Scientific Research Publishing

American Journal of Molecular Biology, 2024, 14, 230-244
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Impact of Genetic Diversity of Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* Strains on the Dissemination of Extended Spectrum Beta-Lactam Resistance Genes in Côte d'Ivoire

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How to cite this paper: Abe, I.A., N'Djetchi, M.K., Traore, M.B., Yao, F., Konan, T.K., Sokouri, P.D., Konate, I. and Koffi, M. (2024) Impact of Genetic Diversity of Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* Strains on the Dissemination of Extended Spectrum Beta-Lactam Resistance Genes in Côte d'Ivoire. *American Journal of Molecular Biology*, **14**, 230-244.
https://doi.org/10.4236/ajmb.2024.144017

Received: August 13, 2024
Accepted: September 26, 2024
Published: September 29, 2024

Abstract

The increase and spread of bacterial resistance to extended-spectrum beta-lactam antibiotics are reported in many infections and are a real public health problem worldwide. Drug pressure is a factor that favors the emergence of a population of better adapted bacteria. However, there is no literature highlighting the genetic diversity and evolutionary structure of *E. coli* and *K. pneumoniae* in an environment with high selection pressure in Côte d'Ivoire. The objective of this study was to evaluate the genetic diversity of *E. coli* and *K. pneumoniae* strains circulating at the HKB Hospital in Abobo and at the Daloa Regional Hospital and its impact on the dissemination of extended spectrum beta-lactam resistance genes. A total of 39 strains isolated from the urinary tract of infected patients, including 30 strains of *E. coli* and 9 strains of *K. pneumoniae* were studied. A total of 39 strains isolated from the urinary tract of infected patients, including 30 strains of *E. coli* and 9 strains of *K. pneumoniae* were studied. From genomic DNA extracts, ESBL resistance genes were amplified by PCR and sequenced, in addition to genetic typing by ERIC-PCR. The data obtained were submitted to genetic and bioinformatics analyses. The results have shown a genetic diversity important in *E. coli* and *K.*

pneumoniae with diversity indexs (SID) ranging from 0.5 to 0.77. The genetic structure of the bacterial species studied has shown a clonal distribution of strains with clones expressing TEM-9 and CTX-M-15 variants. Also, this clonal structure was correlated with the spread of resistance genes in *E. coli* and *K. pneumoniae*. The spread of resistant clones is a factor that might limit the fight against antibiotic resistance.

Keywords

Escherichia coli, Klebsiella pneumoniae, Extended-Spectrum *β*-Lactam, Antibiotic Resistance, Genetic Diversity

1. Introduction

As soon as the first bacterial infections appeared, the methods of antibacterial fight were oriented towards antibiotic therapy. This approach has led to a remarkable decrease in mortality and morbidity from bacterial infections worldwide [1]. Thus, antibiotics have been considered powerful weapons against any bacterial infection. However, as and when new antibiotics are developed and used irresponsibly, bacteria progressively accumulate genes in their genetic material that code for resistance to several of these drugs [2] [3]. According to the World Health Organization (WHO), antimicrobial resistance is a real and growing threat to global health [4]. The extended-spectrum beta-lactamase resistance mechanism is the most common in the world and is generally observed in enterobacteria [5]. Indeed, in a hostile environment generated by drug pressure, bacteria adapt by developing mutational resistance mechanisms that might favor the rapid expression of new, more adapted genotypes or resistance acquired by mobile genetic elements such as plasmids, transposons and integrons [6]. This induced antibiotic pressure, exerted differently in different geographical locations, as well as the adaptive value of bacteria, suggest resistance patterns that vary between strains of the same bacterial species. In this way, drug pressure might contribute to the emergence of a genetically diverse bacterial population that could compromise the control of bacterial infections. In this background, the knowledge of the level of genetic diversity and the influence of the evolutionary structure of bacterial species, in particular of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* on the diffusion of extended-spectrum beta-lactam resistance genes are necessary for a good surveillance of antibiotic resistance. In Côte d'Ivoire, previous studies have reported the spread of *E. coli* and *K. pneumoniae* strains phenotypically producing extended-spectrum beta-lactamases (ESBLs) in various infections [7] [8]. However, none attempt to explain the maintenance and spread of antibiotic resistance by the genetic diversity of bacterial strains. Thus, this study aimed to evaluate the genetic diversity of *E. coli* and *K. pneumoniae* strains responsible for urinary tract infections circulating at the Henriette Konan Bedie Hospital in Abobo

and at the Daloa Regional Hospital and its impact on the dissemination of extended spectrum beta-lactam resistance genes.

