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International Journal of Genetics and Molecular Biology

Full Length Research Paper

Genetic typing and *in silico* assignment of smoked and fresh bushmeat sold on markets and restaurants in west-central Côte d'Ivoire

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Poachers and bushmeat traders most of the time modify the wildlife materials before commercialization. Therefore, the morphological identification of species is no longer possible. However, accurate identification of the sale wildlife materials is essential for species identification, quantification and better conservation management. The aim of this study is to take advantages of specific gene sequences and *in silico* analysis to identify smoked and decapitated wildlife materials encountered on bushmeat markets and traditional restaurants in Center-Western Côte d'Ivoire. DNA from 352 bushmeat samples comprising 123 decapitated and smoked specimens was extracted, amplified by polymerase chain reaction (PCR) using the Cytb and COI primers and analyzed *in silico*. Up to 98.37% of the DNA from smoked bushmeat was successfully amplified. Assignment of Cytb and COI query sequences with reference sequences in the NCBI Genbank allowed to correct three misidentifications by traders. Thus, specimens identified as *Heliosciurus* sp, *Phacochoerus aethiopicus africanus* and *Dendrohyrax dorsalis sylvestris* by traders were assigned to *Funisciurus sp*, *Tragelaphus scriptus, Crossarchus obscurus* with 84.94, 100, 98.76% similarity respectively. Morphologically unidentifiable sample coded IP3CS1O was assigned to species *Eidolon helvum* with 99.53% similarity. The study shows the interest of DNA-based identification of bushmeat to circumvent misidentification for better management of wildlife biodiversity.

Key words: smoked bushmeat, misidentification, genetic typing, *in silico* analysis, identification, biodiversity, wildlife conservation.

INTRODUCTION

Remote rural populations have always fed on wild fauna without really impinging on the conservation of biodiversity (Dufour et al., 2015). Today, the exploitation of wildlife products commonly known as bushmeat is much more for profit than for personal animal protein need (Chabi-Boni et al., 2019). The wildlife materials from the hunted animals are commercialized in different amorphous forms, like smoked meat, chunks decapitated,

chopped meat, tanned skin, and so on (Kumar et al., 2014; Nishant et al., 2017). Due to the lack of morphological characters, identification of this wildlife material becomes inconclusive. Therefore, in order to recognize and accurately quantify poached wild animal for biodiversity and conservation purpose, it is important to use more sophisticated techniques which overcome morphological identification that has become impossible. Fortunately, advent of the use of genetic markers such as Cytochrome b, 12S ribosomal RNA, 16S ribosomal RNA, and Cytochrome c oxidase I are promising tools for species identification due to the bearing of nucleotide variation among the species (Hsieh et al., 2001; Panday et al., 2014; Kumar et al., 2019). In addition, millions of gene sequences are presently available online that permitted sequence similarity search to examine DNA data, generated from any biological material (Porter and Hajibabaei, 2018).

In Côte d'Ivoire, the district of Haut-Sassandra-Marahoué is rich in animal biodiversity in view of the existence of the national park of Marahoué and the protected reserves of Haut-Sassandra. However, with urban development, bushmeat preference by the urban populations and the evidence that the bushmeat trade is very lucrative, poachers are deeply attacking wildlife biodiversity (Yéboué et al., 2020). Thus, bushmeat is found on the markets and in restaurants in an amorphous way without identification possibility. However, it is more than essential to know the quantity of animals extracted from the bush to see the real intensity of biodiversity disturbance. The aim of the present study is to accurately identify smoked and decapitated bushmeat encountered on markets and traditional restaurants based on genetic markers and *in silico* analysis of DNA sequences.

MATERIALS AND METHODS

Collection of wildlife sample

352 specimens of bushmeat comprising 123 smoked or decapitated specimens (Figure 1) were collected with traders or on restaurants for accurate identification purpose. Questions were addressed to traders and restaurant owners to have the first information about the identity of smoked or decapitated species.

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Total genomic DNA extraction was performed using DNeasy® Tissue kit (Qiagen) according to manufacturer protocol and checked on 1% agarose gel for quality control. COI and Cytb primers were used (Table 1) to amplify partial mitochondrial DNA genes (Gaubert et al., 2014; Irwin et al., 1991; Kocher et al., 1989) PCR products representative of all species found according to field identification were coded and sent to BGI TECH SOLUTIONS (HONG-KONG) for sequencing.

DNA sequences assignment

DNA sequences provided in ABI format files were cleaned using Chromas Lite® 2.01 software to make them analyzable. The forward and reverse sequences of the genes were assembled and then corrected using DNA Baser Assembler 5.15.0 software and Chromas Lite® 2.01 software. *In silico* analysis of the sequences and phylogenetic characterization of the bushmeat species are made. Query sequences were aligned with reference sequences by submitting them to the public genomic database of the National Center for Biotechnology Information (NCBI) through its BLAST tool (Basic Local Alignment Search Tool; http://www.ncbi.nih.gov) (Altschul et al., 1990). The purpose of this alignment is to search areas of strong similarity between query and reference sequences existing in the public Genbank. For each alignment, identity percentage and a corresponding E-value are generated. The assignment selected is that of the coding sequence with the highest percentage of identity or similarity.

RESULTS

DNA amplification success rate

347 (98.58%) and 346 (98.30%) of the 352 specimens collected were successfully amplified respectively by Cytb and COI primers. Up to 98.37% (121/123) of the DNA from smoked bushmeat collected were successfully amplified (Figure 2).

Sequences assignment for species identification

According to the information gave by the traders and our wildlife knowledge, the 50 representative specimens sent for sequencing are composed of sixteen species divided into eight orders: the order of Rodentia (05 species), Artiodactyla (04 species), Carnivora (03 species), Primates (01 species), Pholidota (01 species), Lagomorpha (01 species), Hyracoïdea (01 species), and Chiroptera (01 species). Alignment of Cytb and COI genes sequences of studied specimens, with reference sequences available in the NCBI Genbank revealed three misidentifications. Thus, the specimens morphologically identified as *Heliosciurus* sp was assigned to *Funisciurus sp*, with 84.94% similarity for the Cytb gene. The specimen of Artiodactyla code VP2PH1C, identified in the field as *Phacochoerus aethiopicus africanus*, was assigned to species *Tragelaphus scriptus* with 100% similarity with the Cytb gene (Table 2). The specimen initially identified as *Dendrohyrax dorsalis sylvestris* was assigned to *Crossarchus obscurus* with 98.76% similarity after alignment of its COI gene sequence. The sample coded IP3CS1O, collected in a smoked decapitated state

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Figure 1. Bushmeat sold on markets and restaurants. A, B and C are smoked and decapitated specimens. D, E and F are whole specimens.

Table 1. Characteristics of primers used for COI and Cytb genes amplification.

Gene	Amorces	Primers sequences (5’- 3’)	References	Amplicon size (pb)
COI	COI-R	TCAGGGTGTCCAAARAAYCA	Gaubert et al. (2014)	658
	COI-F	CACAAACCACAAAGAYATYGG		
Cytb	Cytb-R	CTCAGAATGATATTTGTCCTCA	Irwin et al. (1991)	402
	Cytb-F	GATATGAAAAACCATCGTG	Kocher et al. (1989)	

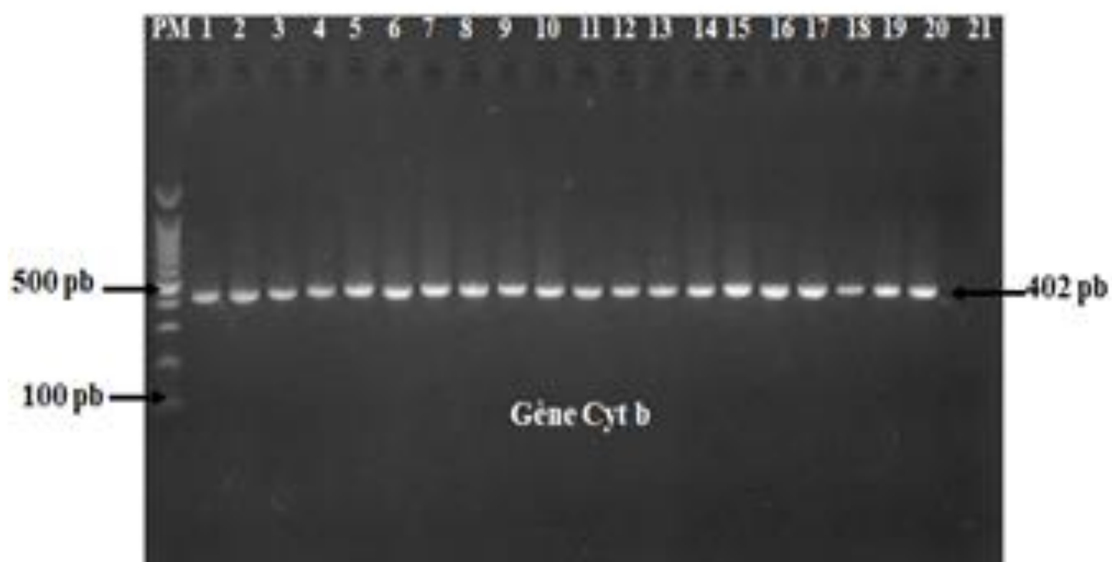

Figure 2. Electrophoresis gel showing PCR amplification products observed with the genetic markers Cytb. From left to right: well N° 1=PM (Molecular weight marker, 100 bp), wells N° 2 to 20=specimens Cytb 402 bp DNA product and well N° 21=PCR negative control.

Table 2. Taxonomic assignment of bushmeat specimens based on DNA sequences similarity.

Oders	Assignment of species according to cytb and COI gene sequences					
	Species identified empirically	**N**	**Cytb**	**% Ident**	**COI**	**% Ident**
Rodentia	*Atherurus africanus*	3	*Atherurus africanus*	98.75	*Atherurus africanus*	98.44
Rodentia	*Thryonomys swinderianus*	8	*Thryonomys swinderianus*	99.5	*Thryonomys swinderianus*	99.69
Rodentia	*Cricetomys gambianus*	4	*Cricetomys gambianus*	99.50	*Cricetomys gambianus*	99.53
Rodentia	*Heliosciurus* sp	1	*Funisciurus sp*	84.94	NA	-
Rodentia	*Xerus erythropus*	4	*Xerus erythropus*	99.00	*Xerus erythropus*	98.60
Artiodactyla	*Philantomba* sp	5	*Philantomba maxwellii*	100	*Philantomba maxwellii*	100
Artiodactyla	*Cephalophus dorsalis*	1	*Cephalophus* sp	100	*Cephalophus sp*	99
Artiodactyla	*Tragelaphus scriptus*	4	*Tragelaphus scriptus*	100	*Tragelaphus scriptus*	99.84
Artiodactyla	*Phacochoerus aethiopicus africanus*	1	*Tragelaphus scriptus*	100	NA	-
Carnivora	*Civettictis civetta*	5	*Civettictis civetta*	100	*Civettictis civetta*	99.69
Carnivora	Genette sp	3	*Genetta pardina*	100	*Genetta pardina*	100
Carnivora	*Galerella sanguinea*	2	*Galerella sanguinea*	94.09	NA	-
Primates	*Cercopithecus petaurista*	2	*Cercopithecus petaurista*	97.65	*Cercopithecus petaurista*	98.11
Pholidota	*Manis tricuspis*	2	*Manis tricuspis*	100	*Manis tricuspis*	99.69
Lagomorpha	*Lepus sp*	1	*Lepus microtis*	97.25	*Lepus microtis*	98.76
Hyracoidea	*Dendrohyrax dorsalis sylvestris*	1	NA	-	*Crossarchus obscurus*	98.76
Chiroptera	NA	3	*Eidolon helvum*	99,53	NA	-

that could not be identified morphologically was assigned to the species *Eidolon helvum* with 99.53% similarity after alignment of its Cytb gene sequence (Table 2). The remained species were accurately identified by traders according to DNA sequences assignment.

Taxonomic structure of poached species

The phylogenetic relationships of the specimens are described through dendrograms built according to the Neighbor-Joining model on the sequences of the Cytb and COI gene sequences generated. Analysis showed very narrow genetic distances between the specimens, defined in 15 taxonomic units: *Civettictis civetta*, *Genetta pardina*, *Galerella sanguinea*, *Manis tricuspis*, *Cercopithecus petaurista*, *Eidolon helvum*, *Funisciurus sp*, *Atherurus africanus*, *Xerus erythropus*, *Philantomba maxwellii*, *Cephalophus dorsalis*, *Thryonomys swinderianus*, *Tragelaphus scriptus*, *Lepus microtis* and *Cricetomys gambianus*. Specimen coded VP2PH1C, identified as *Phacochoerus aethiopicus africanus*, was found in the taxon *Tragelaphus scriptus* and shares a genetic similarity of 81% with specimen VP3G4R of this taxon (Figure 3).

