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DEPARTMENT OF INTERNAL MEDICINE AND SPECIALTIES

Gut Microbiota Pattern and Kidney Function in Autosomal Dominant Polycystic Kidney Disease Patients in Yaoundé

Thesis written and presented publicly in partial fulfillment of the requirements for the award of a Doctor in Medicine Degree by:

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DEDICATION

TO THE NWAGA's FAMILY.

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Declaration of Geneva adopted by the Geneva Assembly of the World Medical Association in Geneva, Switzerland, September 1948 and amended by the 22nd World Medical Assembly, Sydney, Australia (August 1968)
on admission to the medical
profession:

I will solemnly pledge myself to consecrate my life to the service of humanity

I will give my teachers the respect and gratitude which is their due I will practice my profession with conscience and dignity

The health of my patients will be my first consideration

I will respect secrets confided in me, even after the patient has died

I will maintain by all the means in my power the honour and noble traditions of the medical profession

My colleagues will be my brothers

I will not permit considerations of religion, nationality, race, political party or social standing to intervene between my duty and my patient

I will maintain the utmost respect for human life from the time of conception, even under threat I will not use my medical knowledge contrary to the laws of humanity

ABSTRACT

Background: The gut microbiota which maintains a symbiotic relationship with its host has recently been described to undergo dysbiotic changes with kidney function decline in chronic kidney disease (CKD). CKD has as first genetic cause Autosomal Dominant Polycystic Kidney Disease (ADPKD) characterized by fewer comorbid conditions likely to influence the microflora. A study of the gut microbiota in ADPKD patients could address the need for enhanced preventive efforts in the management of CKD through microbiota-derived and renoprotective short-chain fatty acids.

Objective: to evaluate gut microbiota pattern and kidney function of patients with ADPKD.

Method: We conducted a historical cohort study including individuals with ADPKD who had consulted at the Yaoundé University Teaching Hospital (YUTH) and General Hospital (YGH). The ADPKD group was matched for age and sex to a healthy control (HC) group in a 2:1 ratio. Sociodemographic (age, sex), clinical (blood pressure, complications, family history and cyst distribution of ADPKD), blood (creatinine), urine (proteins, leukocytes) and faecal (Bifidobacterium, Lactobacillus and Enterobacteriaceae) data were collected. Blood and urine specimens were analyzed at the YUTH biochemistry laboratory. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation from serum creatinine. Stool specimens were stored and analyzed at the Yaoundé National Veterinary Laboratory. Microbiota analysis consisted of DNA extraction and real-time PCR quantification of the targets mentioned above. Data were analyzed using the software IBM-SPSS. Statistical significance was set at $p < 0.05$.

Results: We enrolled 44 participants (65.9% were females) with a mean age of 40.6±11.9 (20 – 65) years among which 29 had ADPKD. Concerning ADPKD patients, 18 (62.1%) had flank pain, 14(48.3%) had hypertension, 17 (58.8%) had a family history of ADPKD, 11 (37.9%) had extrarenal cysts and 11 (38%) were CKD G3-5. The median eGFR was 74.4[51.2-94.6] ml/min/1.73m^2 and 94.5[77.3-111.7] ml/min/1.73m^2 respectively in ADPKD and HC ($p = 0.022$). All stool samples had Enterobacteriaceae while Lactobacillus and Bifidobacterium were absent in 2 (100% ADPKD) and 10 (60% ADPKD) respectively. Lactobacillus was less abundant in ADPKD with CKD G3-5 compared to CKD G1-2 ($p=0.047$) and HC ($p=0.043$) while Enterobacteriaceae was significantly more abundant in ADPKD with CKD G3-5 compared to CKD G1-2 ($p=0.048$) and HC ($p=0.045$). No difference was observed among groups and subgroups for Bifidobacterium.

Conclusion: There was a decrease in symbiont Lactobacillus and an increase in pathobiont Enterobacteriaceae with kidney function decline in the ADPKD population.

Keywords: Gut microbiota, kidney function, ADPKD

RESUME

Contexte : Le microbiote intestinal, qui maintient une relation symbiotique avec son hôte, a récemment été décrit comme présentant des changements dysbiotiques avec le déclin de la fonction rénale dans la maladie rénale chronique (MRC). La MRC a comme première cause génétique la polykystose rénale autosomique dominante (PKRAD), caractérisée par peu de comorbidités susceptibles d'influencer le microbiote. Une étude du microbiote intestinal chez les patients atteints de PKRAD pourrait répondre à la nécessité d'améliorer les efforts de prévention dans la gestion de la MRC grâce à des acides gras à chaîne courte dérivés du microbiote ayant un effet rénoprotecteur.

Objectif : évaluer le profil du microbiote intestinal et la fonction rénale des patients atteints de PKRAD.

Méthode : Nous avons mené une étude de cohorte historique incluant des individus atteints de PKRAD ayant consulté au Centre Hospitalier et Universitaire de Yaoundé (CHUY) et à l'Hôpital Général de Yaoundé (HGY). Le groupe PKRAD a été apparié pour l'âge et le sexe à un groupe de témoin sain (TS) dans une proportion 2:1. Des données sociodémographiques (âge, sexe), cliniques (tension artérielle, complications, antécédents familiaux et distribution des kystes de la PKRAD), sanguines (créatinine), urinaires (protéines, leucocytes) et fécales (Bifidobacterium, Lactobacillus et Enterobacteriaceae) ont été collectées. Les échantillons de sang et d'urine ont été analysés au laboratoire de biochimie du CHUY. Le débit de filtration glomérulaire estimé (DFGe) était calculé à l'aide de l'équation de la Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) basée sur la créatinine sérique. Les échantillons de selles ont été conservés et analysés au Laboratoire Vétérinaire National de Yaoundé. L'analyse du microbiote a consisté en une extraction d'ADN et une quantification par PCR en temps réel des cibles mentionnées ci-dessus. Les données ont été analysées à l'aide du logiciel IBM-SPSS. La significativité statistique a été fixée à $p < 0,05$.

