Scientific Research Publishing

Advances in Microbiology, 2024, 14, 416-429
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Prevalence and Study of the Clonality of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* Strains in Neonatology at the University Hospitals of Abidjan by the Pulsed Field Gel Electrophoresis and the Quantitative Antibiogram

Valérie M'Bengue Gbonon[1,2], Sidjè Arlette Afran[1,3], Stanislas Assohoun Egomli[4], Djeda Franck Arnaud Gnahoré[2,5], Aboubakar Sylla[6], N'Golo David Coulibaly[2,6], Nathalie Guessennd[1], Assanvo Simon-Pierre N'Guetta[3], Mireille Dosso[1,2,6]

[1]Antibiotics, Natural Substances and Anti-Infectious Resistance Surveillance Unit, Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire
[2]Molecular Genetics Platform, Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire
[3]Genetics and Species Improvement Laboratory, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire
[4]Mechanics and Computer Sciences Laboratory, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire
[5]Biology and Health Laboratory, Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire
[6]Molecular Biology Platform, Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire
Email: valeriecarole@yahoo.fr

How to cite this paper: M'Bengue Gbonon, V., Afran, S.A., Egomli, S.A., Gnahoré, D.F.A., Sylla, A., Coulibaly, N.D., Guessennd, N., N'Guetta, A.S.-P. and Dosso, M. (2024) Prevalence and Study of the Clonality of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* Strains in Neonatology at the University Hospitals of Abidjan by the Pulsed Field Gel Electrophoresis and the Quantitative Antibiogram. *Advances in Microbiology*, **14**, 416-429.
https://doi.org/10.4236/aim.2024.149030

Received: June 27, 2024
Accepted: September 8, 2024
Published: September 11, 2024

Abstract

Background: ESBL-producing strains of *Klebsiella pneumoniae*, one of the main causes of nosocomial and hospital-acquired infections, are commonly associated with therapeutic impasses. Surveillance of these multidrug-resistant pathogens is a crucial tool for controlling and preventing infections. This surveillance involves the use of appropriate molecular and phenotypic typing techniques. The choice of techniques is based on criteria such as discriminatory power, intra- and inter-laboratory reproducibility, epidemiological concordance, ease of use and cost. The aim of our study was to identify clusters of Extended-Spectrum Beta-Lactamase-producing *Klebsiella pneumoniae* (ESBL-*K. pneumoniae*) strains circulating in neonatology using quantitative antibiogram (QA) and Pulsed Field Gel Electrophoresis (PFGE). **Materials and Methods**: This cross-sectional study included 55 *K. pneumoniae* strains isolated from a total of 513 samples. These various samples are taken from newborns, healthcare personnel, and the environment. *K. pneumoniae*

identification followed standard bacteriological procedures and was confirmed using the Vitek® 2 (bioMérieux). The detection of the ESBL phenotype was performed using the synergy test. QA and PFGE were used to identify clonal relationships between the various strains isolated. Concordance between these two methods was assessed by calculating Cohen's KAPPA coefficient and Simpson's diversity index. **Results**: Among the 55 *K. pneumoniae* strains included in this study, 58.2% (32/55) were found to be Extended-Spectrum Beta-Lactamase (ESBL) producers. Most of these strains were isolated from neonatal samples (blood samples and rectal swabs). The quantitative antibiogram method applied to 28 out of the 32 ESBL-producing strains revealed that the isolates were grouped into 5 clusters. Pulsed Field Gel Electrophoresis performed on a total of 16 ESBL-producing strains showed the existence of four profiles. A perfect concordance was observed between the two methods. **Conclusion:** The results of this study highlighted the existence of clonal strains of various origins within neonatology units.

Keywords

Resistance-Clone-*Klebsiella pneumoniae*-Pulsed Field Gel Electrophoresis-Quantitative Antibiogram

1. Introduction

In 2017, the World Health Organization (WHO) classified Extended-Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (ESBL-E) as priority pathogens, urgently requiring new antibiotics (WHO, 2017) [1]. Among these ESBL-E, *Klebsiella pneumoniae* (*K. pneumoniae*) takes a prominent position, being implicated in nosocomial and community-acquired infections (Tufa *et al*., 2022 [2]; Tang *et al*., 2023 [3]; Oliveira *et al*., 2022) [4]. Like other ESBL-producing *Enterobacteriaceae*, ESBL-producing *Klebsiella pneumoniae* (Kpn-ESBL) strains exhibit increased resistance to the majority of beta-lactams, fluoroquinolones, and aminoglycosides reducing the therapeutic options and even leading to therapeutic impasses. In high-risk hospital units such as neonatology, ESBL-producing *K. pneumoniae* strains often cause outbreaks, posing a threat to the survival of hospitalized newborns (Melati *et al*., 2022 [5]; Pillay *et al*., 2021) [6].