Considering the COI gene, phylogenetic analysis of DNA sequences allowed the definition of 13 taxonomic groups comprising *Philantomba maxwellii*, *Cephalophus dorsalis*, *Tragelaphus scriptus*, *Xerus erythropus*, *Cricetomys gambianus*, *Dendrohyrax arboreus*, *Atherurus africanus*, *Manis tricuspis*, *Civettictis civetta*, *Genetta pardina*, *Lepus microtis*, *Thryonomys swinderianus* and *Cercopithecus petaurista* (Figure 4). Phylogenetic trees generated by the COI and Cytb genetic markers were in agreement in the assignment of the species observed except for the two species which could not be analyzed with the COI marker. A low bootstraps value is sometimes observed within the same taxonomic group due to intraspecific genetic diversity (Figures 3 and 4).

DISCUSSION

The precise identification of the wild species present on the bushmeat markets is a crucial concern for actors involved in the control and

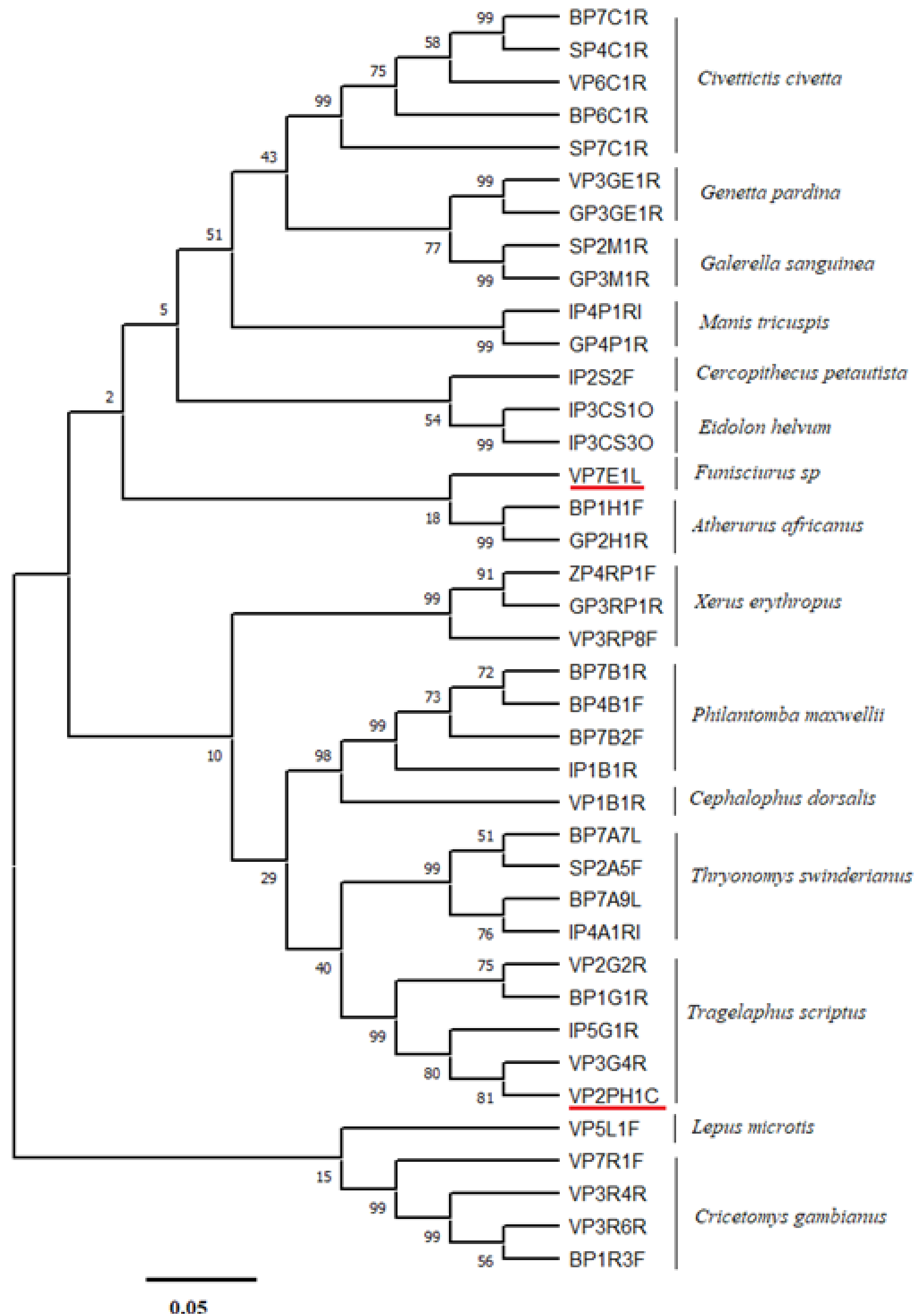

Figure 3. Phylogenic tree generated according to the Neighbor-Joining model on the sequences of the mitochondrial gene Cytb, showing the genetic diversity and the genetic relationships between the identified species.

management of animal biodiversity (Ogden et al., 2009). Indeed, a large part of the bushmeat is sold in the form of carcasses or pieces of cut or smoked meat, which makes it difficult if not impossible to identify the species.

Underestimating the diversity of species sold in bushmeat markets in different forms results in underestimating the number and biomass of game species impacted by illegal hunting activity and trade. The possibility of identifying

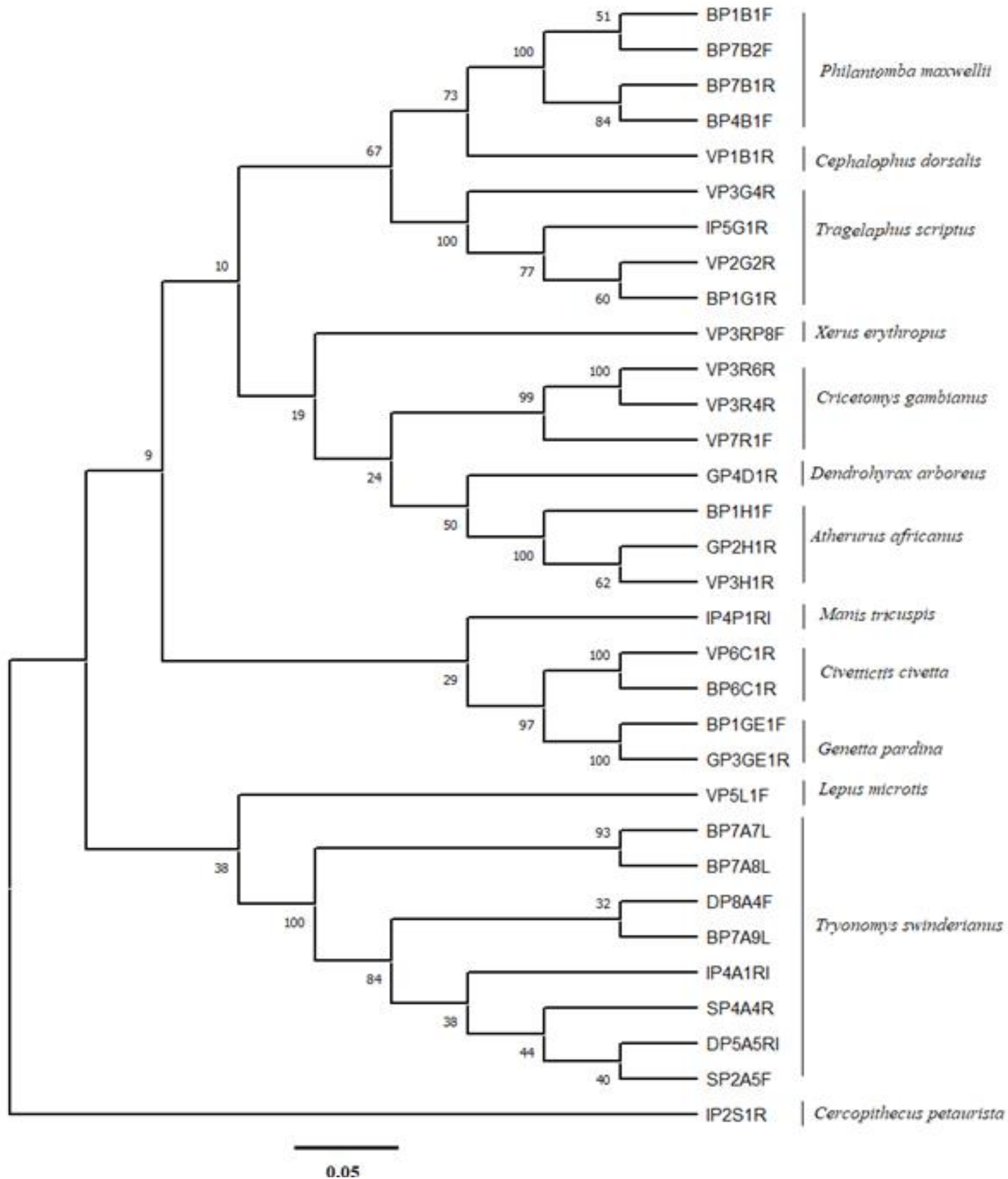

Figure 4. Phylogenic tree generated according to the Neighbor-Joining model on the sequences of the COI mitochondrial gene, showing the genetic diversity and the genetic relationships between the species identified.

products of wild fauna via molecular genetic tools, with reference to global genomic databases (Ross et al., 2003; Ratnasingham and Hebert, 2007) is an innovation in the quantification of danger to wildlife in the reserves and national parks of Côte d'Ivoire.

In this study, molecular analysis using mitochondrial gene markers, COI and Cytb yielded an amplification success rate over 90% as predicted by Gaubert et al., (2014). One of the main steps in developing an accurate protocol for genetic analysis is choosing an appropriate area of the genome for analysis, which will ensure a reliable diagnosis. In the mitochondrial genome of animal species, COI and Cyt b are more slowly evolving genes in terms of non-synonymous mutations, making them very suitable for the diagnosis of unique genetic signatures of species (Silva-Neto et al., 2016). In this

study 15 species of mammals were discriminated with the Cyt b gene and 13 species with the COI gene. These results show that the Cytb gene is the most resolute marker. Similar studies have shown that the Cytb marker is the most efficient in molecular identification of mammalian species (Eaton et al., 2010; Olayemi et al., 2012; Naidu et al., 2012; Gaubert et al., 2014). However, the COI marker, in complementarity with Cytb, made it possible to identify certain species with a very high rate of similarity. Inaccuracies in species allocation have been observed in *Cephalophus*. Indeed, *Cephalophus dorsalis* identified morphologically was simply confirmed to belong to the genus *Cephalophus*. As mentioned by Munch et al. (2008) in their study, this lack of accuracy could be explained by the low representation of intraspecific diversity in Genbank. The discrepancies observed in this study could be due to several factors such as the insufficiency of genomic data on certain species in the public databases which should be updated and strengthened, the inaccurate identification of the species by the sellers and the smoked state. Molecular typing has proven to be very useful in resolving taxonomic confusion of smoked specimens, cryptic species and correcting misidentification in the field. The specimen VP2PH1C smoked and sold in our study area as *Phacochoerius aethiopicus africanus* was assigned after nucleotide sequence analysis to *Tragelaphus scriptus* (Bushbuck). These misidentifications find their justification in the cultural beliefs of the populations of west-central Côte d'Ivoire. Indeed, the Bushbuck is perceived as a prohibited species for these populations because it would be the preferred species for mystical practices. Thus, restaurants, to increase their income margin, sell their cargo at the price of other game. This assertion is supported by the work of Gaubert et al., (2014). These authors in their study suspected that traders increase their income by selling domestic animals (pigs) at the cost of wild game to Muslims in Southwestern Nigeria. Other discrepancies between morphological and molecular identification were also observed in this study with smoked specimens difficult to distinguish with the naked eye from closely related species. This is the case of *Heliosciurus* sp morphologically identified and *Funisciurus sp* attributed by mtDNA. This result could be explained by natural hybridization between species, incomplete sorting of lines or poor delineation of boundaries between species (Gongora et al., 2011). This study revealed another case of discrepancy; that of a morphologically unidentified specimen assigned to the species *Eidolon helvum* by Cyt b DNA marker. The same event was highlighted by the study by Olayemi et al., (2012) in *Cricetomys gambianus* with the Cytb gene. Phylogenetic analysis of the COI and Cytb gene sequences successfully grouped the collected bushmeat specimens into target taxa. In addition, genetic variability was observed within the taxa obtained. This variability is believed to be due to the probable origin of many taxa that formed the targets of this study or to the hybridization of sister taxa. These results are in agreement with those of Ekrem et al. (2007) who experienced non-monophyllous taxa by phylogenetic analysis.