Résultats : Nous avons recruté 44 participants (65,9% de femmes) avec un âge moyen de 40,6±11,9 (20 - 65) ans parmi lesquels 29 souffraient de PKRAD. En ce qui concerne les patients atteints de PKRAD, 18 (62,1 %) avaient des douleurs au flanc, 14 (48,3 %) souffraient d'hypertension, 17 (58,8 %) avaient des antécédents familiaux de PKRAD, 11 (37,9 %) avaient des kystes extrarénaux et 11 (38 %) étaient atteints de MRC G3-5. Le DFGe médian était de 74,4 [51,2-94,6] ml/min/1,73m^2 et de 94,5 [77,3-111,7] ml/min/1,73m^2 respectivement pour PKRAD et TS ($p = 0,022$). Tous les échantillons de selles contenaient la famille des

Enterobacteriaceae, tandis que Lactobacillus et Bifidobacterium étaient absents dans 2 (100% PKRAD) et 10 (60% PKRAD) respectivement. Les Lactobacillus étaient moins abondants chez le groupe PKRAD avec MRC G3-5 que chez le groupe MRC G1-2 (p=0,047) et TS (p=0,043), tandis que les Enterobacteriaceae étaient significativement plus abondantes dans le groupe PKRAD avec MRC G3-5 que dans le groupe avec MRC G1-2 (p=0,048) et TS (p=0,045). Aucune différence n'a été observée entre les groupes et les sous-groupes pour Bifidobacterium.

Conclusion : Une diminution du symbiote Lactobacillus et une augmentation du pathobionte Enterobacteriaceae ont été observées avec la baisse de la fonction rénale dans la population PKRAD.

Mots-clés : Microbiote intestinal, fonction rénale, PKRAD

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LIST OF ABBREVATION

ACEI	Angiotensin converting enzyme inhibitor
ADPKD	Autosomal dominant polycystic kidney disease
AGE	Advanced Glycation End Products
Akt	AKT serine/threonine kinase
ARB	Angiotensin receptor blocker
BMI	Body mass index
BSS	Bristol stool scale
BP	Blood presssure
BUN	Blood urea nitrogen
cAMP	Cyclic adenosine monophosphate
CDCA	Cilia-dependent cyst activation
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
DCT	Distal convoluted tubule
DNA	Deoxyribonucleic acid
GALT	Gut-associated lymphoid tissue
eGFR	Estimated glomerular filtration rate
GIT	Gastrointestinal tract
HPA	Hypothalamic-pituitary-adrenal axis
IBM-SPSS	International business machines- statistical package for the social sciences
IBS	Irritable bowel syndrome
KDIGO	Kidney disease improving global outcomes
MRC	*Maladie rénale chronique*
mTOR	Mammalian target of rapamycin

NSAID	Non-steroidal anti-inflammatory drugs
OTU	Operational taxonomic units
PC1	Polycystin 1
PC2	Polycystin 2
PCR	Polymerase chain reaction
PCT	Proximal convoluted tubule
PKD	Polycystic kidney disease
PKRAD	Polykystose rénale autosomique dominante
PLD	Polycystic liver disease
RAAS	Renin-angiotensin-aldosterone system
RNA	Ribonucleic acid
SCFA	Short chain fatty acids
UTI	Urinary tract infection

CHAPTER I: INTRODUCTION

I.1 Background and rationale

The intestinal microflora which is the collection of all microbes coexisting within the gastrointestinal tract maintains a symbiotic relationship with its host under normal conditions, but its imbalance has recently been associated with several diseases such as cardiovascular diseases and chronic kidney disease [1,2]. CKD remains a major threat to the health of Africans [3–5]. Its major etiologies are diabetes mellitus, hypertension, chronic glomerulonephritis and autosomal dominant polycystic disease (ADPKD) [6]. ADPKD is responsible for about 10% of kidney failure and it is the fourth cause of CKD [4].

Gut microbiota is altered in patients with kidney disease and those on dialysis [7]. In chronic kidney disease (CKD), dysbiotic intestinal microflora has been reported with an increase in pathogenic flora compared to symbiotic flora [8]. It is not clear yet what bacterial composition changes are due to the renal insufficiency per se, and what are in result of the accompanying interventions and comorbid conditions. Most studies analyzed gut microbiota in patients with diabetic nephropathy, hypertensive nephropathy and glomerulonephritis which might have directly influenced the microbiome regardless of alterations in kidney function. A decrease in kidney function is associated with biochemical and biophysical changes notably: the retention of uremic toxins promoting the growth of proteolytic microbes, an alteration in intestinal pH and an increment in colonic transit time [12]. These changes lead to a deterioration of the gut microbiota which plays several roles like: protection from pathogenic microbes, participation in energy metabolism, regulation of the immune system and production of short-chain fatty acids which exert renal protective effects [12, 13]. Additionally, the vast majority of therapeutic strategies and dietary restrictions provided to patients with CKD can independently affect the composition of the gut microbiota [9].

Compared to patients with kidney failure due to conditions such as diabetic nephropathy or glomerulonephritis, patients with ADPKD have less major co-morbid medical conditions or associated medical interventions such as antimicrobial or anti-inflammatory therapies that could potentially alter the gut microbiota. A distinct gut microbiome with decreases in both Lactobacillaceae and Prevotellaceae families has been associated with CKD [7]. Yacoub *et al.* at mount Sinai who studied the gut microbiota of PKD patients reported step-wise changes associated with kidney function decline [9]. However, little is known about the gut microbiota and kidney function in patients with ADPKD in Cameroon, we considered the potential aggravation of kidney function due to gut dysbiosis and the differences between local and

"western" dietary habits (higher energy and ultra-processed food content with low fruit and vegetable content) [10]. In light of this, we carried out a study entitled Gut Microbiota Pattern and Kidney Function in Autosomal Dominant Polycystic Kidney Disease patients in Yaoundé.

I.2 Research question

What is the gut microbiota pattern with respect to kidney function in patients with ADPKD in Yaoundé?

I.3 Research hypothesis

There is a dysbiotic gut microbiota pattern with decline in kidney function in patients with ADPKD.

I.4 Research objectives

I.4.1 General objective

To evaluate the gut microbiota pattern and kidney function in patients with ADPKD.

I.4.2 Specific objectives

1. To quantify Bifidobacterium, Lactobacillus and Enterobacteriaceae in faecal samples of study participants.
2. To determine the glomerular filtration rate in the ADPKD group.
3. To assess the association between Bifidobacterium, Lactobacillus and Enterobacteriaceae and kidney function.