The prevalence of ESBL-producing *K. pneumoniae* strains varies significantly from one region to another. In Africa, studies conducted particularly in Ghana in 2023 and Kenya in 2021 reported respective rates of 9.5% and 95% of ESBL-producing *K. pneumoniae* strains in diverse infections (Saïdou *et al*., 2023 [7]; Michodigni *et al*., 2021) [8]. In Côte d'Ivoire, according to data from the National Reference Center for antibiotics (CNR-ATB), the rate of ESBL production in human-origin *K. pneumoniae* was 35.16% in 2021 (PAN-RAM, 2021) [9].

Surveillance of these pathogens is a crucial tool for controlling and preventing infections, requiring the use of molecular and phenotypic typing methods. The choice of methods is a fundamental aspect of this surveillance (Simar *et al*., 2021)

[10], often based on criteria such as discriminatory power, intra- and inter-laboratory reproducibility, epidemiological concordance, ease of use, and cost (Van Belkum *et al*., 2007) [11].

The antibiogram is one of the phenotypic techniques traditionally used to test a bacterium's sensitivity to a selected panel of antibiotics. However, due to the lengthy execution time and high cost of reagents in common molecular techniques, a quantitative antibiogram (an approach based on calculating Euclidean distances from the diameters of inhibition zones for several antibiotics between pairs of strains) has been implemented. This tool has proven effective in successfully determining epidemiological clusters and controlling hospital epidemics. It also shows concordance with techniques such as Pulsed Field Gel Electrophoresis, MLST, and RAPD (Sloos *et al*., 1998 [12]; Moore et Goldsmith, 2001 [13]; Bendary *et al*., 2016 [14]; Jain *et al*., 2018 [15]; Morin-Le Bihan *et al*., 2023) [16].

The objective of our study was to identify clusters of ESBL-producing *K. pneumoniae* strains circulating in neonatology using quantitative antibiogram (QA) and Pulsed Field Gel Electrophoresis (PFGE).

2. Materials and Methods

Study Period, Type, and Site

From September 2020 to June 2021, a cross-sectional study was conducted in the Neonatology units of the Pediatric Services at the University Hospitals of Abidjan (Treichville (CHU-T), Cocody (CHU-C)) and at the Pasteur Institute of Côte d'Ivoire (IPCI).

Sampling

A total of 55 strains of *K. pneumoniae* isolated from a total of 513 samples were included in this study. These various specimens consisted of 150 blood samples for blood culture, 125 rectal swabs in newborns; 26 nasal swabs and 26 hand swabs from healthcare personnel; and 186 environmental swabs (medical equipment, inert surfaces).

In newborns, blood samples for blood culture were taken from newborns with suspected infections by venipuncture or heel stick. These newborns were then subjected to rectal swabbing by inserting a swab previously moistened with sterile physiological water into the rectum. In healthcare personnel with written consent, the fingers, palms and nasal walls were swabbed using a swab previously moistened with sterile physiological water. In the environment, square portions of 10 × 10 cm^2 of flat, dry surfaces such as tables, workbenches, the inner and outer walls of incubators and bed covers were swabbed using perpendicular and parallel striations. Other environmental components (taps, door handles, oxygen masks and probes, blood pressure cuffs and aspirators) were swabbed by friction. All these samples were collected using swabs moistened with sterile physiological water.

Methods

Bacterial Identification and Detection of ESBL Production

Identification of *K. pneumoniae* strains followed classical bacteriological procedures and was confirmed using the Vitek® 2 system (bioMérieux). The method of detection of ESBL production consisted of the use of the synergy between two discs on the standard antibiogram, *i.e.* a disc of cefotaxime, ceftazidime and a disc containing clavulanic acid (e.g., amoxillin/clavulanic acid: AMC) 30 mm apart from the cephalosporin discs on Mueller-Hinton agar. The presence of an ESBL is expressed by the appearance of a "champagne cork" synergy between the disc containing clavulanic acid and the cephalosporin (CASFM/EUCAST).

Clusters Analysis of *K. pneumoniae* Using Quantitative Antibiogram

The quantitative antibiogram (QA) was conducted in two steps, involving Principal Component Analysis (PCA) and Hierarchical Agglomerative Clustering (HAC) using R software. Euclidean distances were calculated from the diameters of inhibition zones for a panel of 15 antibiotics (amikacin, amoxicillin/clavulanic acid, aztreonam, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, nalidixic acid, and trimethoprim/sulfamethoxazole) for *K. pneumoniae* strains. Smaller Euclidean distances indicate the resemblance between strains while greater distances the dissimilarity between them. The Ward method was applied to the distance matrix to generate dendrograms.

Pulsed Field Gel Electrophoresis (PFGE)

Pulsed Field Gel Electrophoresis was performed following the PulseNet Protocol, 2017 (CDC, 2017) [17]. Bacterial suspensions were prepared from a suspension buffer (100 ml 1M Tris, pH 8, 200 ml 0.5M EDTA, pH 8, and 1000 ml ultrapure water). A volume of 400 µl of bacterial suspension was mixed with 20 µl proteinase K and 400 µl 1% low-melting-point agarose LFTM/PFGE, previously melted. The mixture was poured into mold wells, forming plugs that were submerged in a cell lysis buffer (50 ml 1 M Tris, pH 8, 100 ml 0.5 M EDTA, pH 8, 100 ml 10% N-lauroylsarcosine, sodium salt, and 1000 ml ultrapure water) and incubated for 1 hour and 30 minutes at 54˚C.