Conclusion

The study of poached and smoked wild animals in the central-west of Côte d'Ivoire aimed to identify species, morphologically unidentifiable, found in bushmeat markets and restaurants by genetic typing in order to validate these molecular tools in quantifying the loss of biodiversity linked to poaching in Côte d'Ivoire. Over 90% amplification success was observed with the COI and Cytb markers. The smoky condition of the specimens did not influence the amplification in any way. This study shows that the Cytb gene is the best marker for molecular identification of mammalian species. However, the COI marker, in complementarity with Cyt b, makes it possible to identify certain species at a very high rate of similarity, thus giving the opportunity to correct certain discrepancies in the identification of the species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Molecular characterization and *in silico* analysis of mutations associated with extended-spectrum beta-lactamase resistance in uropathogenic *Escherichia coli* and *Klebisiella pneumoniae* in two hospitals, Côte d'Ivoire

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Escherichia coli **and** ***Klebsiella pneumoniae*** **are pathogens frequently involved in urinary tract infections with high epidemic potential. The increase and spread resistance of these microbes to broad spectrum beta-lactam antibiotics are usually reported and is a real public health concern in Côte d'Ivoire but information on genetic variants and intragenic mutations encoding these resistances are scarce. The aim of this study is to characterize genetic variants and describe the intragenic mutations underlying resistance to broad-spectrum beta-lactam antibiotics in uropathogen** ***E. coli*** **and** ***K. pneumoniae*** **in HKB and CHR hospitals with different epidemiological facies in Côte d'Ivoire. 39 strains comprising 30 of** ***E. coli*** **and 9 strains of** ***K. pneumoniae*** **were isolated from which DNA was extracted, amplified and sequenced. ESBLs genes were detected by polymerase chain reaction in 58.8 % of strain analysis. No significant difference was observed between ESBL from HKB and CHR hospitals although HKB and CHR sites present 50 and 56.8% of ESBL respectively. Nucleotide sequences subjected to BLASTn for sequences similarity and homology revealed diversity of resistance genes with dominance of the gene encoding the extended-spectrum β-lactamase CTX-M-15 and the emergence of a new** ***bla*****TEM-9 gene in Côte d'Ivoire. The significant co-expression of ESBLs might impact 3rd generation cephalosporin multi-resistance among pathogenic bacteria infecting patient population. Routine antibiogram practice could guide the choice of optimal antibiotic therapy for successful treatment and delay the occurrence of multidrug resistance in enterobacterial infections.**

Key words: Urinary tract infection, extended-spectrum β-lactamase, gene variants, mutations, antibiotic resistance, Côte d'Ivoire.

INTRODUCTION

Escherichia coli and *Klebsiella pneumoniae* are commensal enterobacteria of the intestinal microflora of humans and warm-blooded animals (Ardakani and Ranjbar, 2016). These microbes are ability used to colonize the extra-intestinal tracts of the host by acquisition virulence factors and caused a pathological state in this one (Massot et al., 2016). Several studies showed that *E. coli* and *K. pneumoniae* are the pathogens frequently involved in hospital and community-acquired urinary tract infections (Hyun et al., 2019; Yadav et al., 2019). Because of their high frequency in infections, they are constantly subjected to antibiotics used in irrational, empirical and probabilistic ways (Jena et al., 2017) and broad-spectrum antibiotics, mainly β-lactam molecules, are the most recommended by clinicians (Lima et al., 2020). Given its low cost and availability which result in over use (Ventola, 2015), β-lactam antibiotic experienced resistance leading to therapeutic failures (Friedman et al., 2016). Indeed, bacteria, in a hostile environment generated by drug pressure, develop resistance mechanisms or acquire mobile genetic elements (Munita and Arias, 2016). The most common mechanism of resistance in Enterobacteriaceae is related to expression of extended-spectrum beta lactamases (ESBLs) (Shaikh et al., 2015; Teklu et al., 2019). This resistance now extends to the latest generation of cephalosporins and carbapenems (Kim et al., 2017). *bla*TEM, *bla*SHV and *bla*CTX-M are the main variants of genes coding for resistance in Enterobacteriaceae with the latest one (Ahmad and Khadija, 2019). In Côte d'Ivoire, recent studies have reported the spread of ESBL-producing strains of *E. coli* and *K. pneumoniae* in various infections (Tahou et al., 2017; Gadou et al., 2018) although the setup since 2002 of an observatory of antimicrobial resistance is dedicated to actions contributing to fight against the spread of resistant or multi-resistant bacteria (Ouédraogo et al., 2017). In addition, information on genetic variants and intragenic mutations encoding these resistances are scarce. This study aim to determine gene variants as well as nucleotide and peptide mutations involved in resistance to broad-spectrum beta-lactam antibiotics in uropathogenic *E. coli* and *K. pneumoniae* in Côte d'Ivoire.

MATERIALS AND METHODS

Study sites description

This study was carried out in two hospitals belonging to different epidemiological facies: the Henriette Konan Bedié Hospital in Abobo (HKB-Abobo) located in Abidjan 03'86'501 N; 06'01'275 and the Regional Hospital Center of Haut-Sassandra (CHR-Daloa) located in Daloa 07'82'734 N; 07'61'563 W (Figure 1). At HKB hospital, medical management of bacterial infections is carried out according to an empirical and probabilistic model due to the lack of antibiograms implementation which could lead to an accurate diagnosis and the prescription of the correct antibiotic. In addition, this hospital is located in one of the most populated municipalities of Abidjan, with an estimated population of about 1.03 million inhabitants (RGPH, 2014) with common practice of self-medication. These factors actively contribute to the spread of a wide variety of bacterial pathogens that are resistant to a large number of antibiotics. CHR hospital in Daloa remains the only hospital in the Haut-Sassandra region which has a medical microbiology laboratory which carries out routinely microbial tests. Consequently, it receives patients from all four geographical points of the region for bacterial infection cases, showing a good distribution of bacterial infection cases in the region.

Ethics statement

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. This study is part of the ESTHER project which aims to do a better diagnosis of urinary tract infections in outpatients in addition to microbial surveillance of uropathogens and antimicrobial resistance. 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.

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 at CHR Daloa. During this period, a total of 39 strains including 30 strains of *E. coli* and 9 strains of *K. pneumoniae* were isolated from fresh urine samples from patients with urinary tract infections.

Isolation and identification of bacterial strains

The uropathogenic bacterial strains were isolated on CHROMAgar Orientation (Becton Dickinson, Cockeysville, MD), chromogenic medium (Manickam et al., 2013). 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 unique source of carbon, motility, lysine deaminase and lysine decarboxylase production (Tandon and Bhargava, 2019). Samples collection details are presented by the algorithm in Figure 2.

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Figure 1. Geographic location of sampling sites.

Phenotypic detection of uropathogenic ESBL-producing strains

The uropathogenic *E. coli* and *K. pneumoniae* strains, producing extended-spectrum beta-lactamases (ESBL) were phenotypically detected by double synergistic method on Müller Hinton agar (Jarlier et al., 2000). This testing consisted in appearance of "champagne cork" characteristic image within an antibiotic disc containing a beta-lactamase inhibitor such as amoxicillin + clavulanic acid (AMC) and third-generation cephalosporin discs such as ceftazidime (CAZ), ceftriaxone (CRO), aztreonam (ATM) and cefotaxime (CTX).

Molecular characterization of ESBL-producing strains

DNA extraction

The extraction of genomic DNA from *E. coli* and *K. pneumoniae* strains was performed using the phenol-chloroform method described by Chan and Goodwin (1995).

Genotypic detection 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 1 µl of each primer of 10 pmol/µl (Eurogentec, Blegium), 5 µl of $MgCl_2$ PCR buffer 10 x (Qiagen), 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 5 min at 94°C followed by 30 cycles consisting of denaturation at 94°C for 45 s, hybridization at 60°C for 1 min and elongation at 72°C for 1 min followed by final elongation at 72°C for 10 min.

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).

Bioinformatics analysis

Nucleotide sequences were compared to reference sequences in Genbank genomic database of National Center for Biotechnology

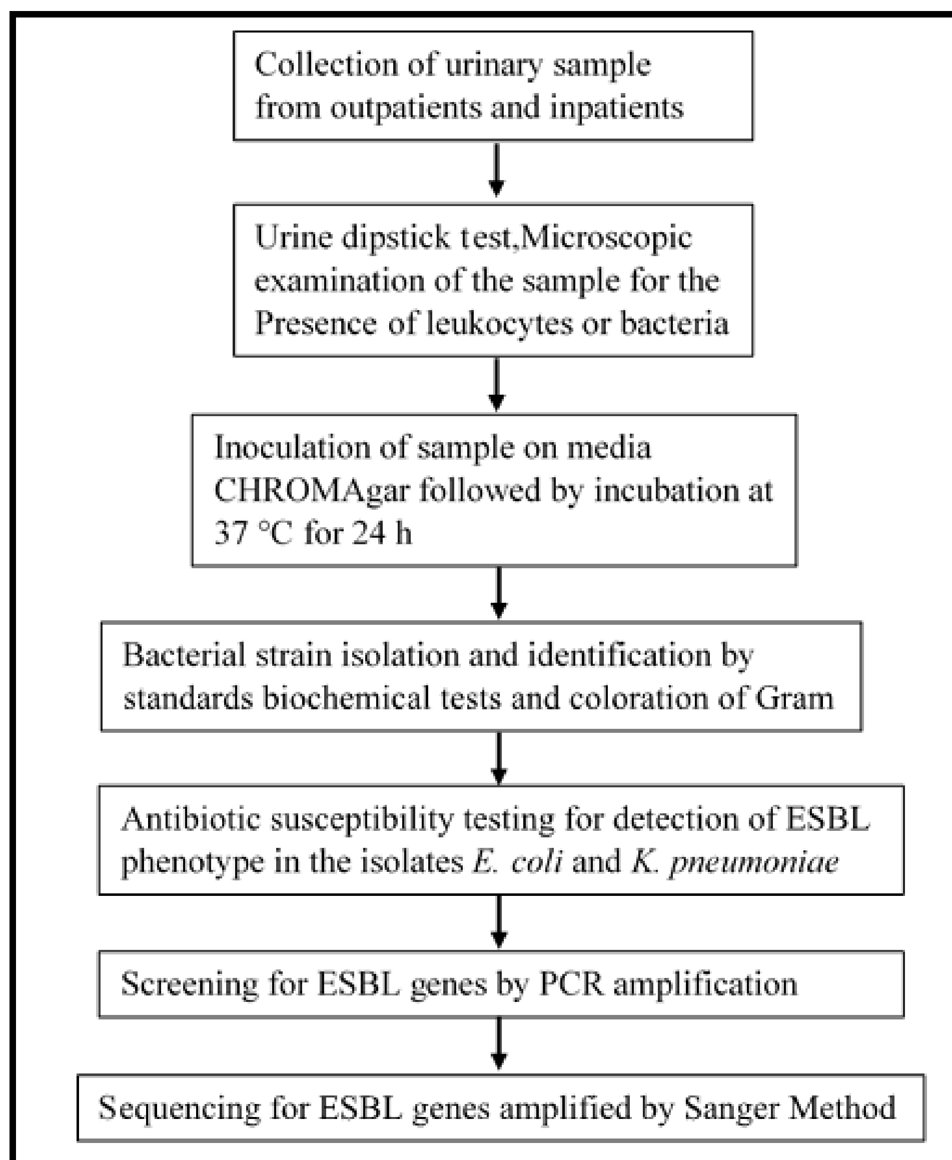

Figure 2. Algorithm for sampling and analysis of collected samples.

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

Genes	Primers Sequences (5'- 3')		References	Amplicon size (bp)
*bla*TEM	R F	CTCAAGGATCTTACCGCTGTTG TTCCTGTTTTTGCTCACCCAG	Zhu et al. (2013)	112
*bla*CTX-M	R F	TTTATCCCCCACAACCCAG AATCACTGCGTCAGTTCAC	Zhu et al. (2013)	701
*bla*SHV	R F	CGCAGATAAATCACCACAATG TCGCCTGTGTATTATCTCCC	Zhu et al. (2013)	768

Table 2. Distribution of broad-spectrum beta-lactamase-producing isolates according to the collection site and the species studied.

		ESBL-Phenotypes				
		N	ESBL + (%)	ESBL - (%)	X^2	*P*
Sites						
	Abobo HKB	22	11(50)	11(50)	0.05	0.82[a]
	Daloa CHR	17	10 (58.8)	7 (41.2)		
	Total	39	21 (53.8)	18 (46.2		
Species						
	E. coli	30	17 (56.7)	13 (43.3)	----	0.70[b]
	K. pneumoniae	9	4(44.4)	5 (55.6)		
	Total	39	21(53.8)	18 (46.2)		

N: total number of isolates; ESBL+: extended-spectrum beta-lactamase production; ESBL-: no extended-spectrum beta-lactamase production; X2: Chi-square constant; p: probability associated with the statistical test; a: independence Chi-square approximation; b: Fisher's exact test.

Information (NCBI) using the BLASTN local alignment search tool available online (http://www.ncbi.nih.gov). Protein sequences derived from the genes were aligned using DNA Baser Assembler 5.15.0 and analyzed in order to identify the CTX-M and TEM type according to sequences leading to detection of the mutations underlying resistance to ESBLs. The statistical Chi-square test of independence and the Fisher exact test were performed to compare the proportions of ESBLs strains. Significant differences are observed when the probability value (*p*) associated with the statistical tests is strictly less than 0.05.