2. Material and Methods

2.1. Study Sites

This cross-sectional study was carried out in two hospitals belonging to different epidemiological facies: the Henriette Konan Bedie Hospital (HKB-Abobo) in Abobo municipality, located in north Abidjan and the Regional Hospital Center of Haut-Sassandra (CHR-Daloa) located in Daloa department in western Côte d'Ivoire. These two sites were chosen in order to compare and understand the trend of drug selection pressure on uropathogens in different epidemiological facies.

2.2. Ethics Considerations

The study protocol was reviewed and approved by the National Ethics Committee of Life Sciences and Health in Côte d'Ivoire with the number: N/Ref: 106-18/MSHP/CNESVS-KM, US DPT OF HHS REGISTRATION: IORG00075 on 30th July 2018. The consent was obtained from patients and/or guardians after explaining the objective of the study. The laboratory results were communicated to patients via physicians for better antibiotic prescription.

2.3. Study Design and Sample Collection

The sampling of this prospective and descriptive study was performed from October 2018 to April 2019 at HKB and from May to October 2019 fat CHR Daloa. During this period, a total of 39 strains including 30 strains of *Escherichia coli* and 9 strains of *Klebsiella pneumoniae* were isolated from fresh urine samples from patients with urinary tract infections.

2.4. Isolation and Identification of Bacterial Strains

The uropathogenic bacterial strains were isolated on CHROMAgar Orientation (Becton Dickinson, Cockeysville, MD), chromogenic medium [9]. The identification of *E. coli* and *K. pneumoniae* species was performed using Gram staining tests and classical biochemical tests such as indole, oxidase, catalase, urease, tryptophan deaminase, glucose and lactose fermentation, production of gases from glucose fermentation, degradation of hydrogen peroxide by the production of hydrogen sulfide, use of citrate as the sole source of carbon, motility, lysine deaminase and lysine decarboxylase production [10].

2.5. Molecular Characterization of Strains Collected

2.5.1. DNA Extraction

The extraction of genomic DNA from *E. coli* and *K. pneumoniae* strains was performed using the standard phenol-chloroform extraction and ethanol precipitation methods.

2.5.2. Genotyping of ESBLs

The following primers *bla*TEM, *bla*SHV and *bla*CTX-M were used to amplify specific DNA sequences involved in resistance to broad-spectrum beta-lactam antibiotics by PCR (Table 1). Total reaction volume of 50 µL containing 1µL of each primer of 10 pmol/µL (Eurogentec, Belgium), 5 µL of PCR buffer (10×) with $MgCl_2$, 2.5 µL of deoxyribonucleoside triphosphates (dNTPs, 200 µM), 0.1 µL of *Taq polymerase* (Qiagen), 37.4 µL of ultrapure water and 3 µL of bacterial genomic DNA was mixed. This PCR mixture was performed in a thermal cycler under the following conditions: initial denaturation for five minutes at 94˚C followed by 30 cycles consisting of denaturation at 94˚C for 45 seconds, hybridization at 60˚C for one minute and elongation at 72˚C for one minute followed by final elongation at 72˚C for 10 minutes. Products of this PCR amplification were migrated on 2% agarose gel for 45 minutes under an electric voltage of 100 volts. This part has already been published in a scientific journal.

Table 1. Primers used for the screening of genes coding for the production of broad-spectrum beta-lactamases.

Genes		Primers Sequences (5'- 3')	Amplicon size (bp)
*bla*TEM	R	CTCAAGGATCTTACCGCTGTTG	112
	F	TTCCTGTTTTTGCTCACCCAG	
*bla*CTX-M	R	TTTATCCCCCACAACCCAG	701
	F	AATCACTGCGTCAGTTCAC	
*bla*SHV	R	CGCAGATAAATCACCACAATG	768
	F	TCGCCTGTGTATTATCTCCC	

2.5.3. Genotyping of *E. coli* and *K. pneumoniae* Strains by ERIC-PCR

Genotyping of bacterial strains was performed using the ERIC-PCR technique to estimate the genetic diversity of *Escherichia coli* and *Klebsiella pneumonia*. This technique uses universal primers, which are intergenic repetitive consensus sequences (ERIC) naturally present in several copies in the genome of enterobacteria and which variation takes into account the strain and its geographical origin. The primers used for this purpose are ERIC-R: ATG-TAA-GCT-CCT-GGG-GAT-TCA-C et ERIC-F: AAG-TAA-GTG-ACT-GGG-GTG-AGC-G described by [11]. The amplification was performed in a final volume of 25 µL including 1.3 µL of each primer of 10 pmol/µL concentration (Eurogentec, Belgium), 2.5 µL of PCR buffer (10×) with $MgCl_2$ (Qiagen), 1.6 µL of dNTPs (200 µM), 0.1 µL of Taq polymerase (Qiagen), 2.5 µL of bacterial genomic DNA (30 ng/µL) and 15.6 µL of ultra-pure water. PCR was performed under the following conditions: an initial five minutes' denaturation at 94˚C followed by 30 cycles including denaturation at 94˚C for 45 seconds, hybridization at 60˚C for one minute and elongation at 72˚C for one minute. An elongation at 72˚C for 10 minutes completed the amplification. Products of this PCR amplification were migrated on 3% agarose gel for two hours 30 minutes under an electric voltage of 80 volts.