The agarose blocks were then subjected to two successive washes with ultrapure water and four washes with TE buffer (10 ml 1 M Tris, pH 8, 2 ml 0.5M EDTA, pH 8, and 1000 ml ultrapure water). Each plug was cut into two equal-length slices, subsequently digested with *Xba* I restriction enzyme (10 U/ml) (Promega Corporation). The slices were loaded into a 1% low-melting-point agarose LFTM/PFGE gel and migrated for 15 hours in the CHEF Dr III system (BIORAD) with a tank filled with 2.2 L of 0.5X TBE. The migration conditions included an initial pulse time of 2.2 s, a final pulse time of 54.2 s, a voltage of 6 V/cm, and an included angle of 120˚.

After migration, the gel was stained in a mixture of 400 ml ultrapure water and 40 µl Syber Safe 10,000 X for 30 minutes, then destained in 500 ml ultrapure water for 20 minutes. Gel images were captured using the Gel Doc EZ Imager system (BIORAD). Interpretation followed the criteria established by Tenover *et al*., 1995 [18].

Statistical Methods

Concordance Test

The concordance between the two techniques was assessed using the calculation of Cohen's Kappa coefficient (K). The interpretation of Kappa values was as follows:

- <0: No concordance
- 0 - 0.20: Slight concordance
- 0.21 - 0.40: Possible concordance
- 0.41 - 0.60: Moderate concordance
- 0.61 - 0.80: Significant concordance
- ≥0.81: Perfect concordance

Simpson's Diversity Index

The discriminative power of the two methods was evaluated by calculating the Simpson's Diversity Index (D). A higher value of D indicates greater discriminative power, with values closer to 1.

Ethical Considerations

This study obtained approval from the National Committee of Ethics in Life and Health Sciences (CNESVS) under the reference IRB000111917. For hand and nasal swabs, all participants provided written consent. Regarding newborns, the collected samples adhered to ethical guidelines, ensuring informed consent was obtained.

General Results

Among the different strains of *K. pneumoniae*, 32.7% (18/55) and 29.1% (16/55) were respectively isolated from rectal swabs and blood samples in newborns (Table 1). The presence of synergy between AMC and one of the G3C disks (indicating ESBL production) was observed in 58.2%, or 32/55 of the strains, with 87.5% (28/32) from CHU-C and 12.5% (4/28) from CHU-T. The highest prevalences were observed in clinical strains (28.1%) and rectal carriage strains (46.9%) in neonates (Table 2).

Table 1. Distribution of *K. pneumoniae* strains by type of sample.

Types of samples	Newborns		Healthcare personnel		Environment	Total
	Blood samples	Rectal swabs	Hand swabs	Nasal swabs	Swabs	
n (%)	16 (29.1%)	18 (32.7%)	4 (7.3%)	8 (14.5%)	9 (16.4%)	55 (100%)

Table 2. Distribution of ESBL-producing *K. pneumoniae* strains by type of sample.

Types of samples	Newborns		Healthcare personnel		Environment	Total
	Blood samples	Rectal swabs	Hands swabs	Nasal swabs	Swabs	
n (%)	9 (28.1%)	15 (46.9%)	1 (3.1%)	2 (6.3%)	5 (15.6%)	32 (100%)

Quantitative Antibiogram

All strains of ESBL-producing *K. pneumoniae* (ESBL-*K. pneumoniae* strains) were selected for quantitative antibiogram analysis. However, due to limitations in the software used, only strains from the neonatology unit of CHU-C could be discriminated. The results revealed the existence of five clusters during the study period (Figure 1).

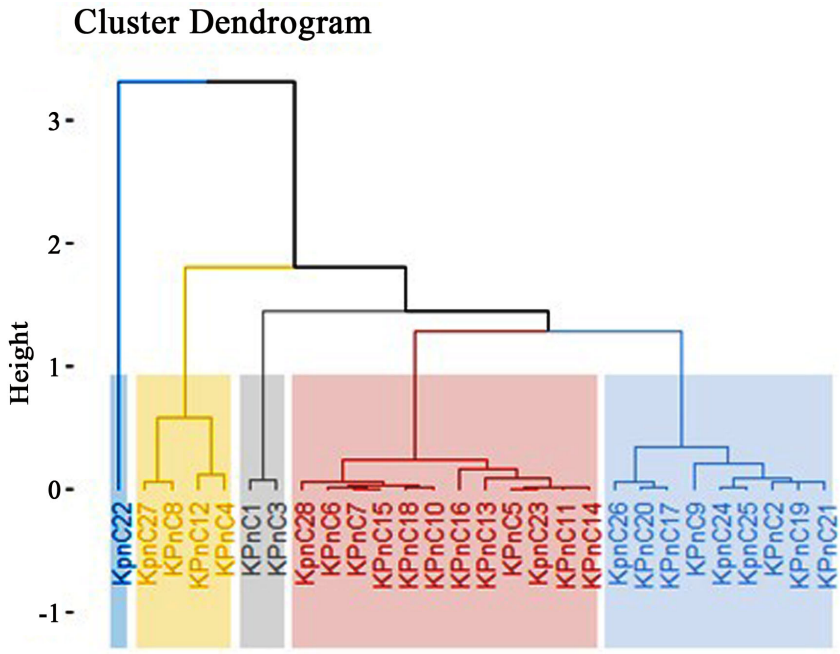

Figure 1. Dendrogram representing similarities among ESBL-producing *K. pneumoniae* strains from the Cocody University Hospital Center.