RESULTS

Phenotypic determination of ESBLs

Figure 3 shows the phenotypic detection of a broad-spectrum beta-lactamase-producing bacterial strain using the double-synergy method on Müller Hinton agar. ESBLs production is detected by the appearance of a particular champagne cork image. The distribution of broad-spectrum beta-lactamase-producing isolates is indicated in Table 2. Results showed that 21 (53.8%) out of 39 study strains produced ESBL. *E. coli* and *K. pneumoniae* species expressed 56.7 and 44.4% at least one ESBL respectively. At the HKB hospital in Abobo, 50 % of strains analyzed were BLSE-producing positive, while this phenotype was expressed in 58.8 % of the strains at the CHR-Daloa. Although the prevalence of ESBL strains is slightly higher at CHR hospital in Daloa, the difference was not statistically significant (P = 0.82). This result showed that ESBL production was not significantly associated with bacterial specie in this study (Table 2).

Molecular detection of ESBLs resistance

The genes coding for resistance to ESBL, namely *bla*TEM, *bla*SHV and *bla*CTX-M were tested for the 21 strains with the ESBL phenotype. The electrophoretic profiles of the detected BLSE genes are shown in Figure 4. Genotyping revealed the presence of two types of ESBL genes: The *bla*TEM gene encoding the TEM enzyme and the *bla*CTX-M gene encoding the CTX-M enzyme. The *bla*TEM and *bla*CTX-M genes are characterized by DNA fragments of 112 and 701 base pairs respectively (Figure 4a and b), but the *bla*SHV gene was not detected in this study.

Distribution of broad-spectrum beta-lactamases resistance genes in *E. coli* and *K. pneumonia*

The distribution of the *bla*CTX-M and *bla*TEM genes encoding resistance to the broad-spectrum beta-lactamases detected in *E. coli* and *K. pneumoniae* in this study are shown in Figure 5. In the population of *E. coli* producing extended-spectrum beta-lactamase, the *bla*TEM gene was detected in nine strains (52.94 %), including six strains from HKB Abobo Hospital (66.7%) and three strains (33.3%) from CHR Daloa. Concerning the *bla*CTX-M gene, an overall frequency of occurrence of 70.6% is obtained in these bacterial species. In the collection from Abobo HKB hospital, the proportion of *E. coli* strains expressing the *bla*CTX-M gene was 58.3% (7/12), while at Daloa CHR, the proportion was 41.7%(5/12). Among the four ESBL-producing strains of *K. pneumoniae*, an overall occurrence frequency of 75 %

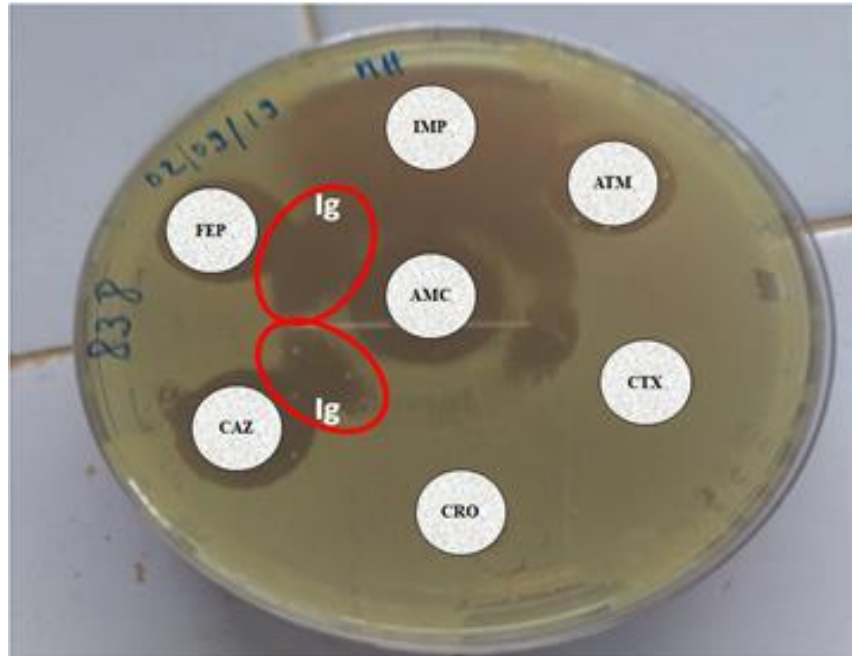

Figure 3. Synergistic image between third generation cephalosporins showing the phenotypic production of extended spectrum beta-lactamase in the bacterial species studied. CAZ: ceftazidime; CRO: ceftriaxone; ATM: aztreonam; CTX: cefotaxime; IMP: imipenem; FEP: cefepime; AMC: amoxicillin + clavulanic acid; Ig: image of synergy.

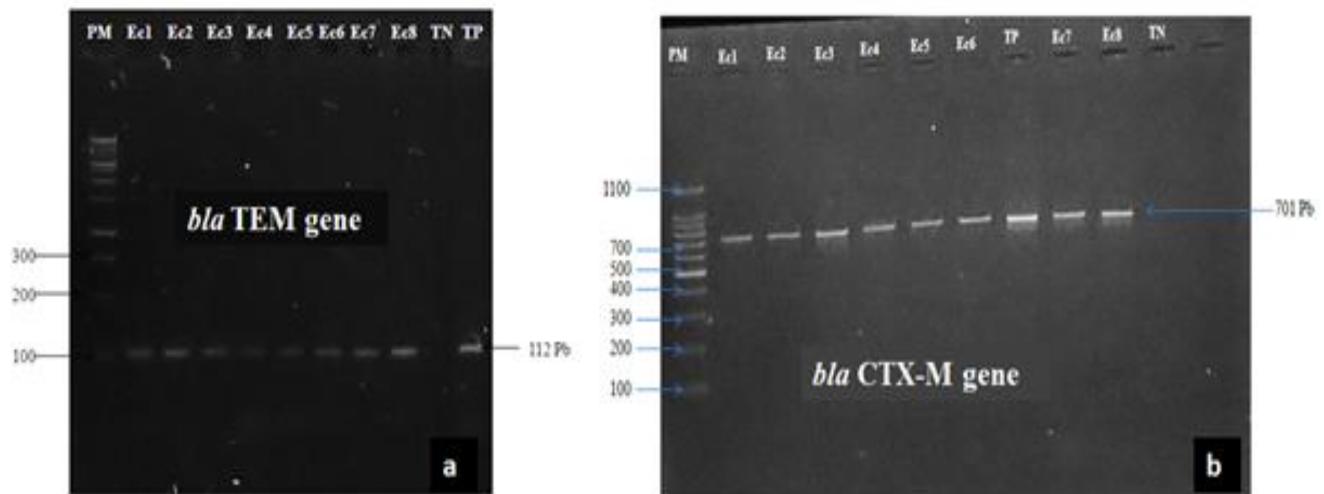

Figure 4. Electrophoretic profile on 1.5 % agarose gel of the *bla*TEM (a) and *bla*CTX-M (b) amplified genes. PM: Molecular weight marker (100bp, Invitrogen); Ec1 to Ec8: Samples tested positive to *bla*CTX-M; TP: Positive control (KCCY 1816); TN: Negative control.

(3 strains) is obtained for the *bla*TEM gene. This gene was detected in all ESBL-producing strains from HKB hospital in Abobo (100%). However, *K. pneumoniae* strain isolated at CHR Daloa did not carry the *bla*TEM gene. The *bla*CTX-M gene was detected in 3 strains of which two were from HKB hospital in Abobo. The unique strain

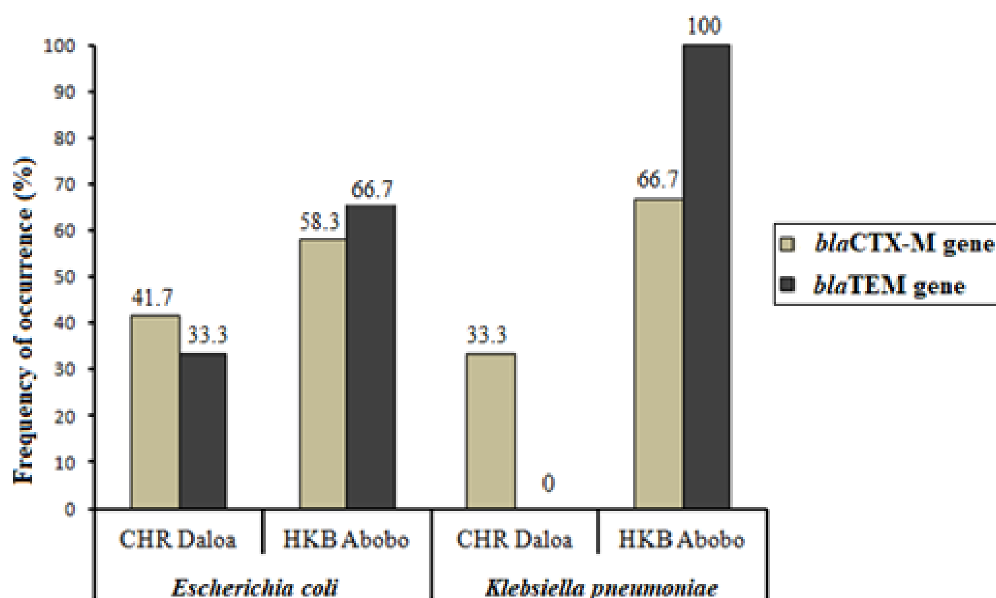

Figure 5. Frequency of occurrence of *bla*TEM and *bla*CTX-M genes detected in *E. coli* and *K. pneumonia.*

of *K. pneumoniae* collected at CHR Daloa was a carrier of the *bla*CTX-M gene, with 33.3% frequency of occurrence. In addition, co-occurrences for these two genes (*bla*TEM and *bla*CTX-M) were observed in this study in four strains of *E. coli* (66.7%) and two strains of *K. pneumoniae* (33.3%) (Figure 5).

Bioinformatics Analysis of *bla*TEM and *bla*CTX-M genes

Out of twenty-one strains expressing ESBL genes detected and sent for sequencing, fifteen were successfully sequenced. These strains consisted of three strains of *K. pneumoniae* and twelve strains of *E. coli*. Analysis of TEM and CTX-M genes sequences were done to determine their relationship to other TEM and CTX-M gene sequences available in Gen-Bank database using BLAST nucleotide algorithm (http://www.ncbi.nlm.nih.gov/). Variants of the *blaTEM* sequences analysis revealed that among the seven strains (*E. coli*1, *E. coli*5, *E. coli*14, *E. coli*6, *E. coli*11, *E. coli*9 and Kleb2), only one strain (14.3 %) isolated at HKB hospital produced the TEM-1 enzyme (Table 3). The corresponding nucleotide sequence was identified to be 97 % homologous to the coding sequence of the 66 base pair length TEM-1 allele, available in the Genbank under accession number KT415643. Alignment of this sequence revealed two base point mutations with changes in amino acid at position 38; the adenine of the CCA codon in the reference sequence was substituted by cytosine (A38C) and at position 48 the guanine of the TGT codon in the reference sequence was substituted by thymine (G48T). These nucleotide mutations induced an amino acid change in the translated peptide sequence. Accordingly, at position 13 of this sequence, glutamine mutated to proline (Gln13Pro) and at position 16 tryptophan mutated to cysteine (Trp16Cys) in the newly formed sequence (Figure 6).

Apart from TEM-1 enzyme, the others strain 85.7% revealed the production of the TEM-9 enzyme with 95.2 to 100 % similarity to the reference TEM-9 allele (accession number KY271103) available in Genbank. As illustration, the nucleotide sequence of the *E. coli* _6 isolate shows 98.6 % homology with the reference TEM-9 allele of the *bla*TEM gene (Figure 7, Table 3). A point mutation of bases was detected in position 3 regarding nucleotide alignment where A base in reference sequence was replaced by a C in the query sequence. However, after translation into amino acid sequences, no mutation was observed (Figure 7).

With the *E. coli*_1 strain, alignment results showed a 95.2% of similarity between the nucleotide sequence of the *bla*TEM gene and the reference sequence coding for the TEM-9 allele (accession number KY271103). The alignment of these two sequences revealed a substitution at position 4 of the thymine TGT codon by adenine (T4A) in the query sequence. A gap extension at positions10

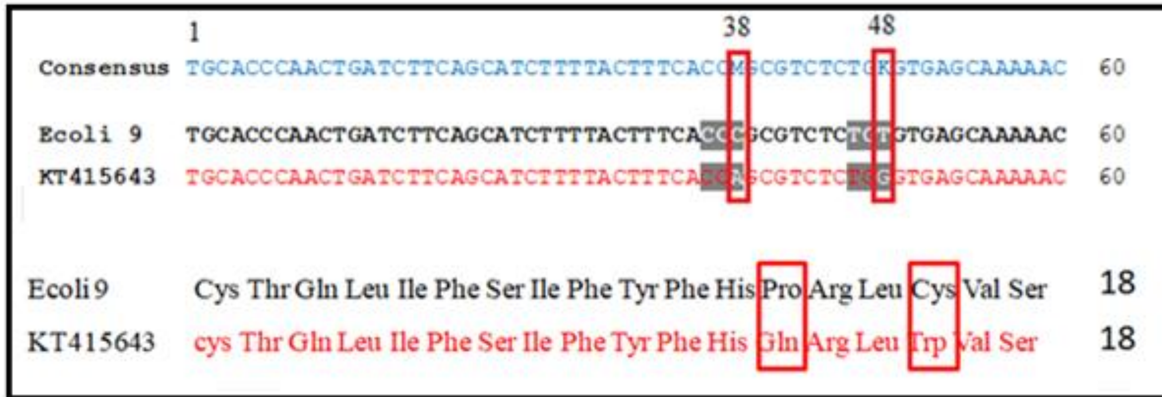

Figure 6. Alignment of the nucleotide and peptide sequences of the *bla*TEM gene of the *E. coli* 9 isolate in black with that of the synthetic reference clone of accession number KT415643 in red. In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

Figure 7. Alignment of the nucleotide and peptide sequence of the *bla*TEM gene of the *E. coli* 6 isolate in black with that of the reference strain *Escherichia coli* ECO0584 of accession number KY271103 (in red). In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

and 11 and an insertion of cytosine at position 55 were also observed in *E. coli*_1 strain sequence (Figure 7). The peptide sequence resulting from this alignment showed numerous amino acid changes from position 2 to 19 and from 21 to 27 (Figure 8). For *E. coli*_5, E. *coli*_11, E. *coli*_14 and Kleb_2 isolates a 100% similarity was obtained with the reference TEM-9 allele of the *blaTEM* gene accessible at KY271103 in the Genbank. The TEM-1 and TEM-9 variants of the *bla*TEM gene detected in this study are mainly produced by strains from HKB hospital.