2.5.4. Sequencing of ESBL resistance genes

Bidirectional sequencing of BLSE-positive PCR products (*bla*TEM, *bla*CTX-M

and *bla*SHV) was performed by BGI TECH SOLUTIONS (HONG-KONG) using the ABI PRISM 3730 automated sequencer (Applied Biosystems).

3. Data Analysis

3.1. Sequences Analysis

Nucleotide sequences were compared with reference sequences in the Genbank database of the National Center for Biotechnology Information (NCBI) using the local alignment program BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi), to identify blaCTX-M and blaTEM gene variants detected as causing ESBL resistance. This part has already been published in a scientific journal [12].

3.2. Simpson's Index of Diversity

Simpson's diversity index (SID) was used to determine the level of diversity in the bacterial populations studied, particularly in populations of uropathogenic *Escherichia coli*. This index measures the probability that two independent isolates are of different genotypes.

This diversity was measured using the following equation:

$$SID = \frac{1-\sum n(n-1)}{N(N-1)} \quad (1)$$

where N is the total number of different genotypes in the population and n is the total number of profiles with the same allele type [13].

3.3. Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) was performed to estimate the distribution of genetic variation between and within different populations of *Escherichia coli* to determine the origin of the estimated genetic variability [14]. This analysis was performed using GenALEX version 6.5 software [15].

3.4. Cluster Analysis by UPGMA (Unweighted Pair Group Method with Arithmetic Mean)

A classification of bacterial strains in the form of a dendrogram was made according to the method based on the grouping of unweighted pairs with the arithmetic mean (UPGMA). This method was carried out from the similarity coefficients of Jaccard similarity coefficients obtained. In this study, the 85% threshold was arbitrarily chosen to group the strains studied. Thus, two strains were considered to belong to the same group when they shared a similarity of at least 85%. The packages ade4 and Vegan of the software R version 4.1.2 [16] were used to calculate Jaccard's similarity index and the realization of the dendrograms. However, the package heatmap3 were used to realize the heatmaps.

3.5. Statistical Tests

Student's t-test was used to investigate the effect of site on the variation in the

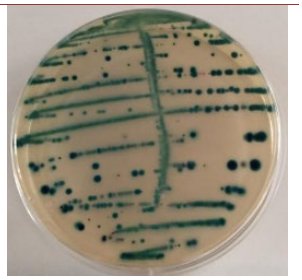
number of ERIC bands in *E. coli*. And the influence of site on the variation in the number of ERIC bands in *E. coli*. Significant differences are observed when the probability value (p) associated with the statistical tests is strictly less than 0.05.

4. Results

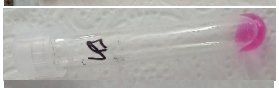
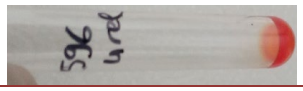
4.1. Identification of *Escherichia coli* and *Klebsiella pneumoniae* Isolates

Escherichia coli and *Klebsiella pneumoniae* isolates were identified on the basis of their cultural and biochemical characteristics. Table 2 shows the results of the biochemical tests performed. culture on CHROMAgar chromogenic medium shows that *E. coli* isolates are distinguished by large, dark pink colonies with or without a halo, while *K. pneumoniae* is characterized by medium-sized colonies colored metallic green. Tests carried out on Kligler Hajna agar indicate that *E. coli* and *K. pneumoniae* strains are bacteria capable to use glucose and lactose as a carbon source. This capacity translates into the fermentation of lactose and glucose, characterized by an acidification that turns phenolic red to yellow on the slope and in the bottom. Gas production by these two species of bacteria is evidenced by the detachment of the agar in the tubes in Table 2. In iron Lysine agar, *E. coli* and *K. pneumoniae* are able to produce lysine decarboxylase, characterized by the absence of a violet to yellow turn in the agar bottom. The difference between *E. coli* and *K. pneumoniae* can be seen in tests performed in Simmons' citrate, urea and indole media. Indeed, *E. coli* strains that positive for indol test (Indol+) are unable to produce urease (Urea−) and use citrate as a source of carbon and energy for their growth (Cit−). However, *K. pneumoniae* strains that are negative to the indol test (Indol−), are able to produce urease (Urea+) and use citrate as a source of carbon and energy for their growth (Cit+) (Table 2).