Kpn C1, Kpn C2, KpnC3, …Kpn C28: K. pneumoniae strains isolated.

C1, C2, C3, C4, and **C5**: Different QA clusters obtained.

Phenotypic and Epidemiological Characteristics of Clusters Generated by Quantitative Antibiogram (QA)

Cluster 1 was significantly associated with high inhibition diameters for amoxicillin/clavulanic acid and low for levofloxacin ($p < 0.05$). The cluster comprised four clinical strains responsible for neonatal sepsis and eight strains isolated from newborn rectal swabs, nasal swabs and environmental colonization, and collected during the period from February 08 to June 1, 2021. The presence of the cluster was observed in all functional subunits (premature newborn ward, full-term newborn ward and intensive care unit).

Cluster 2 was characterized by strains with high inhibition diameters for levofloxacin, imipenem and nalidixic acid ($p < 0.05$). Within it were grouped two clinical strains and seven strains from rectal swabs and healthcare equipment colonization isolated between January 25 and June 03, 2021. The cluster's presence was reported in the various functional subunits.

Cluster 3 comprised four strains, three of which were responsible for neonatal

septicemia and one for swabbing healthcare equipment (oxygen mask from a neonate). Characterized mainly by high inhibition diameters for trimethroprime/sulfamethoxazole and low for imipenem ($p < 0.05$), the strains were isolated between January 28 and June 08, 2021. In contrast to clusters 1 and 2, cluster 3 was detected only in premature infant wards.

Cluster 4 comprised two clinical strains, all of which caused neonatal septicemia and were collected one day apart (January 25 and 26, 2021). The isolates were significantly associated with high inhibition diameters for gentamycin, cefotaxime and meropenem, and low for amikacin ($p < 0.05$). This cluster was reported in the intensive care unit and in the inpatient ward of full-term newborns.

Cluster 5 comprised a single strain of manuported origin. It was characterized by relatively high inhibition diameters for penicillins ($p < 0.05$).

The epidemiological characteristics of the various clusters are shown in **Table 3**.

Pulsed-field electrophoresis (PFGE) Typing

Pulsed-field electrophoresis was applied to 16 of the 32 ESBL-*K. pneumoniae* strains selected. Of these isolates, nine strains from CHU-C and three from CHU-T were correctly typed. Strains from CHU-C showed three profiles (A1, A2 and B2), as did those from CHU-T, where three genotypes (A1, A2 and B1) were identified, making a total of four profiles (A1, A2, B1 and B2). The number of bands present on these profiles ranged from 11 to 17 (**Figure 2**).

Over the majority of strains (66.7%; 8/12) had the 16-band profile (profile A1), with an almost exclusive presence at CHU-C. These strains were isolated from rectal swabs (50%; 4/8) and blood samples (25%; 2/8) from neonates. This profile was also identified in a nasal carriage strain from a member of the healthcare personnel, and in a strain isolated from healthcare equipment (oxygen mask). The first strain in this cluster (KPnT3) was isolated from a premature neonate born in a peripheral health center and admitted to CHU-T on the day of his birth, with samples taken on day 2 of hospitalization in September 2020. The last strains KPn C28 and KPn C17 isolated respectively from oxygen mask swabs and rectal swabs in another premature newborn, admitted on the first day of hospitalization in May 2021 at CHU-C, exhibited the same clone as the previously described neonate. These findings highlight the presence of a hospital clone characterized by ubiquity, inter-hospital distribution, and persistence over time.

A profile of 17 bands (Profile A2) was identified in two strains from rectal swabs (KPn T1 and KPn C11), isolated six months apart and from different study sites. The other strains (KPn T2 and KPn C23) with respective pulsotypes B1 (13 bands) and B2 (11 bands) from the environment and rectal swab were collected at different times and study sites.

Phenotypically, regardless of the PFGE profiles obtained, the strains exhibited the same antibiotic resistance profile. Sensitivity, however, was observed in the presence of amikacin and carbapenems (imipenem and meropenem).

Table 3. Epidemiological characteristics of QA of ESBL-*K. pneumoniae* strains.

