Limited genetic similarity was observed between a foodborne isolate and a small number of clinical isolates; however, this finding should be interpreted with caution due to the very limited number of non-clinical isolates analyzed. Overall, these results highlight the hospital's role as the primary reservoir of ESBL-producing *K. pneumoniae* in this study and underscore the need to strengthen infection prevention and control measures to limit their spread.

Abbreviations

E. coli	Escherichia Coli
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
ESBL	Extended-Spectrum Beta-Lactamase
K. pneumoniae	<i>Klebsiella Pneumoniae</i>

Author Contributions

Nonfra Marie Tuo: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft

Gnagra Marie-Thérèse Wognin: Writing – review & editing

Abdoulaye Diarrassouba: Supervision

Chantal Akoua-Koffi: Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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