Table 2. Cultural and biochemical characteristics of *Escherichia coli* and *Klebsiella pneumoniae.*

		Escherichia coli		*Klebsiella pneumoniae*	
Biochemical tests	**Test medium**	**Test results**	**Experiential view**	**Test results**	**Experiential view**
Isolation	CHROMAgar	Pink colonies		Green colonies	
Glucose	Kligler Hajna agar	+		+	
Lactose	Kligler Hajna agar	+		+	
Gaze	Kligler Hajna agar	+		+	
H_2S	Kligler Hajna agar	−		−	
LDA	iron Lysine agar	−		−	
LDC	iron Lysine agar	+		+	

Continued

Citrate	Simmons'citrate agar	-		+	
Urease	Urea	-		+	
Indol	After addition of Kovac's reagent	+		-	

LDA: lysine deaminase; LDC: lysine decarboxylase; H_2S: hydrogen sulfide; (+): Positive reaction; (−): Negative reaction

4.2. Polymorphism of ERIC Marker in *E. coli* and *K. pneumoniae*

Thirty strains of *Escherichia coli*, nine strains of *Klebsiella pneumoniae*, collected in the two study sites were genotyped by ERIC-PCR method. Figure 1 shows the genomic fingerprints of *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from urine samples obtained by ERIC-PCR. In *E. coli*, the genomic fingerprints obtained showed highly variable DNA fragments with sizes ranging from 150 to 1050 bp and the number of profile bands ranging from two to five bands (Figure 1(A)). In *K. pneumoniae* the number and size of ERIC bands vary from 100 to 1300 bp with constituted profiles of three to five characteristic bands (Figure 1(B)).

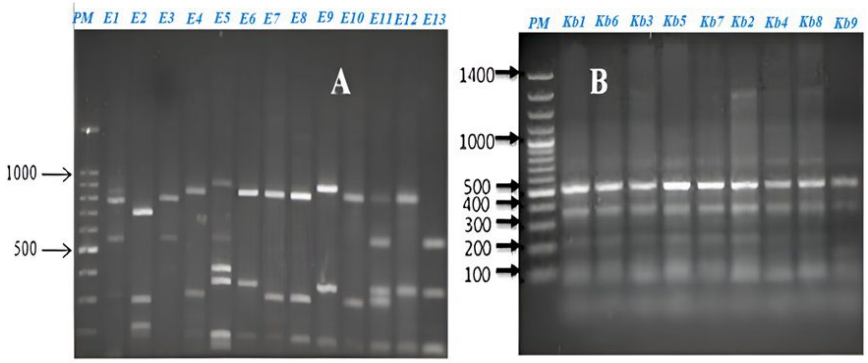


Figure 1. ERIC-PCR genomic fingerprinting of *Escherichia coli* (A) and *Klebsiella pneumoniae* (B) strains isolated from urine samples.

4.3. Measurement of the Genetic Diversity of the Bacterial Species Studied

Parameters for genetic diversity evaluation of bacterial populations studied are shown in Table 3 and Table 4. The genetic variability, characterized by a total number of 17 different bands was revealed in strains of *E. coli*. The number of bands in the genetic profiles varied from 2 to 5 with a mean of 2.9 ± 1.1 bands per genotype, obtained in the overall population of *E. coli* (Table 3). The mean polymorphism rate of the ERIC marker was estimated to 36.54 %. The mean Simpson's diversity index obtained was 0.60 ± 0.1 (Table 3). At the sites level, the results revealed that at the HKB hospital in Abobo, the mean number of ERIC bands obtained in the *E. coli* collection was 3.5 ± 0.5 with a polymorphism rate of

42.31%. The diversity index obtained was 0.70 ± 0.05. In the collection of *E. coli* strains from CHR Daloa, the genetic diversity measured indicated a mean number of 2.2 ± 0.7 bands for a polymorphism rate of 30.77%. The diversity index was 0.51 ± 0.01. According to Student's t-test, the level of genetic diversity of the two *Escherichia coli* populations (HKB Abobo and CHR Daloa) are significantly different ($p < 0.001$). Analysis of molecular variance (AMOVA) has confirmed the existence of significant genetic differentiation ($\Phi PT = 0.367$; $p = 0.001$) between the two populations of *Escherichia coli* (Table 4). *E. coli* strains from the HKB hospital in Abobo (SID = 0.70) have shown a higher level of genetic diversity than those from the CHR Daloa (SID = 0.51).

In the population of *Klebsiella pneumoniae*, the variations observed in the number and size of ERIC DNA fragments reflect significant genetic diversity. Overall, eight bands of different sizes were detected with a mean number of 4.6 ± 1.13 bands per profile. The polymorphism of the ERIC marker obtained in *K. pneumoniae* was 75% with a diversity index estimated to 0.77 ± 0.05 (Table 3).