CHAPTER II: LITERATURE REVIEW

II.1 Recall

II.1.1 The Kidney

II.1.1.1 Anatomy

The kidneys are bean-shaped retroperitoneal organs in the posterior abdominal region. They lie lateral to the vertebral column and extend approximately from vertebra TXII to vertebra LIII. The right kidney is lower than the left due to its rapport with the liver. Both kidneys are covered on their superior poles by the suprarenal glands within renal fat and fascia and are related superiorly to the diaphragm. The right kidney is related to other structures: the liver, the descending part of the duodenum and the right colic flexure. The left is related to: the intraperitoneal stomach, spleen, retroperitoneal pancreas and the left colic flexure.

The kidney has a smooth anterior and posterior surface covered by a fibrous capsule. Medially, a vertical slit known as the hilum enables the passage of renal vessels, lymphatics and nerves **(fig. 1)**. The hilum is continuous with the renal sinus. Each kidney consists of an outer cortex and an inner medulla. The cortex extends into the inner aspect of the kidney as renal columns dividing the medulla into renal pyramids. The apex of each pyramid projects inwards as the renal papilla towards the renal sinus and is surrounded by a minor calyx. Minor calices fuse to form major calices; the latter unite into the renal pelvis which is the superior end of the ureters **(fig. 1).** The renal artery, a branch of the abdominal aorta supplies each kidney as it divides into the anterior and posterior branches. Several renal veins form the left and right renal veins. The kidneys' lymphatic drainage is to the lateral aortic (lumbar) nodes [11].

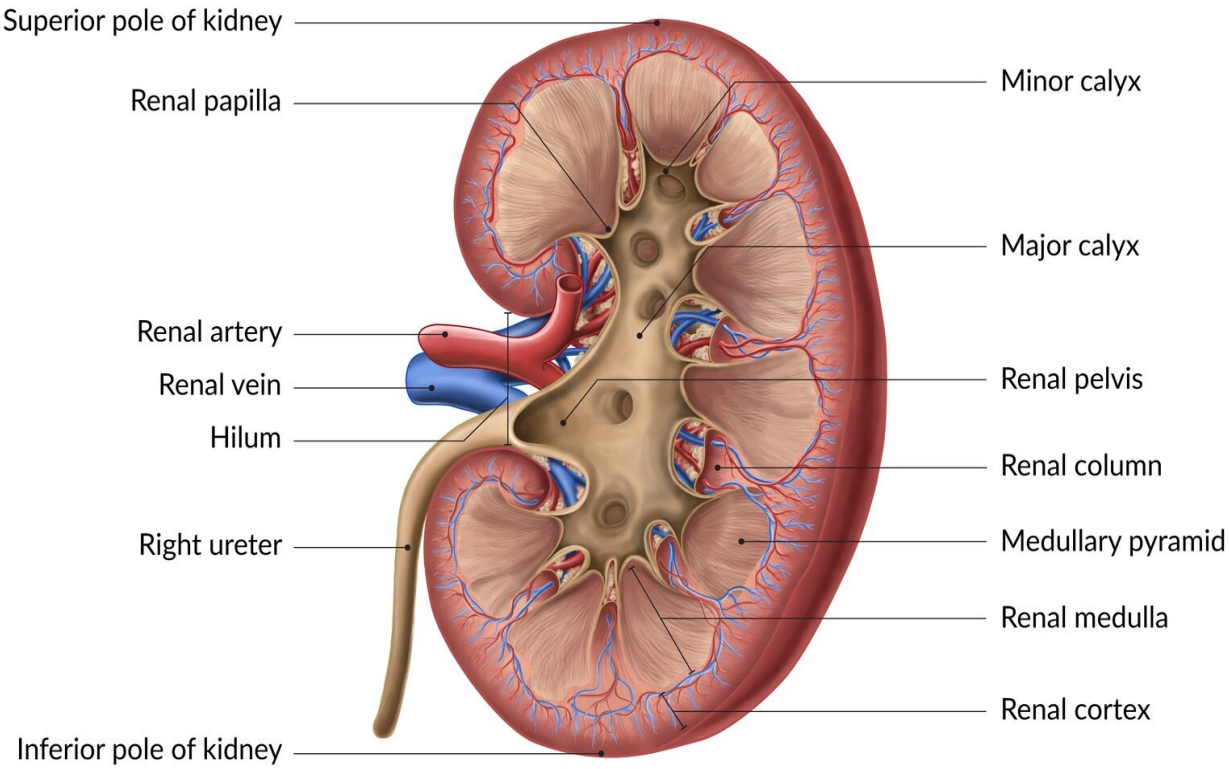

Figure 1: The internal macrostructure of the kidney [12]

Microscopically, the nephron is the functional unit of the kidney as is composed of a renal corpuscle and tubule. There are about 1 million per kidney. The renal corpuscle is located in the renal cortex and it is made up of a network of capillaries known as the glomerulus enclosed by the Bowman capsule. The glomerulus made up of afferent and efferent arterioles forms the vascular pole of the corpuscle while the Bowman capsule forms the urinary pole of the corpuscle. The corpuscle's function is to filter blood across the glomerular filtration barrier.

The renal tubule is made up of the proximal convoluted tubule (PCT), the loop of Henle, the distal convoluted tubule (DCT) and the collecting duct (**fig. 2**). Each segment of the renal tubule has a distinct epithelial lining and function. The renal tubule reabsorbs and secretes solute and water from the ultrafiltrate producing urine. Within the nephron, the juxtamedullary complex located between the DCT and the afferent arteriole maintains GFR by regulating the renin-angiotensin-aldosterone system (RAAS) [12].