Clusters	Strains	Types of samples	Dates of isolation	Admission wards
1 (n = 12)	KPn C5	Blood	February 12, 2021	Intensive care unit
	KPn C6	Blood	April 07, 2021	Intensive care unit
	KPn C7	Blood	April 07, 021	Premature newborn ward
	KPn C10	Rectal swab	February 08, 2021	Premature newborn ward
	KPn C11	Rectal swab	March 25, 2021	Premature newborn ward
	KPn C13	Rectal swab	March 26, 2021	Premature newborn ward
	KPn C14	Blood	April 07, 2021	Premature newborn ward
	KPn C15	Rectal swab	April 07, 2021	Premature newborn ward
	KPn C16	Rectal swab	April 28, 2021	Full-term newborn ward
	KPn C18	Rectal swab	June 01, 2021	Premature newborn ward
	KPn C23	Nasal swab	March 02, 2021	Consultations ward
	KPn C28	Oxygen mask	May 04, 2021	Premature newborn ward
2 (n = 9)	Kpn C2	Blood	February 25, 2021	Intensive care unit
	Kpn C9	Blood	June 01, 2021	Intensive care unit
	Kpn C17	Rectal swab	May 04, 2021	Premature newborn ward
	Kpn C19	Rectal swab	May 31, 2021	Consultations ward
	Kpn C20	Rectal swab	June 03, 2021	Premature newborn ward
	Kpn C21	Rectal swab	June 08, 2021	Full-term newborn ward
	Kpn C24	Nasal swab	March 02, 2021	Premature newborn ward
	Kpn C25	Oxygen mask	June 03, 2021	Premature newborn ward
	Kpn C26	Oxygen mask	June 03, 2021	Premature newborn ward
3 (n = 4)	Kpn C4	Blood	January 28, 2021	Premature newborn ward
	Kpn C8	Blood	June 08, 2021	Premature newborn ward
	Kpn C12	Blood	March 25, 2021	Premature newborn ward
	Kpn C27	Oxygen mask	March 25, 2021	Premature newborn ward
4 (n = 2)	Kpn C1	Blood	January 25, 2021	Intensive care unit
	Kpn C3	Blood	January 26, 2021	Full-term newborn ward
5 (n = 1)	Kpn C22	Hand swabs	March 05, 2021	Consultations ward

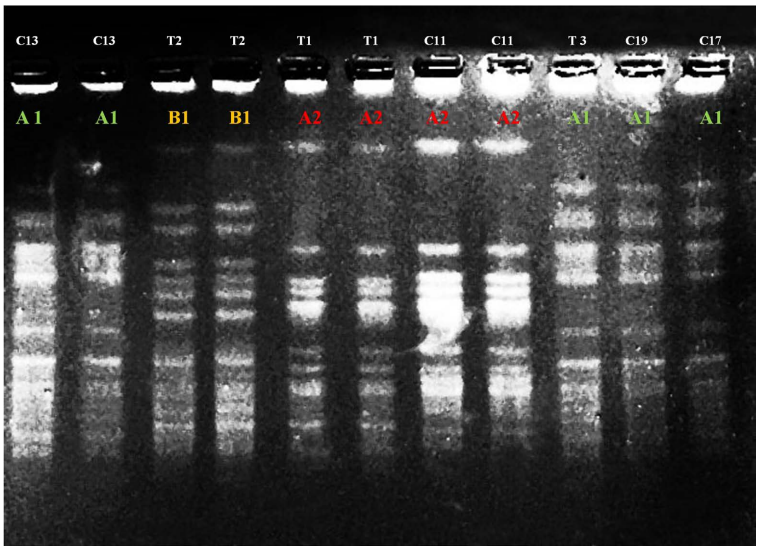

Figure 2. Electrophoretic profile of pulsotypes PFGE obtained of ESBL-*K. pneumoniae* strains.

C13 (KPnC13), **T2** (KPnT2), **T1** (KPnT1), **C11** (KPnC11**), T3** (KPnT3), **C19** (KPnC19) and **C17** (KPnC17**)**: pulsotypes obtained after enzymatic digestion with the *XbaI* restriction enzyme of seven ESBL-*K. pneumoniae* strains.

A1, A2 and B1: different PFGE profiles identified.

Combined and comparative analysis of profiles generated by quantitative antibiogram and pulsed-field electrophoresis

Combined and comparative analyses were carried out on strains with both QA and PFGE profiles. A total of nine strains from CHU-C were considered. The results showed that these isolates were grouped into three QA clusters (Clusters 1, 2 and 4) and presented three PFGE profiles (A1, A2, B2).

Cluster 1 comprised one clinical strain and four carriage strains with different PFGE profiles (A1, A2 and B2), with a high prevalence of the A1 profile (3/5, 60%). Clusters 2 and 4 respectively comprised three carriage strains and one clinical strain with the same PFGE profile (profile A1) (Table 4).

For the comparative analysis, statistical tests showed a similarity between the discriminating power of the two methods using Simpson's diversity index D_{QA} = D_{PFGE} = 0.99). Beyond this similarity, there was perfect concordance between QA and PFGE (K = 1.2).

Table 4. Combined QA and PFGE results of ESBL-*K. pneumoniae* strains.