Alignment of the nucleotide sequences of the *bla*CTX-M gene of the studied strains revealed a single variant of this gene, CTX-M-15 with 100 % identity to the coding reference sequence (accession number MN816278) in Genbank (Figure 9). CTX-M-15 is the only CTX-M-like enzyme produced by the studied strains to resist broad-spectrum beta-lactams. This variant was detected in 10 uropathogenic strains (66.7%) versus seven strains (46.7%) for the *bla*TEM variant. These strains comprised one strain of *K. pneumoniae* from each of the two study sites and eight strains of *E. coli* (four from HKB hospital in Abobo and four from the CHR hospital in Daloa).

DISCUSSION

Faced with drug pressure, bacteria develop resistance mechanisms whose expression is encoded by genetic factors subject to mutations. In this study, 53.8% of strains analyzed produced at least one broad-spectrum beta-lactamase. Of the two bacteria species studied. *E.*

Table 3. Characteristics of the coding sequences obtained after alignment of the amplified sequences to Genbank references.

Isolates	Collection Sites	Reference Organism	Acession No	% GC	Variants	% Identity	E-value	Sequence length (bp)
*E. coli*1	HKB hospital	*E. coli*. ECO0584	KY271103	46.3	bla_{TEM-9}	95.2	$2.11,10^{-25}$	83
*E. coli*5	HKB hospital	*E. coli*.ECO0584	KY271103.1	43.5	bla_{TEM-9}	100	$9.45,10^{-22}$	62
Kleb2	HKB hospital	*E. coli*.ECO0584	KY271103.1	48.9	bla_{TEM-9}	100	$9.97,10^{-27}$	72
*E. coli*14	HKB hospital	*E. coli*.ECO0584	KY271103	42.9	bla_{TEM-9}	100	$3.82,10^{-22}$	63
*E. coli*6	HKB hospital	*E .coli*.ECO0584	KY271103	46.6	bla_{TEM-9}	98.6	$5.72,10^{-26}$	73
*E. coli*11	HKB hospital	*E. coli*.ECO0584	KY271103	42.9	bla_{TEM-9}	100	$2.71,10^{-22}$	63
*E. coli*9	HKB hospital	Clone synthétique	KT415643	45.5	bla_{TEM-1}	97	$2.71,10^{-22}$	66
*E. coli*6	HKB hospital	*E. coli*.LM8	MN816278	55.1	$bla_{CTX-M-15}$	100	0	604
*E. coli*11	HKB hospital	*E. coli*.LM8	MN816278	55.1	$bla_{CTX-M-15}$	100	0	590
Kleb8	HKB hospital	*E. coli*.LM8	MN816278	55.1	$bla_{CTX-M-15}$	100	0	604
*E. coli*8	HKB hospital	*E. coli*.LM8	MN816278	55.3	$bla_{CTX-M-15}$	100	0	506
*E. coli*2	HKB hospital	*E. coli*.LM8	MN816278	54.9	$bla_{CTX-M-15}$	100	0	576
*E. coli*29	Daola CHR	*E. coli*.LM8	MN816278	54.6	$bla_{CTX-M-15}$	100	0	577
Kleb9	Daola CHR	*K.pneumoniae*.NC1	MN786379	54.5	$bla_{CTX-M-15}$	100	0	560
*E. coli*24	Daola CHR	*E. coli*.LM8	MN816278	55.3	$bla_{CTX-M-15}$	100	0	602
*E. coli*27	Daola CHR	*E. coli*.LM8	MN816278	54.5	$bla_{CTX-M-15}$	100	0	571
*E. coli*20	Daola CHR	*E. coli*.LM8	MN816278	55.1	$bla_{CTX-M-15}$	100	0	604

% GC: Guanine+Cytosine content, bp : bases pairs, HKB : Henriette Konan Bedie, CHR : Regional Hospital Center.

coli is the one which presents the greatest prevalence of resistance (56.7%) even if the 44% presented by *K.* pneumoniae are not negligible. The high prevalence of ESBLs in this study, as well as some reported in Côte d' d'Ivoire (Guessennd et al., 2011; Gadou et al.. 2018) and in other parts of Africa (Togo et al., 2014; Saravanan et al., 2018; Ouchar et al., 2019), show that the spread of ESBL-producing Enterobacteriaceae in an African context remains a very worrisome phenomenon. The high proportion of uropathogenic strains producing ESBL is thought to be related to the widespread use of third-generation cephalosporins and lack of adequate urinary tract infection (UTI) treatment regarding dosing regimens (Halaji et al., 2020). Indeed, the prevalence of the resistant phenotype is often an accurate reflection of antibiotic prescribing habits (Llor and Bjerrum, 2014). The molecular profile of the 21 strains is characterized by the expression of two main types of ESBL enzymes, namely CTX-M and TEM. The *bla*CTX-M gene was the most involved in ESBL resistance in *E. coli*, while, *bla*TEM gene was detected at 75% in ESBL resistance *in K. pneumoniae*. Expression of these genes may be the cause of failures observed in UTI treatment, particularly using extended-spectrum beta-lactam drugs (Hagel et al., 2013). The concomitant expression of *bla*TEM and *bla*CTX-M genes revealed in this study could be due to cross-resistance to penicillins and acquired cephalosporins, suggesting the multi-resistant character of these strains (Ahmad and Khadija, 2019).

In silico analysis of the DNA and protein sequences of the *bla*TEM gene revealed two enzyme variants, TEM-1 and TEM-9. The TEM-1 variant observed with proline/glutamine substitutions is an original plasmid-mediated beta-lactamase from which all other TEM variants are derived by mutation of one or more amino acids (Ur Rahman et al., 2018). Commonly found in *E. coli*, TEM-1 is able to hydrolyze penicillins and narrow spectrum cephalosporins such as cephalothin (Ur Rahman et al., 2018; Galindo-

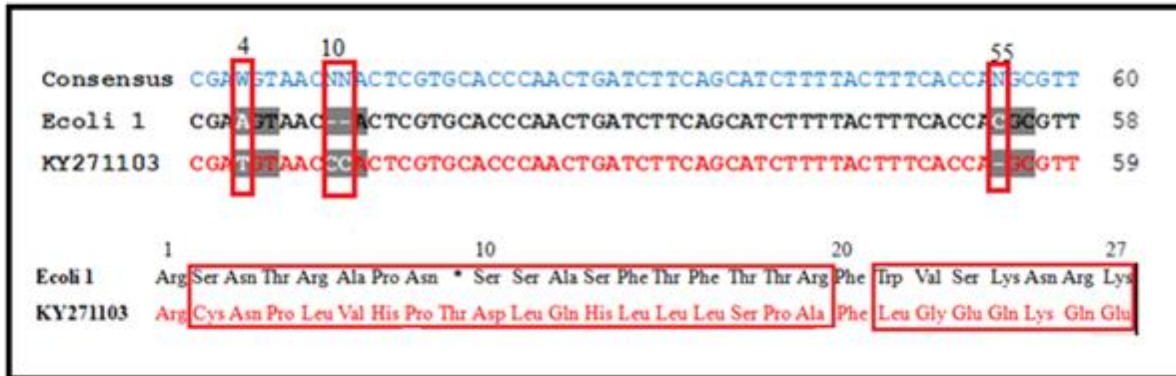

Figure 8. Alignment of the nucleotide and peptide sequence of the *bla*TEM gene of the *E. coli*_1 isolate in black with that of the reference strain *Escherichia coli* ECO0584. accession number KY271103 (in red). In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

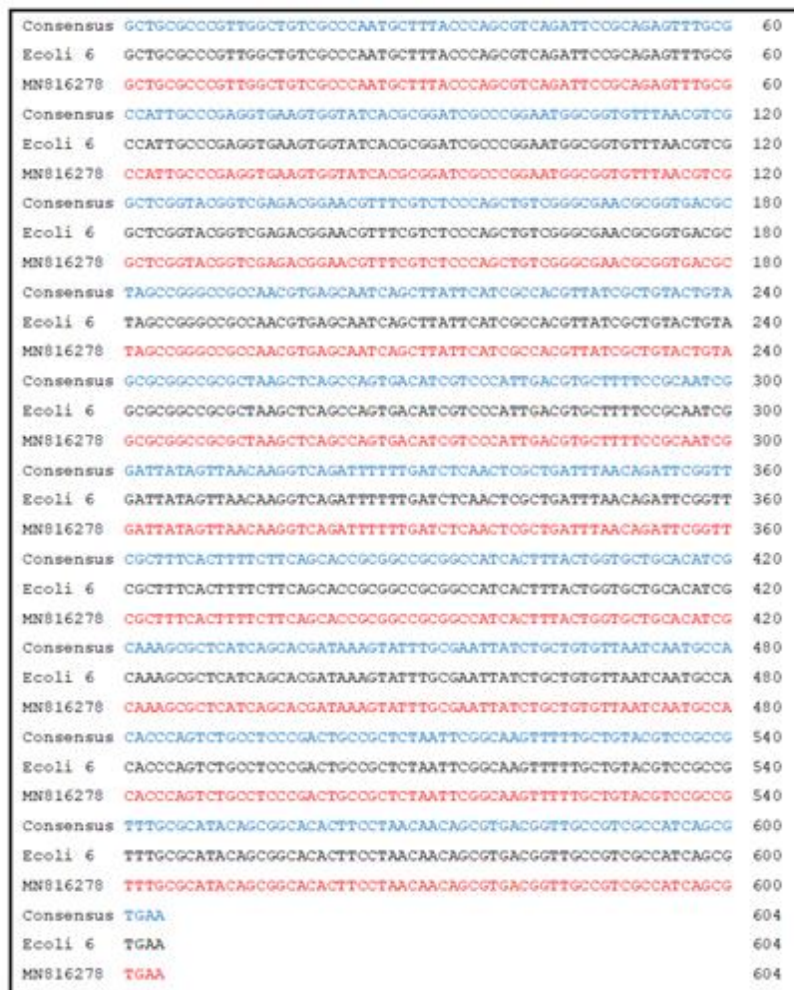

Figure 9. Alignment of *E. coli* 6 nucleotide sequence of *bla*CTX-M with that of the reference strain *E. coli* LM8 (accession number MN86278).

Méndez, 2020). TEM-1 has also been reported in Côte d'Ivoire from previous studies (Guessennd et al. 2008; Tahou et al., 2017) in *Klebsiella pneumoniae*. According to the scientific literature, the TEM-9 variant is detected for the first time in Côte d'Ivoire. This variant was derived from TEM-1 by four amino acid substitutions and was first characterized by Mabilat et al. (1990) in a clinical strain of *K. pneumoniae* at the Royal Hospital in Hallamshire in England. This mutation leads to resistance to third-generation cephalosporins.particularly ceftazidime (Jahani et al., 2017). In addition to *bla*TEM, *bla* CTX-M was also detected with the CTX-M-15 variant being the only one observed in this study. Initially reported in India in 1999, this variant predominates the current molecular epidemiology of ESBLs in *K. pneumoniae* and *E. coli* involved in both community UTI and nosocomial infections (Chong et al., 2018). The co-expression of the TEM-9 and CTX-M-15 variants, observed in the *E. coli1*_1 strain in this study is evidence of the multi-drug resistant nature of the strains circulating in the African region, due to the ability of this organism to hydrolyze larger substrates and an extended transmission of these genetic resistance factors (Parajuli et al., 2016).