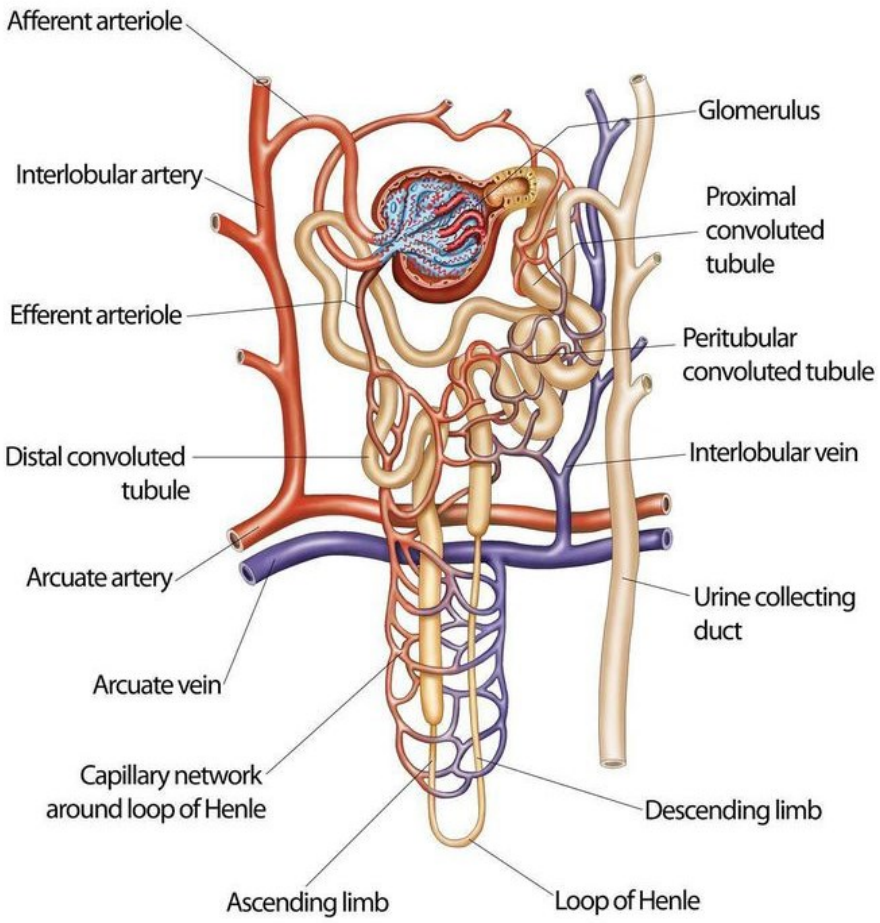

Figure 2: The structure of a nephron[13]

II.1.1.2 Physiology

Production of urine

- Excretion of metabolic waste and end-products of metabolism
- Maintenance of electrolyte and acid-base equilibrium
- Regulation of blood pressure and blood volume

Hormone synthesis

- Erythropoietin
- Calciferol
- Renin

II.1.1.3 Embryology

The kidney originates from the intermediate mesoderm cells that migrate caudally to form the mesonephric duct at the 4th week of embryonic development. The mesonephric duct gives rise to the nonfunctional pronephros, partially functional mesonephros and metanephros which persists as the permenant kidney (**fig. 3)**. The metanephros is made up of the ureteric bud (metanephric diverticulum), the metanephric mesenchyme and the ureteropelvic junction. The ureteric bud is an outgrowth of the caudal portion of the mesonephric duct that migrates and invades the undifferentiated metanephric mesenchyme. Reciprocal signaling leads to metanephric mesenchyme differentiation (mesenchymal condensation and nephron induction) and ureteric bud branching and elongation. The process of branching morphogenesis is under the control of certain growth factors: glial-derived neurotrophic factor (GDNF) and fibroblast growth factors such as FGF-7. The ureteric bud differentiates into the collecting ducts, minor and major calices, renal pelvis and ureters (collecting duct system). The metanephric mesenchyme differentiates into the nephrons: glomerulus, PCT, loop of Henle and DCT. The ureteropelvic junction forms the junction between the ureter and renal pelvis [14,15].

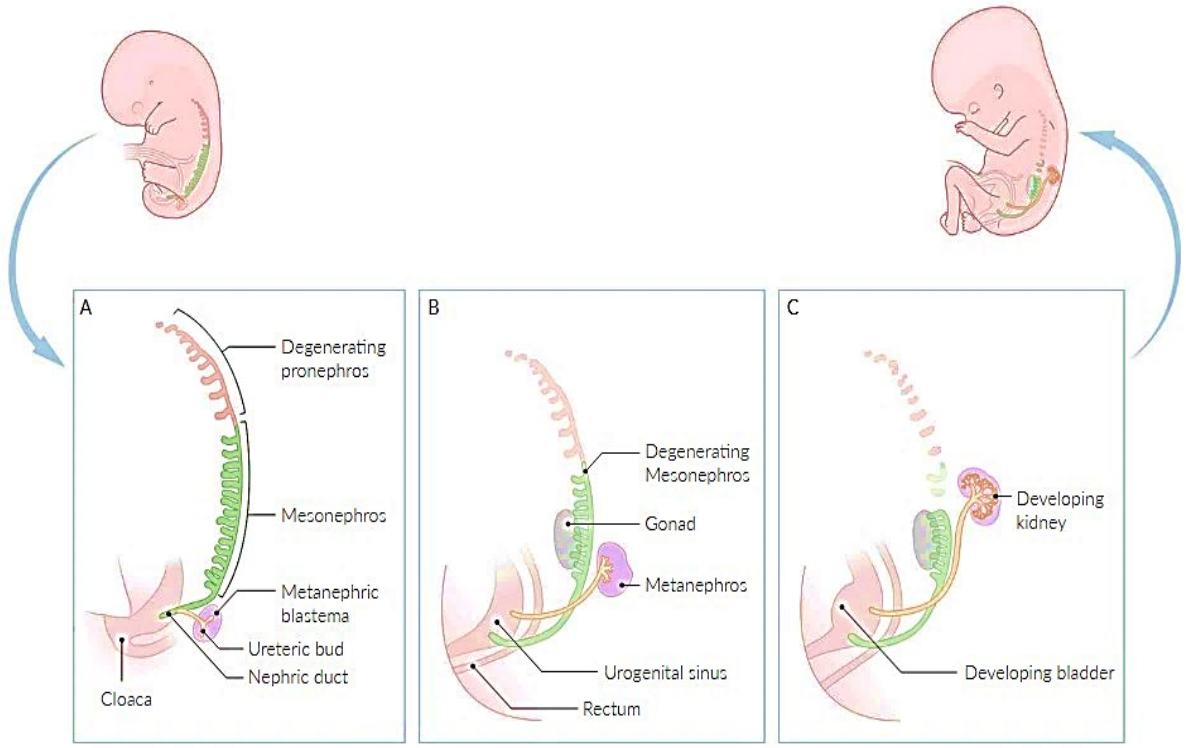

Figure 3: Schematic representation of kidney embryology [12]

II.1.2 Autosomal Dominant Polycystic Kidney Disease

II.1.2.1 Definition

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is an inherited kidney disease due to mutations in *PKD1* and *PKD2* genes that encode respectively for polycystin-1 (PC-1) and polycystin-2 (PC-2) proteins. It is characterized by the bilateral growth and development of cysts causing kidney enlargement and impaired [4].