Table 3. Measuring genetic diversity of uropathogenic *E. coli* and *K. pneumoniae* strains at the two collection sites.

Species studied	Sites	N	Nbfr	SID	Nbp	P (%)
E. coli	HKB Abobo	14	10	0.70 ± 0.05	3.5 ± 0,5	42.31
	CHR Daloa	16	7	0.51 ± 0.05	2.2 ± 0,7	30.77
	Total	30	17	0.60 ± 0.1	2.9 ±1,1	36.54 ± 577
	p value			0.0266	< 0.001	--
K. pneumoniae	HKB/CHR	9	8	0.77 ± 0.05	4.6 ± 1.13	75

N: number of strains collected; **Nbfr**: number of frequent bands; **SID**: Simpson's diversity index; **Nbp**: mean number of bands per ERIC profile; **P**: polymorphism of the ERIC marker; **p:** probability associated with Student's statistical test for two independent samples

Table 4. Molecular analysis of variance (AMOVA) within and between the two populations of uropathogenic *Escherichia coli*.

Source of variation	Df	SS	MS	VC	% of variation	Φ_{PT}	p value
Interpopulation	1	17.80	17.80	1.069	37		
Intrapopulation	28	51.59	1.843	1.843	63		
Total	29	69.40	--	2.911	100	0.367	0.001

Df: Degree of freedom; **SS**: Sum of squares**; MS**: Mean of squares; **VC**: Variance components; **Φ_{PT}**: genetic differentiation; **p**: associated probability

4.4. Genetic Structure and Distribution of Detected ESBL Gene Variants

Sequences analysis of the amplified *bla*TEM and *bla*CTX-M genes was revealed three variants: TEM-1, TEM-9 and CTX-M-15. Strains are considered to belong to the same group when they show at least 85% similarity. Thus, in *E. coli*, the results showed a clonal structure of strains with eleven clones and 13 different ERIC profiles. Six complexes (complex I to VI) were defined in the *E. coli* population. The strains from the HKB Abobo hospital in red (Figure 2), distributed for the most part in complexes II and III where they form clonal groups (four clones),

seem to be easily distinguishable from those of the CHR Daloa in blue (Figure 2) which are composed of six clones. The Complex II consisted of a single clone with the ERIC3 profile. Strains with this genetic profile produce TEM-9 type extended spectrum betalactamases (ESBL). In complex III, three clones of genetic profile ERIC4, ERIC5 and ERIC6 were obtained. The ERIC4 profile was related to the ERIC5 profile with 60% similarity. These clones were linked to the ERIC6 profile with 28.57% similarity. Thus, the ERIC5 profile, including 50% (4/8) of Complex III strains, consisted of ESBL-producing strains.