AQ Profiles	Strains	PFGE Profiles	Types of samples	Dates of isolation	Admission wards
Cluster 1 (n = 5)	Kpn C13	**A1**	Rectal swab	March 26, 2021	Premature newborn ward
	Kpn C28		Oxygen mask	May 04, 2021	Premature newborn ward
	Kpn C14		Blood	April 07, 2021	Premature newborn ward
	Kpn C11	**A2**	Rectal swab	March 25, 2021	Premature newborn ward
	Kpn C23	**B2**	Nasal swab	March 02, 2021	Consultations ward
Cluster 2 (n = 3)	Kpn C19	**A1**	Rectal swab	May 31, 2021	Consultations ward
	Kpn C17		Rectal swab	May 04, 2021	Premature newborn ward
	Kpn C24		Nasal swab	March 02, 2021	Premature newborn ward
Cluster 4 (n = 1)	Kpn C3		Blood	January 26, 2021	Full-term newborn ward

3. Discussion

The use of molecular typing methods is essential in the epidemiological surveillance of Multidrug-Resistant Organisms (MDROs) and in determining the phylogenetic relationships between strains. The selection of one or more of these methods depends on discriminative power, stability, and reproducibility, as well as the problem to be addressed and the epidemiological context in which the method (s) will be applied (Sabat *et al*., 2013) [19].

PFGE is one of the most widely applied techniques for typing nosocomial and community pathogens (Pegah and Rashid, 2023 [20]; Elahi *et al*., 2019) [21]. Until the emergence of whole-genome sequencing, this technique was considered the *gold standard* of molecular methods (Neoh *et al*., 2019) [22]. Based on the study of restriction patterns in total bacterial DNA fragments, PFGE had a considerable

impact on the field of molecular biology, making it possible to separate and migrate large molecules. Despite its success, due in large part to its discriminatory power and high epidemiological concordance, several constraints on its use have been cited: long run times, high cost of reagents, non-standardized protocols and the need for highly skilled personnel.

Considered an alternative to molecular techniques and developed in the USA in 1982 by Flournoy (Flournoy, 1982) [23], QA has successfully typed strains of *E. coli*-ESBL, *K. pneumoniae*-ESBL, MRSA, *Staphylococus epidermidis* (Sloos *et al*., 1998 [12]; Moore and Goldsmith, 2001 [13]; Bendary *et al*., 2016 [14]; Morin-Le Bihan *et al*., 2023 [16]). Its advantages include the molecular characterization of bacterial species, especially in confirming suspected epidemics, ease of implementation, and accessibility to all laboratories (Moore and Goldsmith, 2001) [13].

Although it is an easy and fast method to implement, its execution requires, among other things, qualitative data from antibiograms, software to generate dendrograms using this data, and epidemiological data for cluster analysis. For some authors, factors such as the choice of antibiotics, restriction to strains with well-known epidemiological context, and the estimation of a cutoff threshold are crucial in obtaining better results (Blanc *et al*., 1994) [24]. In another study, these same authors suggested selecting antibiotics based on the following criteria: (i) the determinants of antibiotic resistance must be independent and (ii) the number of antibiotics selected must be minimal (Blanc *et al*., 1996) [25].

In this study, QA performed on ESBL-*K. pneumoniae* strains in the neonatology unit of the Pediatrics department at CHU-C showed the existence of five clusters composed of 12, nine, four, two, and one strains during the period from January 25 to June 8, 2021. In Clusters 1 and 2, strains were found in all functional subunits and in various types of samples. In Clusters 3, 4, and 5, isolates were mostly of blood origin, with their presence detected either exclusively in the hospital wards of premature newborns (cluster 3), in intensive care units and in the hospital wards of full-term newborns (cluster 4), or in consultations wards (Cluster 5).

Although QA is useful for discriminating bacteria, some authors recommend combining it with other molecular typing methods to validate the obtained results (Blanc *et al*., 1996) [25]. Applying this approach in our study, pulsed-field gel electrophoresis (PFGE) identified four profiles. It was also found that eight out of the 13 typed strains had profile A1. These strains were isolated from a rectal swab at CHU-T on September 15, 2020, and from various types of samples at CHU-C from January 13 to June 08, 2021. This situation could be explained by a potential inter-hospital spread of strains facilitated by the transfer of patients between hospitals. However, during the study period, there were no transfers of newborns between the two hospital structures. The most likely explanation would be the exchange of healthcare personnel. Profiles B1 and B2 have been identified in two and one strains, respectively, all of them carriage strains. Their distribution could be described as sporadic.

In order to assess a potential correlation between our two methods, we conducted comparative and combined analyses of the results obtained from strains tested with both AQ and PFGE. A total of nine strains were involved, among which three clusters and three different profiles were described. Our results showed that AQ was equally discriminant as PFGE. Furthermore, we observed that AQ grouped seven out of eight strains presenting the same PFGE profile into each of the different clusters. This result is interesting as these clusters showed a closer proximity to each other on the dendrogram generated by AQ.

This observation highlights the existence of variations in the size of the diameters of the zones of antibiotic inhibition in similar strains. The presence of strains with different PFGE profiles (A1, A2 and B2) was noted within the same cluster (Cluster 1). This result is probably due to the fact that, according to the criteria of Tenover and colleagues, the isolates would be closely related, and the number of bands obtained in the B2 profile (11 bands) would be linked to genetic alterations (Tenover *et al*., 1995) [18].