Conclusion

This study showed a high proportion of ESBL-producing strains in UTI involving *E. coli* and *K. pneumoniae* species. Molecular epidemiology and *in silico* analysis of antibiotic resistance in these two uropathogens is characterized by a diversity of beta-lactam resistance genes with the emergence for the first time of *bla*TEM-9 gene variant in Côte d'Ivoire and the dominance of the gene encoding ESBL CTX-M-15, confirming the high diffusion of this variant worldwide. Furthermore, the study highlighted significant co-expression of ESBLs imparting 3rd generation cephalosporin resistance among pathogenic bacteria infecting patient population. Routine antibiogram practice could guide the choice of optimal antibiotic therapy for successful treatment and delay the occurrence of multidrug resistance in Enterobacterial infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABO and Rh (D) blood group distribution among blood donors: A Study of Natural and Computational Science Faculty graduating class students at Woldia University, Ethiopia

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The ABO and Rh (D) blood type distributions vary from one ethnic group to another. However, they play an essential part in population genetics studies, blood transfusion practices and certain medico-legal cases. Thus, this study is aimed to assess ABO and Rh (D) blood group distribution patterns among Natural and Computational Science faculty graduating class students in Woldia University and to document a blood group database and creating awareness among blood donors for safe transfusion. To this end, blood samples from 429 (N=560) volunteer students were taken during the period of 1st February 2020 to 28th April 2020 blood donation campaign and observational cross sectional study design was performed. ABO and Rh (D) typing was done by antigen-antibody agglutination test using commercially available and standard anti-sera. Descriptive statistical measures on phenotypic frequency of ABO and Rh (D) blood types were described in simple percentages. The allelic and genotypic frequencies of the ABO and Rh blood groups were estimated using the assumption of Hardy–Weinberg equilibrium. The ABO and Rh (D) blood phenotypes respectively were found to be A (28.20%) > B (25.40%) > AB (24.70%) > O (21.67%) and Rh^+ (60.13%) > Rh- (39.86%) and the combined ABO/Rh(D) blood groups were in the order A^+ (16.31%) > B^+ (15.85%) > O^+ (14.21%) > AB^+ (13.75%) and A^- (11.88%) >AB^- (10.95%) > B^- (9.55%) > O^- (7.45%). The allelic frequencies of IA (P), IB (q), IO(r) respectively were 0.314, 0.2939 and 0.4655. The allelic frequencies for ID = 0.3687 and for Id = 0.6313 were found. A genotypic frequency of IAIO was the most frequent (0.2923) and IBIB was the least frequent (0.0863). Whereas, I^DI^d was most frequent (0.4655) and IDID was the least frequent (0.1359). The observed and expected frequencies of individuals having ABO blood group showed no significant difference ($\chi2$ = 35.4381, df = 3; P < 0.05) and Rh (D) blood phenotypes of individuals were significantly different ($\chi2$ = 0.000011, df = 1; P < 0.05), thus, not fitting Hardy-Weinberg assumptions. The data obtained in the present study could be used as important input for blood bank services to have better blood management and safe blood transfusion practices in different regions of Ethiopia and abroad in the future.

Key words: ABO, allele, blood donors, blood groups, distribution, genotype, Rh (D), Woldia University.

INTRODUCTION

Blood at all times had a special mysteriousness in that lives of individuals depend on it. Over the past hundred or above years, the entire blood groups had been supposed to be identical and terrible costs during blood

transfusion were unstated. However, later in their discovery ABO and Rh (D) blood groups exhibit genetic polymorphism in different populations (Tekade et al., 2011). Human ABO blood type was the first to be discovered by Landsteiner in 1901(Garraty et al., 2000) and the Rh (D) blood group identified later in 1941 by Landsteiner and Wiener (Rahman and Lodhi, 2004). Since then these two systems considered as the most important blood group from the point of view of blood transfusion purposes. Blood group systems are common to all human populations; even though they differ in frequencies of specific types. The occurrence of ABO and Rh (D) groups shows a discrepancy among different races, ethnic groups, and socio-economic classes in many parts of the world (Sidhu and Sidhu, 1980). Approximately 700 red cell antigens are characterized and categorized into 30 blood group systems by the International Society of Blood Transfusion; in addition, ABO and Rh (D) are significantly recognized (ISBT, 2008). The antigens of ABO and Rh (D) blood group are important gene products and useful in population genetics studies, migration patterns among populations, in assessing the likelihood of being hemolytic in the newborn, for forensic determinations as well as for medico-legal purposes, predominantly in doubtful fatherliness; they have enormous applications in blood transfusion practices and organ replacement in that the giver blood type must fit that of the receiver (Kassahun et al., 2015). Different types of blood groups are hereditary and determined by the presence or absence of surface antigens and an antibody (Daniel and Clark, 2007) that could play a vital role during blood donation and transfusion. Humans however, have different types and combinations of these molecules (Daniels, 2002). Blood group substances (antigens) are controlled by a genetic locus consisting of a different number of alleles e.g. A, B, and O in the ABO system (Murray et al., 2003; Daniels, 2002). The ABO blood group system is controlled by a particular gene with IA, IB and IO alleles located on the long arm of chromosome 9 (Zahid et al., 2016). The IA and IB alleles are Co-dominant but IO allele is recessive to both in the situation when either of A or B allele affects the expression of it in the same gene locus (Murphy et al., 2003). Surface antigens on RBCs determine the ABO blood group system that is, antigen A determines blood group A, antigen B determines blood group B, or both antigens (A and B) determines blood group AB and no antigen determines blood group O. This in turn categorizes all human blood in one of the following groups; B, AB, A and O (Zahid et al., 2016) and the genotypes were; OO, AO, BO, AA, BB and AB (Khalid et al., 2013). Rh(D) is another clinically significant blood type that causes hemolytic disease of the fetus and newborn, which is controlled by a gene found on the short arm of chromosome 1 (Murphy et al., 2003). It is also di-allelic; consisting of D (dominant) and d (recessive) alleles. People who have RBCs with D-antigen on their surface are known as Rh^+ (Rhesus-positive) and represent DD or Dd genotype, while those without D-antigen on their RBCs are called as Rh^- (Rhesus-negative) and represent dd genotype (Knowles and Poole, 2002). Till now, just about 49 Rh (D) antigens are identified and defined as highly immunogenic (Rayhana et al., 2013). Contrasting the ABO group, anti-D antibodies are only formed in Rh- individuals when they are exposed to Rh+ blood, rather than commonly existing in the blood (Saladin, 2003). The two genes, RHD and RHCE encode Rh blood group antigens. These genes are highly homologous and have close related genomic sequences, each comprising 10 coding exons placed in different alignment on chromosome one (Daniels, 2002). Antibodies to D negative individuals can cause transfusion reactions during blood transfusion or pregnancy. If an Rh- individual receives an Rh+ blood, the receiver produces anti-D (Bethesda, 2005); hence D antigen is the other notable structure. A similar disorder can happen when an Rh- woman is expecting an Rh+ fetus. The first gestation period is likely to be safe because the placenta typically averts motherly and fetal blood from blending. Though, during delivery or miscarriage, placental failure may leak anti-D antibodies from mother to Rh^+ fetal blood. After all, fetal erythrocytes would become agglutinated and hemolyzed. Thus, the new born baby is being hemolytic with severe anemia called Erythroblastosis fetalis (Saladin, 2003). For this reason, the Rh status is regularly checked in blood givers, blood receivers, and in mothers-to-be (Bethesda, 2005). In view of ABO and Rh systems together, 8 blood types could be found as indicated in Figure 1. “B+” is to mean somebody has B antigen and Rhesus factor on RBCs and could receive blood from same B+ type donor and vice versa.

On one side, in the ABO blood group distributions, type AB (7%) < type B (20%) < type A (27%) < type O (46%) in ascending order were common among African-Americans and the same distribution patterns also seen in Caucasians in the United State, as type AB (3%) < type B (9%) < type A (41%) < type O (47%). Additionally, type AB (3%) < type B (9%) < type A (42%) < type O (46%) were also observed among Western Europeans, what is more, type O has high distribution pattern while AB was the least (Pramanik and Pramanik, 2000; Adeyemo and Soboyejo, 2006). On the other side, Rh+ is recorded as 95% in African-Americans, 100% in Africans while Rh- is 5.5% in South India, 5% in Nairobi, 7.3% in

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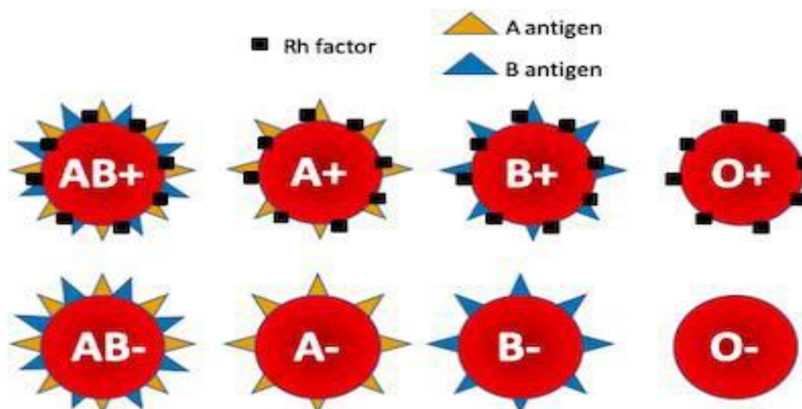

Figure 1. The eight blood types when ABO and Rh systems are in combination.
Source: ISBT (2008).

Lahore, 4.8% in Nigeria (Mwangni, 1999; Omotade et al., 1999). According to Atire (2015) agglutination is the mass piling of red blood cells with their antigens by antibody molecules and form bridges between them. When antigens on RBCs combined with their specific antibodies aggregates of the red blood cells were formed. Hence, agglutination occurs and this can happen in two stages. Sensitization- the first stage, antibodies found in the serum bind to the corresponding antigen on the red blood cell surface, a red blood cell catch-up by antibodies thus, being sensitized. The physical agglutination (clumping) is the second stage, occurs when an antibody potentially binding to a large number of antigens on RBCs producing a net or mesh that holds the cells together. If the aggregates formed from these cells were too large they can be seen with unaided eye. However, there are also aggregates which cannot be seen without the aid of biological tool. Successful blood transfusion can be achieved when the contributor and receiver blood group antigen systems being compatible and depends on the ability of agglutinins of these blood groups to cause haemolytic transfusion reaction and haemolytic disease of the fetus and newborn (Knowles and Poole, 2002; Murphy et al., 2003). Blood transfusion safe-guarded the lives of many individuals. However, faulty procedures will cost severe and delayed complications; even become life-threatening (Wakgari and Getahun, 2019). Knowing the distribution data of ABO and Rh (D) blood group is vital for enrollment of voluntary contributors as required for each region across the country, to take precautionary actions counter to the diseases linked with diverse blood types and for proper management of blood in the blood bank. Blood Transfusion Service (BTS) is an important practice of modern health care system without which emergency cases will not be mitigated efficiently. The main purpose of a BTS is to deliver faultless and safe blood as well as blood products and possibly to meet patient's requirement (Kotwal et al., 2014). Even though, the incidence of the ABO and Rhesus blood types described in Ethiopia was insufficient, nothing has been done in Woldia University. Thus, the general objectives of the present study are: to assess the distribution patterns of ABO and Rh (D) blood groups among Natural and Computational Science faculty graduating class students in Woldia University and the specific objectives are: (a) to estimate the phenotypic, allelic and genotypic frequencies of ABO and Rh (D) blood groups. (b) to create awareness and document a blood group database for safe blood transfusion.

MATERIALS AND METHODS

Description of the study area

Ethiopia is a country that establishes dynamic growth oriented higher educational institutions and Woldia University is among the higher institutions in Ethiopia (Figure 2). It was founded in Woldia town, the capital of North Wollo Zone Amhara regional state through the council of ministers regulation No 223/2011 issued on May 26, 2011 and formally recognized by Ministry of Education, Ethiopia. Foundation stone for building accomplishments of the university was put down on October, 26, 2008 by his Excellency Ato Ayalew Gobeze, previous head state of the Amhara Region and his Excellency Dr. Adhana Haile, previous secondary state minister of education. At present, the overall area of the university is 200 hectares of acreage. The university has two grounds, namely, the main campus called Woldia University and the second one is Mersa campus of Agriculture. It is 30 kms distance from the main campus. The first allocation of students, add up to 599, has been known to the university on December 10/2011 in excellent welcome ceremony including invited visitors, town inhabitants, representatives of different zone and Woreda administrative offices and university’s community. Students have been assigned into four faculties and 12 departments. In its second operation, the university accepted over 1457 new students who come across different regions of the country, that is, from Amhara, Oromia, Tigray, Southern Nation Nationalities and peoples, Gambela, Afar, Somali, Harari and Benishangul gumz regions. The amount of faculties grew into six including Natural and Computational Sciences; the two newly added being the faculty of Agriculture

Figure 2. Partial view of Woldia University.
Source: A photo taken by camera.

and pedagogical and behavioral science faculty. Likewise, the number of departments doubled into 24 and different courses and programs have been offered, leading it to nationally competent and highly renowned higher education in several areas of study. Nowadays, the university consists of over 5500 student populations.