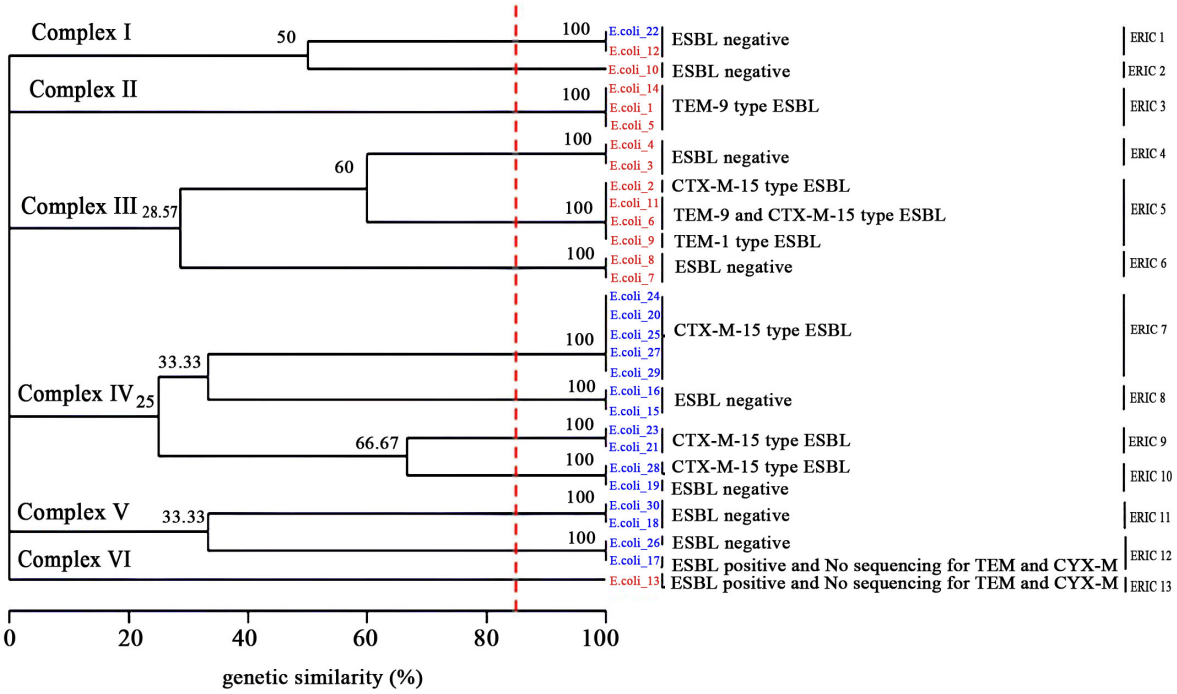


Figure 2. Dendrogram showing the genetic structure of the *Escherichia coli* population and the distribution of ESBL genes obtained by UPGMA method. Strains collected at HKB Abobo Hospital (red) and strains collected at Daloa Hospital (blue). The red line indicates the 85 % threshold.

Among these, *E. coli*_2 was a CTX-M-15 ESBL producer, *E. coli*_9 was a TEM-9 ESBL producer and *E. coli*_6 and *E. coli*_11 were producers of both TEM-9 and CTX-M-15 ESBL. In the *E. coli* population of CHR Daloa in blue, the genetic structure has showed a clonal distribution of strains in complexes IV and V. The complex IV was the most representative with four clones: ERIC7 (n = 5 strains), ERIC8 (n = 2 strains), ERIC9 (n = 2 strains), and ERIC10 (n = 2 strains). Strains of ERIC7 and ERIC9 profiles were all CTX-M-15 ESBL producers. In ERIC10 profile, only *E. coli*_28 was CTX-M-15 ESBL producer. In the complex I, the *E. coli*_12 strain from the HKB hospital in Abobo was genetically similar to the *E. coli*_22 strain from the CHR Daloa with an ERIC1 profile. However, they were not producers of extended spectrum beta-lactamases. In the overall population, 93.3% (28/30) of uropathogenic *Escherichia coli* strains studied were clones. However, the variation in ERIC profiles observed between clones showed genetic

variability in this *E. coli* population, and the distribution of ESBL gene variants appears to be clonal. In *K. pneumoniae*, the phylogenetic structure showed six ERIC profiles from 1 to 6 with the presence of two clones (ERIC 1 and ERIC 3), corresponding to 55.6 % of the strains and four unique ERIC profile (Figure 3). The strains collected at the HKB hospital in Abobo clustered in a clonal complex with 50% similarity. In ERIC 1 profile, the Kleb_5 or Kb_5 strain has shown the *bla*TEM and *bla*CTX-M resistance genes and the Kleb_8 or Kb_8 strain have produced a CTX-M-15 beta-lactamase (Figure 3). For the ERIC 3 profile, the Kleb_2 or Kb_2 strain has produced a TEM-9 ESBL. The unique strain from CHR Daloa differs from those of HKB hospital in Abobo.