The KAPPA test and Simpson's diversity index used to compare the data obtained indicated a perfect concordance between the two techniques. Other authors have also showed a high correlation between quantitative antibiogram and the performed molecular typing method (Bearson *et al*., 2004 [26]; Sloos *et al*., 2000) [27].

Limitations

In this study, we encountered problems in subculturing of *K. pneumoniae* strains, which explains why only 16 isolates out of a total of 28 could be typed. Other difficulties, such as obtaining a suitable weight marker to assess the size of the obtained bands, were also encountered.

4. Conclusion

The results of this study highlighted the existence of clonal ESBL-*K. pneumoniae* strains from various sources within the neonatology units. The data provided by quantitative antibiogram showed a perfect concordance with the results of pulsed-field gel electrophoresis. The combined approach of these two methods could significantly contribute to epidemiological surveillance and the management of nosocomial infections.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Organisation Mondiale de la Santé (2017) Who Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

[2] Tufa, T.B., Mackenzie, C.R., Orth, H.M., Wienemann, T., Nordmann, T., Abdissa, S.,

et al. (2022) Prevalence and Characterization of Antimicrobial Resistance among Gram-Negative Bacteria Isolated from Febrile Hospitalized Patients in Central Ethiopia. *Antimicrobial Resistance & Infection Control*, **11**, Article No. 8. https://doi.org/10.1186/s13756-022-01053-7

[3] Tang, P., Chen, X., Xiao, F., Hu, Y., Hao, J., Tian, J., Liu, W., Zhang, Y. and Liu, P. (2023) Multidrug-Resistant Gram-Negative *Bacilli* in Neonatal Intensive Care Unit in South China. https://doi.org/10.21203/rs.3.rs-3106796/v1

[4] Oliveira, R., Castro, J., Silva, S., Oliveira, H., Saavedra, M.J., Azevedo, N.F., *et al*. (2022) Exploring the Antibiotic Resistance Profile of Clinical Klebsiella Pneumoniae Isolates in Portugal. *Antibiotics*, **11**, Article 1613. https://doi.org/10.3390/antibiotics11111613

[5] Melati, D., Utomo, M.T., Arif, M.T., Etika, R., Puspitasari, D. and Aden, T.Y. (2022) Outbreak of Extended-Spectrum Beta-Lactamase-Producing Klebsiella Pneumoniae and Multidrug-Resistant Acinetobacter Baumannii in the Neonatal Intensive Care Unit, Dr. Soetomo General Hospital, Indonesia: A Case Series. *Sri Lanka Journal of Child Health*, **51**, 294-298. https://doi.org/10.4038/sljch.v51i2.10138

[6] Pillay, D., Naidoo, L., Swe Swe-Han, K. and Mahabeer, Y. (2021) Neonatal Sepsis in a Tertiary Unit in South Africa. *BMC Infectious Diseases*, **21**, Article No. 225. https://doi.org/10.1186/s12879-021-05869-3

[7] Saïdou, M.S.F., Ousmane, A., Inoussa, A., Bachir, A.M., Boureima, H., Gora, L., *et al*. (2023) Prevalence of Extended-Spectrum Beta-Lactamase-Producing Strains Isolated at Zinder National Hospital (ZNH) in 2021 and Their Antibiotic Susceptibility Profile. *Open Journal of Medical Microbiology*, **13**, 43-51. https://doi.org/10.4236/ojmm.2023.131004

[8] Michodigni, N.F., Nyachieo, A., Akhwale, J.K., Magoma, G. and Kimang'a, A.N. (2021) Molecular Identification of Co-Existence of Carbapenemase and Extended-Spectrum β-Lactamase Genes in *Klebsiella pneumoniae* Clinical Isolates, and Their Phylogenetic Patterns in Kenya. *Advances in Microbiology*, **11**, 399-415. https://doi.org/10.4236/aim.2021.118030

[9] Guessennd-Kouadio, N., Diane, K.M., M'Bengue Gbonon, V. and Dadié, A.T. (2021) Plan d'Action National multisectoriel de lutte contre les Résistances aux Anti-Microbiens (PAN-RAM) 2021-2025. https://faolex.fao.org/docs/pdf/ivc221871.pdf

[10] Simar, S.R., Hanson, B.M. and Arias, C.A. (2021) Techniques in Bacterial Strain Typing: Past, Present, and Future. *Current Opinion in Infectious Diseases*, **34**, 339-345. https://doi.org/10.1097/qco.0000000000000743

[11] van Belkum, A., Tassios, P.T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N.K., *et al*. (2007) Guidelines for the Validation and Application of Typing Methods for Use in Bacterial Epidemiology. *Clinical Microbiology and Infection*, **13**, 1-46. https://doi.org/10.1111/j.1469-0691.2007.01786.x

[12] Sloos, J.H., Horrevorts, A.M., Van Boven, C.P. and Dijkshoorn, L. (1998) Identification of Multiresistant Staphylococcus Epidermidis in Neonates of a Secondary Care Hospital Using Pulsed Field Gel Electrophoresis and Quantitative Antibiogram Typing. *Journal of Clinical Pathology*, **51**, 62-67. https://doi.org/10.1136/jcp.51.1.62