Study design and sample population

The observational cross sectional study was conducted in Woldia University main campus during the 1st February 2020 to 28th April 2020 blood donation campaign. The study populations were Natural and computational sciences graduating class students of Woldia University. From 560 Natural and Computational Science Faculty graduating class students, a total of 429 voluntary students were taking part in blood donation operations and thus considered as study subjects. From 429 study subjects 237 were males and 192 were female students. The age of the participants were above 18 years and socially represents different ethnic groups as they come across different regions of the country that is, Amhara, Southern Nations Nationalities and Peoples (SNNP), Oromia, Gambela, Tigray, Afar, Harari, Somali and Benishangul gumz regions of Ethiopia.

Specimen collection and laboratory investigation

First of all, the objectives and procedures of blood donation were briefed to all the participants (students) during the campaign over a week at university level. Then donors were screened by Woldia blood bank service professionals and the selection was based on the criteria and guidelines used in blood donation processes. Assessments like body weight which is greater than 45 kg; Hemoglobin levels; for male 13.5 to 17.0 g/dl and for female 12.5 to 16 g/dl, blood pressure; up to 160/90mmHg were performed in order to select candidate donors. In addition, mental readiness, good health status, physical wellbeing and medical fitness of individuals were taken into considerations. Thus, donors who satisfied these criteria were enlisted and accepted. Then the study subjects were asked to fill up registration forms which comprise personal details, socio-demographic details, occupation and medical history. All blood collections were taken from voluntary donors at blood donation camps in Woldia University. After blood donation, antigen-antibody agglutination tests of ABO and Rh blood groups was done using commercially available and standard anti-sera (Bhasin and Chahal, 1996) that is, anti-A, anti-B and Anti-D at Woldia blood bank service by laboratory experts. Anti-sera were actually, validated by national blood bank. During blood typing, agglutination was recorded as a positive reaction. Blood groupings were done by separate glass slides on which blood drops were designated as A, B, and D and are mixed with different disinfected applicator sticks. If aggregates were seen in the blood drop A, then it belongs to blood group A, if aggregates were observed in blood drop B, then it belongs to blood group B, if aggregates were observed in both A and B blood drops, then it belongs to blood group AB and if neither A nor B drops showed aggregates, then it belongs to blood group O. In the same way, aggregates in blood drop D were reflected as Rh+ and no aggregates as Rh- (Avent and Reid, 2000). Finally, the results were observed and documented as B+, A+, O+ AB+ and B-, A-, O- and AB- observation under microscope was also employed when doubtful cases were confirmed.

Statistical analysis

Descriptive statistical measures on phenotypic frequencies of ABO and Rh (D) blood groups were carried out and stated in simple percentages (Tables 1 to 3). The allelic and genotypic frequencies were also estimated from the observed phenotypes by considering Hardy–Weinberg assumptions. The IA, IB, and IO alleles from ABO blood groups were symbolized by p, q, and r, respectively. The frequencies were computed following Sutton (1980); Wakgari and Getahun (2019). Thus, IO allele which is corresponding to $r = \sqrt{O}$, IA allele which is corresponding to $p = 1 - \sqrt{(O + B)}$ and allele IB which is corresponding to $q = 1 - \sqrt{(O + A)}$ were calculated. Then, the genotypic frequencies were computed as: p^2 = IAIA, q2 = IBIB, 2pq = IAIB, 2pr = IAIO, 2qr = IBIO and r^2 = IOIO. The total genotypic frequency could be: $p^2 + 2pq + q^2 + 2pr + 2qr + r^2 = (p + q + r) 2 = 1$ and $p + q + r = 1$(Hanania et al., 2007; Wakgari and Getahun, 2019). The same procedure was applied to calculate dominant allele (D) and recessive allele (d) frequencies of Rh blood group. Thus, Id allele which is corresponding to $q = \sqrt{Rh-}$ and ID allele which is corresponding to $P = 1 – q$ were calculated. The genotypic frequencies were computed from the observed allelic frequency under the assumption of Hardy-Weinberg equilibrium: p^2 = IDID, q^2 = IdId, 2pq = IDId. Thus, the total genotypic frequencies

Table 1. Distribution of ABO blood group systems with respect to ethnic group and gender (%) in the year 2020.

Blood group	Ethnic group and gender																		Total Occurrence rate (%)
	AMHA		OROM		TIGR		SNNP		HARA		GAMB		AFAR		SOMA		BENI		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
A	27(22.13)	14(11.47)	10(8.47)	19(16.10)	10(15.87)	7(11.11)	10(12.65)	9(11.39)	1(16.6)	1(16.66)	2(28.57)	-	4(44.44)	-	2(22.22)	-	4(25)	1(6.25)	121(28.20)
B	15(12.29)	16(13. 11)	11(9. 32)	21(17.79)	8(12. 69)	5(7.93)	10(12. 65)	11(13.92)	1(16.66)	-	2(28. 57)	-	3(33.33)	-	4(44.44)	-	2(12.5)	-	109(25.40)
AB	6(4.91)	7(5.73)	18(15.25)	16(13.55)	12(19.04)	10(15.87)	14(17. 72)	9(11. 39)	1(16.66)	1(16. 66)	2(28. 57)	-	2(22.22)	-	1(11.11)	-	4(25)	3(18.75)	106(24.70)
O	22(18.03)	15(12. 29)	12(10.16)	11(9. 32)	4(6.34)	7(11. 11)	9(11.39)	7(8.86)	-	1(16. 66)	1(14. 28)	-	-	-	1(11.11)	1(11. 11)	2(12.5)	-	93(21.67)
Total	70(57.37)	52(42. 62)	51(43.22)	67(56.77)	34(53.96)	29(46.03)	43(54. 43)	36(45.56)	3(50)	3(50)	7(100)	-	9(100)	-	8(88.88)	1(11. 11)	12(75)	4(25)	429(100)

AMHA=Amhara, OROM= Oromia, TIGR=Tigray, SNNP=Southern Nation Nationalities and People, HARA= Harari, GAMB=Gambela, AFAR=Afar, SOMA=Ethio-Somali, BENI=Benishangul, M=Male, F=Female.

Table 2. Distribution of Rh-blood group systems with respect to ethnic group and gender (%) in the year 2020.

Rh factor	Ethnic group and gender																		Total occurrence rate (%)
	AMH		ORO		TIG		SNNP		HARA		GAMB		AFAR		SOMA		BENI		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Rh+	46(37. 70)	38(31.14)	34(28. 81)	26(22. 03)	22(34. 92)	11(17. 46)	20(25. 31)	26(32. 91)	2(33. 33)	2(33. 33)	5(71.42)	-	8(88.88)	-	7(77.77)	1(11.11)	8(50)	2(12.5)	258(60.13)
Rh-	24(19. 67)	14(11.47)	17(14. 40)	41(34. 74)	12(19. 04)	18(28.57)	23(29. 11)	10(12. 65)	1(16. 66)	1(16. 66)	2(28.57)	-	1(11.11)	-	1(11.11)	-	4(25)	2(12.5)	171(39.86)
Total	70(57. 37)	52(42.62)	51(43. 22)	67(56. 77)	34(53. 96)	29(46. 03)	43(54. 43)	36(45. 56)	3(50)	3(50)	7(100)	-	9(100)	-	8(88.88)	1(11.11)	12(75)	4(25)	429(100)

could be: $p^2 + 2pq + q^2 = (p + q)^2 = 1$ and $p + q = 1$. Chi-square (χ^2) test ($P < 0.05$) was also computed to compare the observed and expected ABO and Rh blood phenotype frequencies were in the Hardy- Weinberg equilibrium. Therefore, $\chi^2 = \Sigma (O - E) 2 / E$ where, E = Expected frequency; O = Observed frequency. Expected phenotypic frequencies for each blood group were calculated following Wakgari and Getahun (2019). (1) Blood group A = f (AA + AO) × 429, (2) blood group B = f (BB + BO) × 429, (3) blood group AB = f (AB) × 429, (4) blood group O = f (OO) × 429, (5) blood group Rh+ = f (DD + Dd) × 429 and (6) blood group Rh- = f (dd) × 429. Where, "f" is frequency.

Ethical considerations

Generally actions implemented in researches concerning human subjects were in agreement with the National and Institutional Ethical standards of the research committee. Approval to conduct the study was decided by the Health Research Ethics Committee of Woldia University. An introduction letter was obtained from the Department of biology which was presented to the provost of the faculty for permission to carry out the research. Informed consent was also obtained from participants and collected data were kept private.

RESULTS AND DISCUSSION

The ABO and Rh (D) blood group distributions among Natural and Computational Science Faculty graduating class students

Tables 1 and 2 respectively showed the ABO blood groups and Rhesus factor distributions with respect to ethnic group and gender among blood donors (students) in Woldia University.

Furthermore, the overall ABO blood phenotypes were highly frequent in male students who come from Amhara, Tigray, SNNP,Gambela, Afar, Somali and Benishangul gumz ethnic groups than female students hence represent the highest distribution. However, the vice versa happened in Oromia region and the ABO blood phenotypes were observed equally in both male and female students from Harari ethnic group. Besides, the same trend holds true for Rh (D) blood phenotypes. Comparatively, Amhara, Oromia, SNNP and Tigray regions respectively have the highest frequency whereas, Benishangul gumz,

Afar, Somali, Gambela and Harari regions in decreasing order represent the least frequency in ABO and Rh(D) distributions. However, the frequencies in each blood group in fact depend on the number of volunteer students who belongs to different ethnic groups in blood donation operations (Tables 1 and 2). The main reasons behind deviations in number among donors were a huge number of female students from the menstruating age groups likely to be found anemic, low body weight, lack of awareness, lack of inspiration and fear of blood donation. Thus, considered as unfit for donating blood and voided during the pre-donation screening and counseling. These reasons in the present result were also shared by Swamy et al. (2012). The health status of the female students however, needs to be improved by providing proper nutritional diet and iron supplements. An effort has to be done with female students to avoid fear regarding blood donation by educating them about the advantages of blood donations. The phenotype and genotype frequencies of ABO and Rh (D) groups vary widely across different races, geographical areas of the world and may not be found in equal numbers in different populations (Kumar et al*.,* 2018; Atire, 2015; Wakgari and Getahun, 2019). The present study also showed that A, B, and Rh positive blood groups are having the highest prevalence rate of 28.20, 25.40, and 60.13% respectively which is relatively contradictory to other studies conducted by Kumar et al. (2018). Another blood group AB is also found to be lowest position in occurrence rate (24.70%) and O is having the last lowest occurrence (21.67%) (Table 1). When comparing Rhesus factors, Rhesus-negative factor shows the lowest prevalence percentage (39.86%) (Table 2). A similar finding by Kumar et al. (2018) revealed Rhesus-negative has lowest prevalence rate. Both ABO and Rh (D) are routinely typed in any blood bank or blood transfusion service (Bakare et al*.,* 2006) but their frequencies were different (Enosolease and Bazuaye, 2008). In the present result, it is also common to get specific blood types with less frequency with respect to ethnic group and gender. However, knowing the distribution patterns of ABO and Rh (D) blood groups is crucial for better blood management (Canizalez-Román et al*.,* 2018).

The result of the present study showed that the frequencies of the ABO and Rh (D) blood types distributions respectively were in the order A (28.20%) > B (25.40%) > AB (24.70%) > O (21.67%) and Rh+ (60.31%) > Rh- (39.86%) (Tables 1 and 2). On the contrary, another study in Oromia region conducted by Wakgari and Getahun (2019) reported that O (41.32%) > A (31.9%) > B (21.4%) > AB (5.4%) and Rh^+ (92.86%) > Rh^- (7.14%). A study done by Kassahun et al. (2015) also shows deviation to the present study in that the prevalence of AB (5.2%), B (21.3%), A (24.5%) and O (41.0%) and 7.94%for Rh- and 92.06% for Rh+ in Silte zone, Ethiopia. According to Nwauche and Ejele (2004) a lot of researches have shown that blood group AB is the least common while O is the most common in different ethnic groups. Even though, O is the most commonly shared blood group in most populations in different parts of the world, blood groups B or A are rarely prevalent (Wakgari and Getahun, 2019). According to the study conducted by Khattak et al*.* (2008) the ABO blood phenotypes distribution patterns were reported to be B (32.40%) > O (29.10%) > A (27.92%) > AB (10.58%) in Swat district, Pakistan. Khalid et al. (2013) also indicated that studies conducted in Bangladesh and India showed AB < A < O < B typical Asian distribution patterns. Some European nations however, showed an AB < B < O < A pattern (Khalid et al*.,* 2013). A hypothesis supposed by Lemu et al*.* (2017) reported that in malaria endemic areas the 'O' blood group is more frequent than non 'O' types and this helps, individuals with blood type 'O' survived better. In these areas however, individuals with both Rh^- and 'O' types thought to be convincing malaria transmission as asymptomatic carriers, this is probably because of the beneficial effects of their phenotype make them resistant to the disease. Having the above scenario in mind, most studies in various parts of the world point out that, the dominance of O blood type could be important to fight against malaria because O blood type is not suitable for rosette formation by *Plasmodium falciparum* (Wakgari and Getahun, 2019). Moreover, since blood type O is universal donor, its higher prevalence again is very important particularly when emergency cases are present. This can also provide better information for proper management and safe transfusion practices in blood banks (Lemu et al*.,* 2017). The present study also revealed that Rhesus-negative frequency was less common (39.86%) than Rhesus-positive (60.31%) indicating that it could be scarcely available in blood banks and unable to meet patients need. Thus, individuals should be motivated and persuaded to give blood and make this blood type more accessible in blood banks. Wakgari and Getahun (2019) indicated that the phenotypic frequency of Rhesus-negative factor is less common in most parts of the world. In Nigeria and Madagascar for example, 6 and 1% respectively were reported as Rh^-. In China, Indonesia, and Japan less than 1% of the population is Rh^- Lemu et al. (2017).