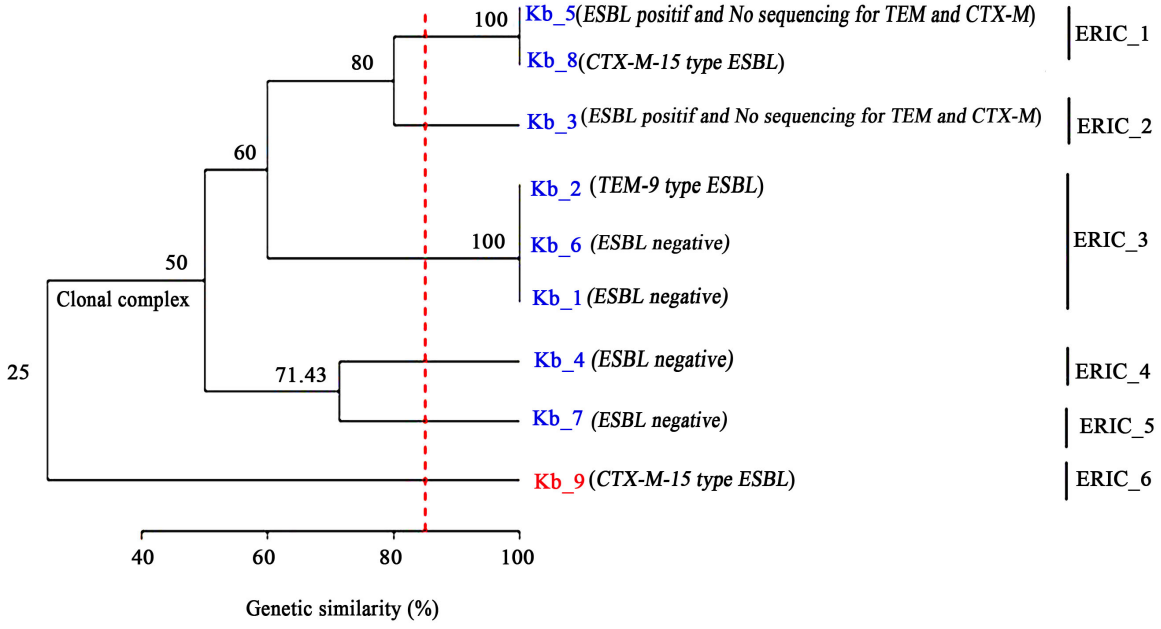


Figure 3. Dendrogram showing the genetic structure of the *Klebsiella pneumoniae* population obtained by UPGMA method. Strains collected at HKB Abobo Hospital (red) and strains collected at Daloa Hospital (blue). The red line indicates the 85 % threshold.

4.5. Correlation between Genetic Diversity and Antibiotic Resistance

On the basis of the results obtained, the null hypothesis that these two variables (the number of antibiotics to which the bacterium is resistant and the number of ERIC bands) are not linked was rejected ($p < 0.05$). The correlation coefficient values $r = 0.83$ for *E. coli* strains (Figure 4(B)) and $r = 0.75$ for *K. pneumoniae* (Figure 4(A)), show that the number of antibiotics to which uropathogenic bacterial strains are resistant and the number of ERIC bands are positively associated. in other words, strains with a high number of ERIC bands were resistant to a large number of antibiotics, and vice versa (Figure 4). Coefficient of determination (R^2) values above 0.50 indicate a good fit of the model applied. They also show that 57% and 69% of the variability in resistance profiles can be explained by the variability of ERIC profiles in *Klebsiella pneumoniae* and *Escherichia coli*, respectively.

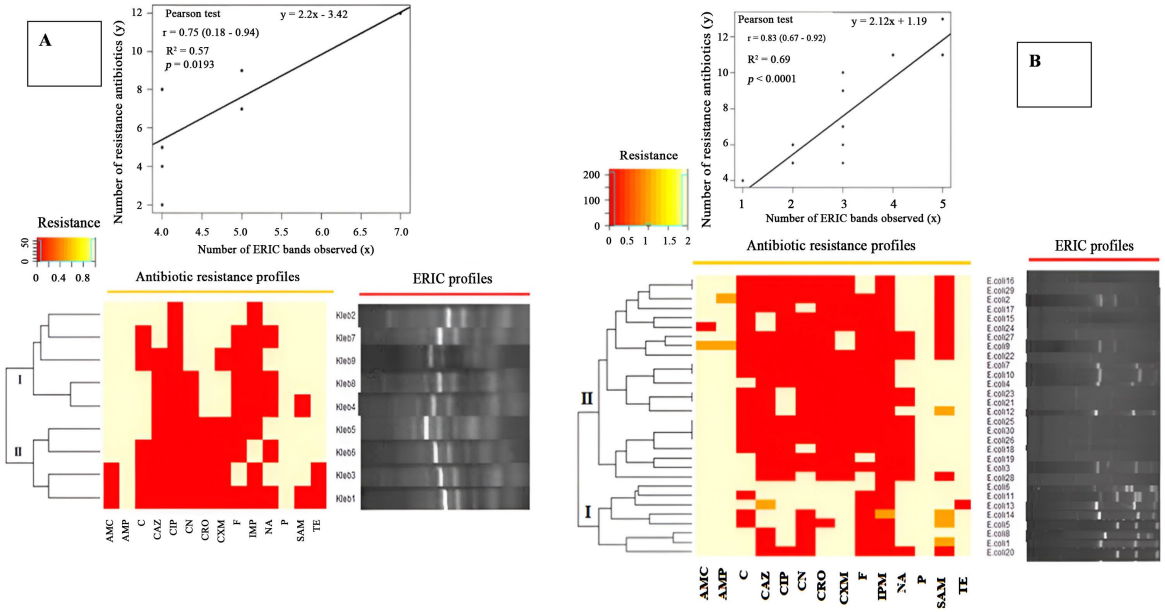


Figure 4. Correlation between genetic variation and resistance profile in *Klebsiella pneumoniae* (A) and *Escherichia coli* (B).