II.1.2.2 Epidemiology

ADPKD is the first genetic cause of CKD and is found in all races and ethnic groups [16]. It occurs in approximately 1 in every 1000 birth [17]. It accounts for about 10% of kidney failure [4]. A prevalence of 1 in 250 was reported in Dakar with hypertension being the main clinical finding in 68% of patients [18]. In Cameroon, a study carried out in 2011 describes 41 patients with ADPKD [19].

II.1.2.3 Etiology and physiopathology

Cystic diseases of the kidney are broadly divided into genetic cysts and non-genetic cysts. A kidney cyst is a fluid-filled sac resulting from dilatation of any part of the nephrons or collecting ducts. The table below summarizes the various types of cystic diseases of the kidney **(Table I).**

II.1.2.3.1 Etiology

ADPKD is due to mutations in *PKD1* and *PKD*2 genes that encode respectively for the proteins polycystin-1 (PC-1) and polycystin-2 (PC-2); they are responsible for 85-90% and 10-15% of cases of ADPKD respectively [16]. This results from an abnormality on chromosome 16 (*PKD1* locus) or chromosome 4 (*PKD2* locus). ADPKD not resulting from either mutations has no identifiable cause. *PKD1* mutation leads to a faster progression to kidney failure compared to *PKD2* [17].

Table I: Classification of cystic diseases of the kidney [16]

Non-genetic	**Genetic**
Acquired disorders	***Autosomal dominant***
Simple renal cysts (solitary or multiple)	Autosomal dominant polycystic kidney disease
Cysts of the renal sinus	Tuberous sclerosis complex
Acquired cystic kidney disease (in patients with chronic kidney impairment)	Von Hippel-Lindau disease
Multilocular cysts	Medullary cystic disease
Hypokalemia-related cysts	Glomerulocystic disease
Developmental disorders	***Autosomal recessive***
Medullary sponge kidney	Autosomal recessive polycystic kidney disease
Multicystic kidney	nephronopthisis
Pyelocalyceal cysts	***X-linked***
	Orofaciodigital syndrome type 1

II.1.2.3.2 Pathophysiology

ADPKD is explained by the two-hit hypothesis in which a mutated dominant allele is inherited from one of the parents. The inheritance of ADPKD is shown on **fig. 4.** Cyst formation occurs only when the normal, wild-type gene undergoes a second 'hit'. Mutations in *PKD1* or *PKD2* gene which encode for polycystin-1 (PC-1) and polycystin-2 (PC-2) respectively lead to the expression of abnormally small and non-functional proteins [20]. PC-1 and PC-2 are transmembrane proteins. PC-1 interacts and forms a complex with PC-2, it is hypothesized to be implicated in the regulation of PC-2. PC-1 and PC-2 work together to regulate cell growth and proliferation, cell migration and cell-to-cell interactions [21].

PC-2 is a divalent cation channel involved in cellular Ca^{2+} signaling in other words it acts as a calcium ion channel at the plasma membrane, endoplasmic reticulum and primary cilia. In the kidney, primary cilia is found in most cells of the nephron projecting into the tubular lumen from the apical surface and function as mechanosensors to sense fluid flow [20].

The cilioplasma is enriched with signaling and transport proteins, which are activated in response to mechanical and chemical stimuli [5].

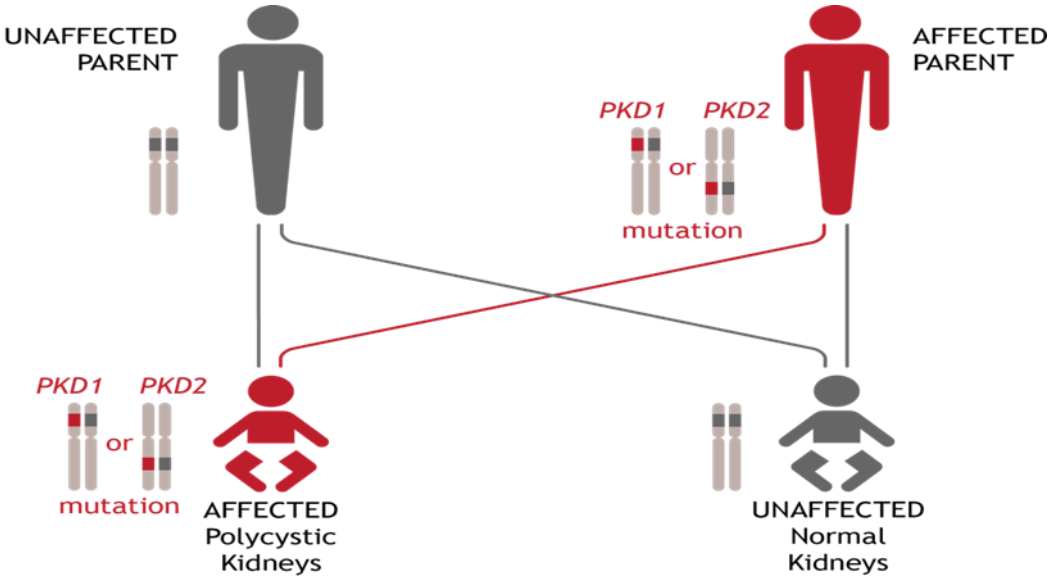

Figure 4: hereditary transmission of Autosomal Dominant Polycystic Kidney Disease [24]

PC-1 is involved in G-protein-coupled signal transduction and is able to induce the arrest of the cell cycle through the activation of the JAK-STAT signaling pathway and likely plays a role in cytoskeletal organization as demonstrated by its presence in E-cadherin complex. Fibrocystin is also part of the complex with PC-2. This complex regulates intracellular Ca^{2+} levels. These proteins (PC-1, PC-2, fibrocystin) are found in the primary cilia [22].