Table 3 shows the distribution pattern of Rhesus factors in each ABO blood types with respect to ethnic group and gender. In ABO/Rh(D) combinations, Rhesus-positive factors distribution pattern was found in the order A+ (16.31%) > B+ (15.85%) > O+ (14.21%) > AB+ (13.75%) and Rhesus-negative factors were observed in the order A- (11.88%) > AB- (10.95%) > B- (9.55%) > O- (7.45%) which is different from overall ABO blood group pattern that is A (28.20%) > B (25.40%) > AB (24.70%) > O (21.67%) (Table 1). With regard to sex, Rhesus-positive and Rhesus-negative patterns respectively were

Table 3. Distribution Pattern of Rhesus factors in each ABO blood group with respect to ethnic group and gender (%) in the year 2020.

ABO	Ethnic group and gender																		
Blood group	AMH		ORO		TIG		SNNP		HARA		GAMB		AFAR		SOMA		BENI		Total
with Rh factor	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Occurrence rate (%)
A^+	16(13.11)	9(7.37)	7(5.93)	6(5.08)	6(9.52)	3(4.76)	3(3.79)	7(8.86)	1(16. 66)	1(16. 66)	2(28. 57)	-	3(33. 33)	-	2(22. 22)	-	3(18. 75)	1(6.25)	70(16. 31)
B^+	9(7.37)	13(10.65)	7(5.93)	6(5.08)	7(11. 11)	2(3.17)	5(6.32)	9(11. 39)	-	-	2(28. 57)	-	3(33. 33)	-	3(33. 33)	-	2(12. 5)	-	68(15. 85)
AB^+	6(4.91)	5(4.09)	11(9.32)	7(5.93)	7(11.11)	3(4.76)	8(10. 12)	5(6.32)	1(16. 66)	-	-	-	2(22. 22)	-	1(11. 11)	-	2(12. 5)	1(6.25)	59(13. 75)
O^+	15(12.29)	11(9.01)	9(7.62)	7(5.93)	2(3.17)	3(4.76)	4(5.06)	5(6.32)	-	1(16.66)	1(14.28)	-	-	-	1(11.11)	1(11.11)	1(6.25)	-	61(14.21)
A^-	11(9. 01)	5(4.09)	3(2.54)	13(11.01)	4(6.34)	4(6.34)	7(8.86)	2(2.53)	-	-	-	-	1(11. 11)	-	-	-	1(6.25)	-	51(11. 88)
B^-	6(4.91)	3(2.45)	4(3.38)	15(12.71)	1(1.58)	3(4.76)	5(6.32)	2(2.53)	1(16. 66)	-	-	-	-	-	1(11. 11)	-	-	-	41(9.55)
AB^-	-	2(1.63)	7(5.93)	9(7.62)	5(7.93)	7(11. 11)	6(7.59)	4(5.06)	-	1(16. 66	2(28. 57)	-	-	-	-	-	2(12. 5)	2(12. 5)	47(10. 95)
O^-	7(5.73)	4(3.27)	3(2.54)	4(3.38)	2(3.17)	4(6.34)	5(6.32)	2(2.53)	-	-	-	-	-	-	-	-	1(6.25)	-	32(7.45)
Total	70(57.37)	52(42.62	51(43.22)	67(56.77)	34(53.96)	29(46.03)	43(54.43)	36(45.56)	3(50)	3(50)	7(100)	-	9(100)	-	8(88. 88)	1(11. 11)	12(75)	4(25)	429(100)

found to be B+ > O+ > A+ > AB+ and AB- > A- > B- > O- in females. However, A+ > B+ = AB+ > O+ and A- > AB- > B- = O- respectively were observed in both Rhesus positive and Rhesus negative patterns in males (Table 3). Similarly the study conducted by Kumar et al. (2018) showed the prevalence of Rhesus-positive patterns in females in the order B+ > O+ > A+ > AB+. In the present study, with respect to number, discrepancies in both male and female sexes were also observed in each Rhesus-positive and Rhesus-negative patterns. However, in the overall Rhesus-positive factors males were more frequent, while females were less frequent and vice versa in Rhesus-negative factors (Tables 2 and 3). With regard to ethnic group, Amhara, Oromia, Tigray, Gambela, Afar, Somali and Benishangul gumz regions were represented by the highest Rhesus- positive factors with more frequent in males than in females, while Harari ethnic group represented by equal number of both sexes and SNNP region showed vice versa. Overall, these regions however, exhibit high Rhesus-positive factor frequency than Rhesus-negative factor frequencies (Tables 2 and 3). In addition, Amhara, SNNP, Gambela, Afar, Somali and Benishangul gumz regions were represented by the highest Rhesus-negative factors and highly frequent in males than in females. Harari showed the same trend as Rhesus-positive factor frequency stated above. But Oromia and Tigray regions showed frequency deviation in both sexes with reference to Rhesus-negative prevalence (Tables 2 and 3). On one hand, the study conducted by Atire (2015) indicated that, O+ (32.43%), A+ (22.97%), B+ (20.27%), AB+ (2.70%) and B+ (5.41%), A+ (4.05%), O+ (2.7%), AB+ (0%) respectively were observed in males and females with Rh-positive. On the other hand, AB- (0%), A- (1.35%), O- (2.70%), B- (4.05%), and B- (0%), AB- (0%), O- (0%), A- (1.35%) respectively were found in Rh-negative males and females who belong to Amhara ethnic group. According to the study of Atire (2015), the order A+ (23.08%), B+ (11.54%), AB+ (1.92%), O+ (30.77%) and A+ (9.62%), B+ (7.70%), AB+ (1.92%), O+ (5.77%) respectively were found in Rh-positive males and females who belong to Oromo ethnic group while Rh-negative males and females respectively show the order A- (0%), B- (1.92%), AB- (0%), O- (1.92%) and A- (0%), B- (1.92%), AB- (1.92%), O- (0%). Another study conducted by Hussain et al. (2013) reported that, out of the 724 tested subjects 613 (84.67%) subjects were Rh+ and 111(15.33%) subjects were Rh-. Patel et al. (2012) also reported the Rhesus grouping on male’s sex; 28.78% were O+

Table 4. The ABO and Rh (D) blood groups Allelic and genotypic frequencies among natural and computational science faculty graduating class students in the year 2020.

Phenotype	**O**	**A**		**B**		**AB**	**Rh+**		**Rh-**
Allele	O(r)	A(p)		B(q)		-	D(p)		d(q)
Frequency	0.4655	0.314		0.2939		-	0.3687		0.6313
Percentage	46.55	31.4		29.39		-	36.87		63.13
Genotype	**IOIO**	**IAIA**	**IAIO**	**IBIB**	**IBIO**	**IAIB**	**IDID**	**IDId**	**IdId**
Frequency	0.2166	0.0985	0.2923	0.0863	0.2736	0.1845	0.1359	0.4655	0.3985
Percentage	21.66	9.85	29.23	8.63	27.36	18.45	13.59	46.55	39.85

Table 5. Comparison of allelic frequencies for ABO and Rh blood type in different studies at national and international levels.

Authors	**Year**	**IA**	**IB**	**IO**	**ID**	**Id**
Present study	2020	0.314	0.2939	0.4655	0.3687	0.6313
Wakgari and Getahun	2019	0.21	0.15	0.64	0.73	0.27
Kumar et al	2018	0.2403	0.2475	0.5122	0.7452	0.2548
Kassahun et al	2015	0.19	0.15	0.65	0.72	0.28
Yassin	2013	0.154	0.249	0.591	0.676	0.324

blood type and the remaining 1.98% was O- blood type.

The ABO and Rh (D) blood groups allelic and genotypic frequencies among Natural and Computational Science Faculty graduating class students

The allelic as well as genotypic frequencies of ABO and Rh (D) groups vary widely across different races and geographical areas of the world (Atire, 2015). In the present study, the results of allelic and genotypic frequencies were estimated by following Hardy-Weinberg assumptions and calculated according to Kumar et al. (2018). The estimated allelic frequencies of the ABO blood types were; 0.314 for IA, 0.2939 for IB and 0.4655 for IO and the calculated genotypic frequencies found to be 0.0985 for IAIA, 0.2923 for IAIO, 0.0863 for IBIB, 0.2736 for IBIO, 0.1845for IAIB and 0.2166 for IOIO. The calculated values revealed that IO and IAIO respectively, exhibit the highest allelic and genotypic frequencies, Whereas, IB and IBIB respectively, found to be the lowest allelic and genotypic frequencies. In the present study, Rhesus blood group allelic and genotypic frequencies were also calculated following Kumar et al. (2018), Wakgari and Getahun, 2019). That is the allelic frequencies for ID = 0.3687 and for Id = 0.6313 were found. The genotypic frequencies found to be 0.1359 for IDID, 0.4655 for IDId and 0.3985 for IdId. Thus, these estimations showed that Id and IDId respectively were the most frequent in allelic and genotypic frequencies. But ID and IDID respectively were the least frequent in allelic and genotypic frequencies (Table 4).

Table 5 indicates that the allelic distribution of Id deviates from the rest of the studies at national and international level. The deviation may be due to allele Id is found in heterozygous form. Genotypic frequencies in Table 5 can also be calculated from allelic frequencies. The frequency dominance of IO in most of the studies in Table 5 may also be due to the fact that A and B blood groups can carry O allele in the heterozygous state, in addition to the homozygous condition.

The ABO and Rh (D) blood groups observed and expected frequencies and Chi-square test

In ABO blood groups, the observed and expected frequencies were not significantly different (Table 6). That is the critical chi-square (0.05, three degree of freedom) = 7.815. Thus, null hypothesis is accepted that it says this population is in Hardy-Weinberg proportions, (χ^2 for ABO = 35.4381, which is greater than the critical value, df = 3; $P < 0.05$). On the contrary, in Rh (D) blood phenotypes the observed and expected frequencies of individuals were significantly different (Table 6). That is the critical chi-square (0.05, one degree of freedom) = 3.841. Thus, the null hypothesis is rejected that it says this population is in Hardy-Weinberg proportions (χ^2 for Rh (D) = 0.000011, which is less than the critical value, df = 1; $P< 0.05$) thus, not fitting Hardy-Weinberg assumptions. However, according to the study of Wakgari and Getahun

Table 6. The ABO and Rh (D) blood groups observed and expected frequency comparisons and Chi-square (χ2) test among blood donors (graduating class students) in the year 2020.

Blood groups	A	B	AB	O	Rh+	Rh-
Observed (O)	121	109	106	93	258	171
Expected (E)	167.6532	154.3971	79.1505	92.9214	258.0006	170.9565
Difference (O-E)	-46.6532	-45.3971	26.8495	0.0786	-0.0006	0.0435
(O-E)2/E	12.9822	13.348	9.1079	0.000066	1.4E-09	0.000011
χ2 = Σ (O - E)2 ⁄ E		χ2 = 35.4381,	Df=3		χ2= 0.000011,	df= 1

(2019), the observed and expected frequencies show no significant difference among individuals having both ABO and Rh blood groups (χ2 test for ABO = 0.4729, df = 3 and χ^2 test for Rh = 0.0145, df = 1; $P < 0.05$).

Conclusions

Blood at all times had a special mysteriousness in that lives of individuals depend on it**.** In the present study the most common blood type is A (28.20%) and O (21.67%) is the least common among the donors (students) in Woldia University. Rh-positive were (60.31%) and Rh-negative were (39.86%). In the present result, it is also common to get specific blood types with less frequency with respect to ethnic group and gender. Individuals should be motivated and persuaded to give blood and make Rh- more available in blood banks. Furthermore, awareness about donation of blood has to be created in order to increase the number of female donors. The allelic and genotypic frequencies were also reported in the present study. However, deviations were observed compared to most studies at national and international levels (Table 5). The difference is may be due to the fact that few alleles are found in heterozygous state in addition to homozygous state. In ABO blood groups, the observed and expected frequencies were not significantly different while Rh (D) blood groups show significance variation (Table 6). The data obtained in the present study could be an important input for blood bank services to have better blood management and safe blood transfusion practices in different regions of Ethiopia and abroad in the future.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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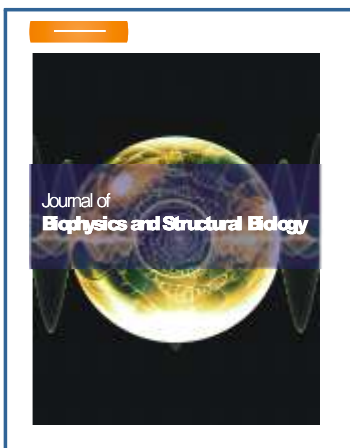

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