5. Discussion

In this study, 30 strains of *E. coli* and 9 strains of *K. pneumoniae* collected were analyzed by ERIC-PCR. DNA band sizes were ranged from 100 to 1300 base pairs. In addition to the variability observed in band sizes, thirteen polymorphic ERIC profiles with a number of bands ranging from 2 to 5 and six (06) profiles with 3 to 7 polymorphic bands were obtained in *E. coli* and *K. pneumoniae* respectively. The polymorphism rates and the variability observed in the number and size of the bands, observed in this study and reported by several authors, indicated that the ERIC-PCR technique seems to be powerful for strains differentiation [17] [18]. Comparable results were reported by Jena et al. in the *E. coli* population [19]. These authors obtained fourteen ERIC profiles consisted from 1 to 7 bands in a collection of 41 strains. Contrary to the results of this study with *K. pneumoniae* strains, in 2013, Barus et al. identified seventeen ERIC profiles in a collection of 61 *K. pneumoniae* isolates [20]. This difference might be justified by the difference in the number of samples analyzed. Indeed, only 9 strains of *K. pneumoniae* were analyzed in our study compared to 61 in the study conducted by these authors. Simpson's diversity index values of 0.60 in *E. coli* and 0.77 in *K. pneumoniae* are lower than those obtained by Gibreel, which ranged from 0.84 to 0.93 in uropathogenic *E. coli* [21]. This difference might be related to the techniques used. Indeed, the molecular typing technique used by this author is the multilocus sequencing typing (MLST) which remains more discriminating than the PCR typing techniques [22]. However, diversity index values upper 0.5 reflect and confirm significant genetic diversity of uropathogenic *E. coli* and *K. pneumoniae* strains in this study. Many studies using the ERIC-PCR method, have also shown a great genetic variability within the strains of these two bacterial species [23] [24]. This important

genetic diversity could be due to the selection pressure of the immediate environment which would force bacteria to adapt by the rapid expression of new genotypes and the emergence of a new population better adapted to its environment [24]. Analysis of molecular variance has indicated the existence of significant genetic differentiation between the two populations of *E. coli* (HKB and CHR) with significantly different in diversity index values. *E. coli* strains from HKB Abobo (SID = 0.70) appeared to be more diverse than those from CHR Daloa (SID = 0.51). These results indicated a geographic effect on the genetic structure of *E. coli* strains that might be explained by the large geographic distance between the two sites, which would limit gene exchange between study sites [23]. These results are consistent with those reported by Gibreel in England [21]. In addition, the differences between epidemiological facies might be in favour of highly variable levels of adaptation of the species studied. A clonal distribution of strains was observed in both sites indicating that the urinary tract infections, involving these two germs, detected in patients in this study were clonally transmitted. These results corroborate those of Durmaz *et al*. which work were focused on a collection of *E. coli* isolates from the Erciyes University Medical School Hospital in Turkey [25]. The results of this study also have revealed different ESBL-producing clones of TEM-9 and CTX-M-15 type [12]. The spread of genes encoding extended-spectrum beta-lactam resistance would be related to clonal transmission of strains [19]. Similar results were reported by [26]. These authors reported that the spread of the CTX-M-15 enzyme was associated with the spread of the *E. coli* ST410 clone, detected in human and animal samples. Several authors have reported the spread of the pandemic CTX-M-15 enzyme-producing *E. coli* O25b: H4-ST131 clone, detected in the environment, food, and animals [27] [28]. The clonal transmission of TEM-9 and CTX-M-15 variants might be the cause of therapeutic failures, which therefore requires special attention in antibiotic resistance surveillance programms. A significantly positive correlation was established between variation in the number of ERIC bands and variation in antibiotic resistance profiles in the uropathogens *E. coli* and *K. pneumoniae.* This strong correlation might be explained by the high selection pressure exerted by drugs, forcing bacteria to adapt by rapidly expressing new genotypes. According to Nguyen et al., the genetic variability of strains bacterial, correlated with antimicrobial resistance and virulence, is one of the limiting factors in the fight against bacterial infections [29].

6. Conclusion

This study showed significant genetic diversity of uropathogenic *E. coli* and *K. pneumoniae* and highlighted inter-population structure in *E. coli*. It also revealed a clonal distribution of uropathogenic strains that expressed extended spectrum beta-lactam resistance genes within the bacterial populations studied. Genetic diversity of bacterial species is therefore a determining factor to be considered in antibiotic resistance surveillance programs. The clonal spread of antibiotic-resistant strains of *Escherichia coli* and *Klebsiella pneumoniae* is a serious threat

worldwide, particularly in Côte d'Ivoire and results obtained should attract urgent action by health policy makers to respond effectively to the spread of these strains.

Acknowledgments

We acknowledge Mr Mel of HKB Hospital and Dr Monto of CHR Hospital for their support in sample collection. We are grateful to Germany's ESTHER Group for their financial support. We extend our gratitude to Professor Mathurin Koffi for providing access to his molecular biology laboratory to carry out the research work for this study. Thank to research group in Molecular Genetics and Epidemiology Research of Jean Lorougnon Guede University, Daloa (Côte d'Ivoire) for their technical support.

Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

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