When the cilia are mechanically stimulated, Ca^{2+} enters the cell via the PC-1/PC-2 complex and causes the release of Ca^{2+} from the endoplasmic reticulum. This increase in Ca^{2+} initiates several intracellular signaling cascades. A decrease in Ca^{2+} levels, disruption in Ca^{2+}-dependent signaling pathways and an eventual accumulation of cyclic adenosine monophosphate (cAMP) are thought to lead to cyst formation. Cyst formation is denoted by increased cell proliferation and apoptosis, a response to cAMP that induces mitosis and fluid secretion (**fig. 5**). Within cells of a normal kidney epithelium, cAMP prevents cell proliferation and favours fluid reabsorption. However, in ADPKD cells, the accumulation of cAMP promotes the opposite events. cAMP buildup stimulates fluid secretion inside the cyst. Chloride ion Cl^- enters the principal cells through the $Na^+/K^+/2Cl^-$ cotransporter and is extruded into the lumen. This causes a negative potential in the lumen which increases the movement of Na^+ and osmotically driven water into the lumen of the cyst [22,23]. Vasopressin receptor-2 (V2R)

stimulated pathways cause cAMP levels to increase thus stimulating the cascades that lead to cyst formation and growth [24]. Therefore, antagonism of V2R signaling pathway lowers cAMP levels and reduces the formation and expansion of kidney cysts. Tolvaptan, a selective antagonist of vasopressin receptor 2 slows ADPKD progression via the above mechanism. Blocking the V2R with tolvaptan can affect the extracellular signal-regulated kinase (ERK) pathway, cAMP production, and Cl^- secretion [5,22].

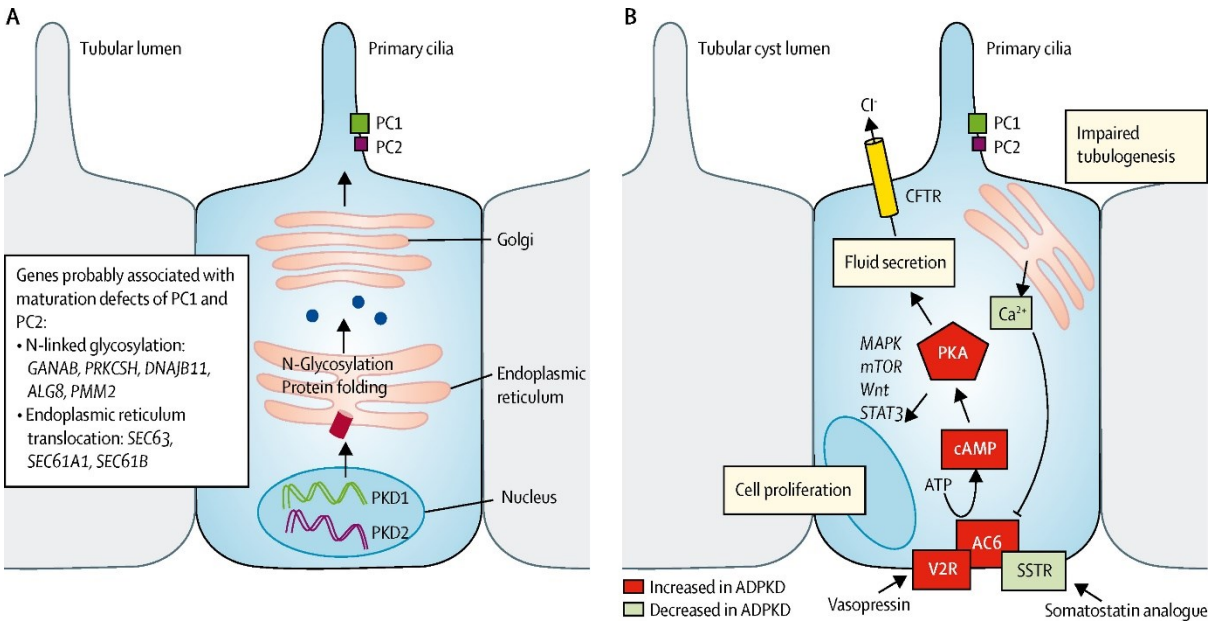

Figure 5: Role of cAMP in the induction of fluid retention in Autosomal Dominant Polycystic Kidney Disease [25,26]

The PC1-PC2 complex in the ciliary membrane inhibits the cilia-dependent cyst activation (CDCA) signal. The dysfunction of PC1-PC2 in ADPKD promotes cyst growth owing to the weakening of the CDCA inhibition. Additionally, ciliary PC1-PC2 plays a role for the maintenance of epithelial cells in differentiated and polarized states. The inhibition of this complex leads to a decreased Ca^{2+} influx into the cell [5].

Moreover, Phosphoinositide 3-kinase (PI3K), AKT serine/threonine kinase (Akt) and mammalian target of rapamycin (mTOR) are kinases that convey signals regulating cellular cycle and survival, cytoskeletal rearrangements and metabolism. In PKD, mTOR cascade promotes cyst growth via the stimulation of kidney tubule metabolism and proliferation. They equally play a role in kidney tubule homeostasis and morphogenesis. Hence the inhibition of this pathway, leads to a reduction in the growth of cysts and kidney size [27].

Additionally, several other pathophysiologic mechanisms of ADPKD are being explored such as epidermal growth factor (EGF) signaling and mitochondria-related signaling pathways [5].

In a nutshell, mutations in *PKD1* and *PKD2* cause abnormal cilia-mediated signaling pathways which lead to dedifferentiation of cystic epithelium, increased cell division, increased apoptosis and loss of resorptive capacity. Cysts growth and expansion compress renal vessels causing intrarenal ischemia and activation of the renin-angiotensin-aldosterone system (RAAS) which leads to progressive cyst growth, increased systemic vascular resistance, sodium retention and renal fibrosis. Despite the progressive destruction of renal parenchyma, compensatory hyperfiltration in the remaining glomeruli maintains renal function within the normal range for decades before the onset of the first symptoms [4,5,20–22,24].

II.1.2.4 Clinical features

Symptoms usually occur after the first four decades of life. They can be classified as renal and extrarenal manifestations. Extrarenal manifestations can further be classified as cystic and non-cystic.