[13] Moore, J.E. and Goldsmith, C.E. (2001) Phenotyping of *Campylobacter jejuni* and *Campylobacter coli* by a Quantitative Antibiogram [MIC] Typing Scheme Using Euclidean Distances [QATED]. *BMC Microbiology*, **1**, Article No. 13. https://doi.org/10.1186/1471-2180-1-13

[14] Bendary, M.M., Solyman, M.S., Azab M.M., Mahnoud N.F. and Hanora A.M. (2016) Genetic Diversity of Multidrug Resistant Staphylococcus Aureus Isolated from

Clinical and Nonclinical Samples in Egypt. *Cellular and Molecular Biology*, **62**, 55-61.

[15] Rajput, M.S., Jain, K. and Gurjar, A.S. (2020) Control of Pseudomonas Aeruginosa Blood Stream Infection Outbreak in Neonatal Intensive Care Unit by Quantitative Antibiogram. *Indian Journal of Microbiology Research*, **5**, 378-381. https://doi.org/10.18231/2394-5478.2018.0079

[16] Morin-Le Bihan, A., Le Neindre, K., Dejoies, L., Piau, C., Donnio, P. and Ménard, G. (2023) Use of the Quantitative Antibiogram Method for Assessing Nosocomial Transmission of ESBL-Producing Enterobacterales in a French Hospital. *Journal of Hospital Infection*, **135**, 132-138. https://doi.org/10.1016/j.jhin.2023.01.023

[17] Centers for Disease Control and Prevention (2013) Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157: H7, *Escherichia coli* non-O157 (STEC), *Salmonella serotypes*, *Shigella sonnei* and *Shigella flexneri*. https://pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf

[18] Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., *et al*. (1995) Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing. *Journal of Clinical Microbiology*, **33**, 2233-2239. https://doi.org/10.1128/jcm.33.9.2233-2239.1995

[19] Sabat, A.J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J.M., Laurent, F., *et al*. (2013) Overview of Molecular Typing Methods for Outbreak Detection and Epidemiological Surveillance. *Eurosurveillance*, **18**, Article 20380. https://doi.org/10.2807/ese.18.04.20380-en

[20] Pegah, R. and Rashid, Z. (2023) Molecular Epidemiology Typing of blaOXA-48 and blaNDM-1 Producing *Klebsiella pneumoniae* Causing Nosocomial Infections. *New Microbiology*, **46**, 278-284.

[21] Elahi, A., Akya, A., Chegene Lorestani, R., Ghadiri, K. and Baakhshii, S. (2019) Molecular Typing of Klebsiella Pneumoniae Isolated from Medical Centers in Kermanshah Using Pulse Field Gel Electrophoresis. *Archives of Pediatric Infectious Diseases*, **7**, e84331. https://doi.org/10.5812/pedinfect.84331

[22] Neoh, H., Tan, X., Sapri, H.F. and Tan, T.L. (2019) Pulsed-Field Gel Electrophoresis (PFGE): A Review of the "Gold Standard" for Bacteria Typing and Current Alternatives. *Infection, Genetics and Evolution*, **74**, Article 103935. https://doi.org/10.1016/j.meegid.2019.103935

[23] Flournoy, D.J. (1982) Quantitative Antibiogram as a Potential Tool for Epidemiological Typing. *Infection Control*, **3**, 384-387. https://doi.org/10.1017/s0195941700057349

[24] Blanc, D.S., Lugeon, C., Wenger, A., Siegrist, H.H. and Francioli, P. (1994) Quantitative Antibiogram Typing Using Inhibition Zone Diameters Compared with Ribotyping for Epidemiological Typing of Methicillin-Resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, **32**, 2505-2509. https://doi.org/10.1128/jcm.32.10.2505-2509.1994

[25] Blanc, D.S., Petignat, C., Moreillon, P., Wenger, A., Bille, J. and Francioli, P. (1996) Quantitative Antibiogram as a Typing Method for the Prospective Epidemiological Surveillance and Control of MRSA: Comparison with Molecular Typing. *Infection Control and Hospital Epidemiology*, **17**, 654-659. https://doi.org/10.2307/30141299

[26] Bearson, B.L., Labarca, J.A., Brankovic, L.E., Cohen, M., Bruckner, D.A. and Pegues, D.A. (2004) Use of Quantitative Antibiogram Analysis to Determine the Clonality of Coagulase-Negative Staphylococcus Species from Blood Culture. *Clinical Microbiology*

and Infection, **10**, 148-155. https://doi.org/10.1111/j.1469-0691.2004.00753.x

[27] Sloos, J.H., Dijkshoorn, L., Vogel, L. and van Boven, C.P.A. (2000) Performance of Phenotypic and Genotypic Methods to Determine the Clinical Relevance of Serial Blood Isolates of *Staphylococcus epidermidis* in Patients with Septicemia. *Journal of Clinical Microbiology*, **38**, 2488-2493. https://doi.org/10.1128/jcm.38.7.2488-2493.2000