Renal manifestations

Cystic

Multiple and bilateral kidney cysts

Non-cystic

- Hypertension (most common)
- Flank pain
- Enlarged kidneys
- Microscopic or gross hematuria
- Recurrent urinary tract infection (UTI)
- Lower back discomfort
- Nephrolithiasis

Extrarenal manifestations

Cystic

- Hepatic (most common)
- Pancreatic
- Thyroid
- Subarachnoid
- Seminal vesicle
- Splenic
- Prostatic
- Epididymal

Non-cystic

- Intracranial aneurysms (most lethal)
- Mitral valve prolapse and aortic valve regurgitation
- Thoracic, iliac, and abdominal aortic aneurysms
- Coronary artery aneurysms
- Vertebral artery aneurysms
- Intracranial arterial dissection and intracranial arterial dolichoectasia
- Megadolichobasilar artery
- Colonic and duodenal diverticula
- Linea alba, inguinal or umbilical hernia [26]

II.1.2.5 Diagnosis

Suggestive Findings

ADPKD should be suspected in patients with the following:

- Multiple bilateral kidney cysts and absence of manifestations suggestive of a different cystic kidney disease
- Cysts in other organs, especially the liver, but also seminal vesicles, pancreas, and arachnoid membrane
- Enlargement of the kidneys or liver on physical examination
- Hypertension in an individual younger than age 35 years
- An intracranial aneurysm

- Family history consistent with autosomal dominant inheritance (e.g., affected males and females in multiple generations). Absence of a known family history does not preclude the diagnosis [28].

Diagnosis

The diagnosis is made using age-specific kidney imaging (**fig. 6)** criteria based on Ravine's criteria and either an affected first degree relative with ADPKD or genetic testing showing a heterozygous pathogenic variant in *PKD1* or *PKD2* [28].

Ultrasound

Table II: Ultrasound-based unified Ravine's criteria for diagnosis or exclusion of Autosomal Dominant Polycystic Kidney Disease [4,29]

Diagnostic confirmation			
Age (years)	**PKD1**	**PKD2**	**Unknown gene type**
15–29	A total of ≥3 cysts *: PPV=100%; SEN=94.3%	PPV=100%; SEN=69.5%	PPV=100%; SEN=81.7%
30–39	A total of ≥3 cysts *: PPV=100%; SEN=96.6%	PPV=100%; SEN=94.9%	PPV=100%; SEN=95.5%
40–59	≥2 cysts in each kidney: PPV=100%; SEN=92.6%	PPV=100%; SEN=88.8%	PPV=100%; SEN=90%
Disease exclusion			
Age (years)	**PKD1**	**PKD2**	**Unknown gene type**
15–29	No renal cyst: NPV=99.1%; SPEC=97.6%	NPV=83.5%; SPEC=96.6%	NPV=90.8%; SPEC=97.1%
30–39	No renal cyst: NPV=100%; SPEC=96%	NPV=96.8%; SPEC=93.8%	NPV=98.3%; SPEC=94.8%
40–59	No renal cyst: NPV=100%; SPEC=93.9%	NPV=100%; SPEC=93.7%	NPV=100%; SPEC=93.9%

NPV= negative predictive value; PPV=positive predictive value; SEN= sensitivity;

SPEC= specificity * unilateral or bilateral

Age = age of first-degree relative at-risk of ADPKD

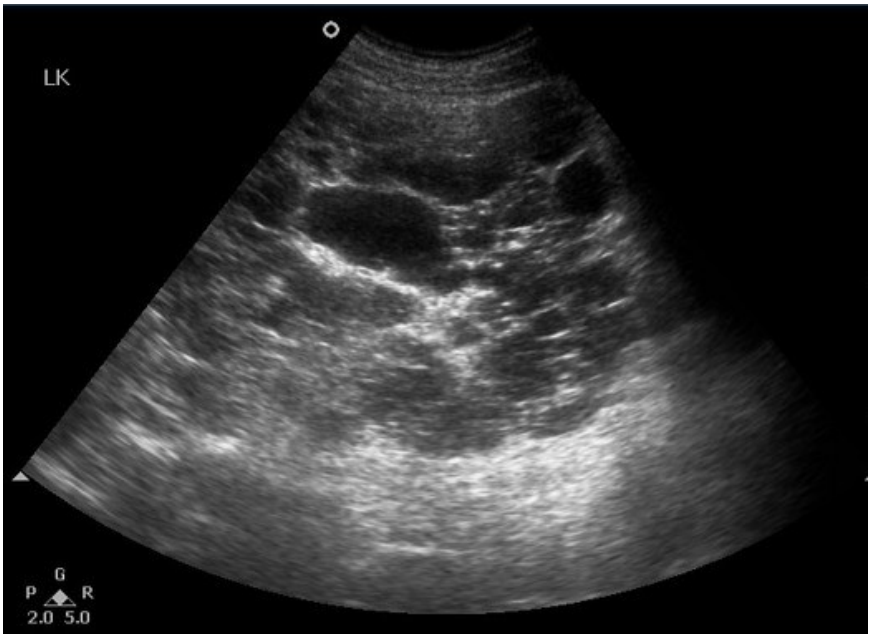

Figure 6: Ultrasound of Autosomal Dominant Polycystic Kidney Disease [30]

Genetic Testing

DNA linkage analysis to identify ADPKD1 and ADPKD2 on chromosome 16 (*PKD1* locus) or chromosome 4 (*PKD2* locus).

II.1.2.6 Pathology

An ADPKD kidney presents macroscopically with progressive cystic dilatation of the tubular system (**fig. 7**). Scanning electron microscopy of ADPKD kidneys show compression of tubules near existing cysts. Cysts were lined by cells typical of the collecting duct, proximal tubule or glomerular visceral epithelium. [31,32].

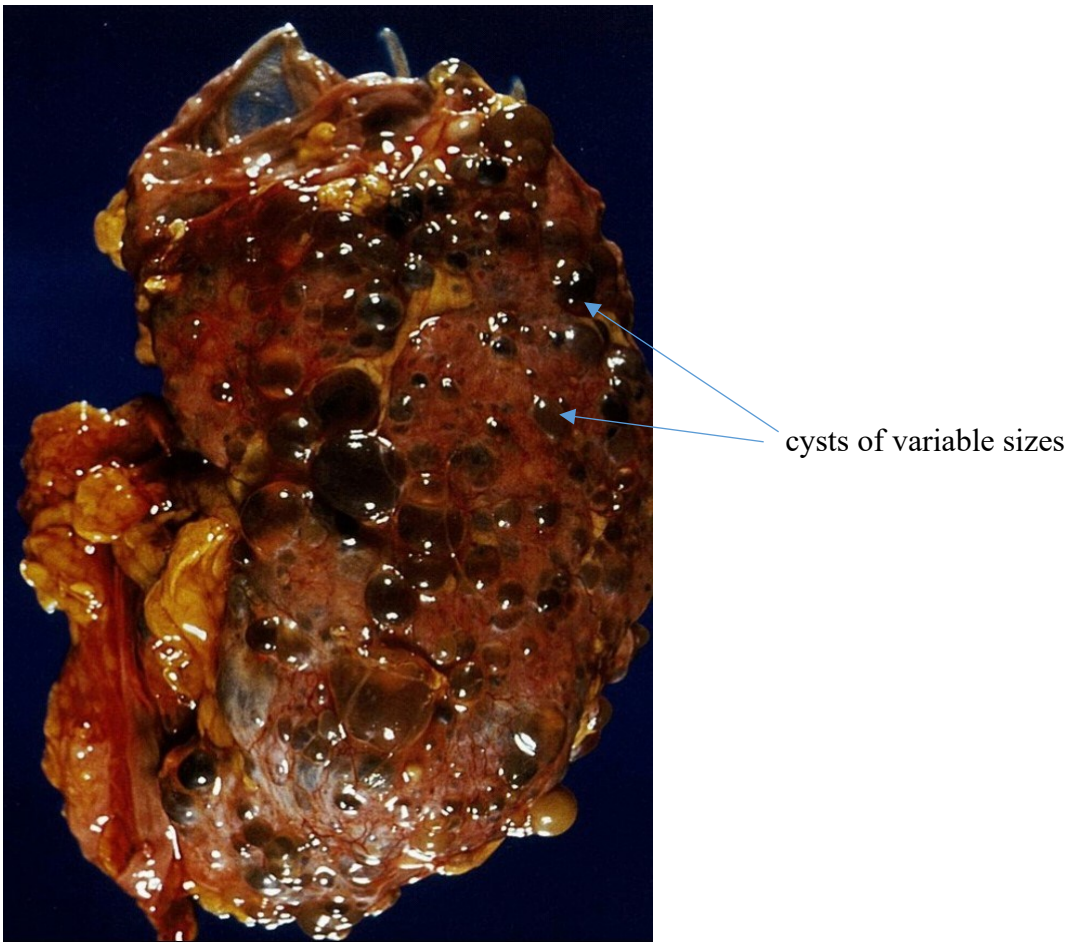

Figure 7: Multiple cysts in kidney with Autosomal Dominant Polycystic Kidney Disease [33]

II.1.2.7 Treatment

General measures [4,20,28,33]

- Rest for flank pain
- High fluid intake to prevent kidney stone formation and to possibly slow cyst progression.
- Low osmolar intake with moderate sodium and dietary protein restriction.
- Avoidance of nephrotoxic substances (e.g NSAIDs, sulfonamide antibiotics, aminoglycosides).
- Early treatment of UTIs to prevent renal cyst infection.
- Analgesics such as tricyclic depressants, non-opoid drugs and narcotics (reserved for acute episodes) for the management of flank pain.

Prevention of Progression [4,20,23,28]

Vasopressin V2 Receptor Antagonists

Tolvaptan has been shown to slow the growth of kidney cysts, delay decline in kidney function and preserve estimated glomerular filtration rate (eGFR) [4].

Anti-hypertensives

The target in the management of hypertension is a blood pressure less than 140/90mmHg. Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are used to counter the effects of the RAAS, reduce proteinuria and ESRD.

mTOR inhibitors

They slow the growth of cysts by inhibiting the stimulation of kidney tubule metabolism and proliferation.

Octreotide

It slows the progression of ADPKD by inhibiting the production of cAMP which promotes fluid retention and cyst growth.

ESRD management

- Kidney transplantation which is the only curative option.
- Hemodialysis or peritoneal dialysis
- Nephrectomy is reserved for affected individuals with a history of infected cysts, frequent bleeding, severe hypertension or massive kidney enlargement.

Table III: Novel therapies in Autosomal Dominant Polycystic Kidney Disease [15]

Drug name/class	Mechanism of action	Supporting data
mTOR inhibitors	Inhibition of mTOR signaling cascade, disrupting renal epithelial cell proliferation	Randomized single-blind study showing significant slowing of renal size in patients treated with rapamycin and telmisartan, compared to telmisartan alone
Vasopressin receptor antagonists	Disruption of binding of vasopressin to V2 receptors, inhibiting cAMP-associated signaling cascades	Phase 2 results show tolvaptan to be safe and well-tolerated; phase 3 studies in progress
Octreotide	Inhibition of cAMP production	Shown in a randomized, cross-over, placebo-controlled trial to significantly slow renal volume expansion; no effect on GFR

mTOR = mammalian target of rapamycin; cAMP = cyclic adenosine monophosphate

II.1.3 The Gut Microbiota

II.1.3.1 Overview

The human body is made up of a vast number of bacteria, archaea, viruses, and unicellular eukaryotes. The collection of all microbes coexisting within their host is known as the **microbiota**. It is equally referred to as the **microflora** or **normal flora**. The combined genetic material of these microorganisms is known as the microbiome.

The microbiota contains about 10^{14} bacteria cells which is 10 times more than the total number of human cells [34]. The microbiota colonizes virtually every part of the human body that is exposed to the external environment with the greatest microbial population found in gastrointestinal tract (GIT). The GIT has an estimated surface area of $200m^2$ which can accommodate a large number of microbes. It equally contains nutrients for these microbes, hence it is a preferred colonization site [34].

Colonization of the human gut by microbes begins at birth with varying abundance per segment. During the passage through the birth canal, neonates are colonized by a rich microbial population (**fig. 8**). Evidence of this has been shown in studies demonstrating similarities between the infant gut microbiota and mother's vaginal microbiota [35]. Equally, there is a difference in microflora community between children born by caesarean section and those born vaginally. Other factors such as the host genetics may influence the normal flora [34].

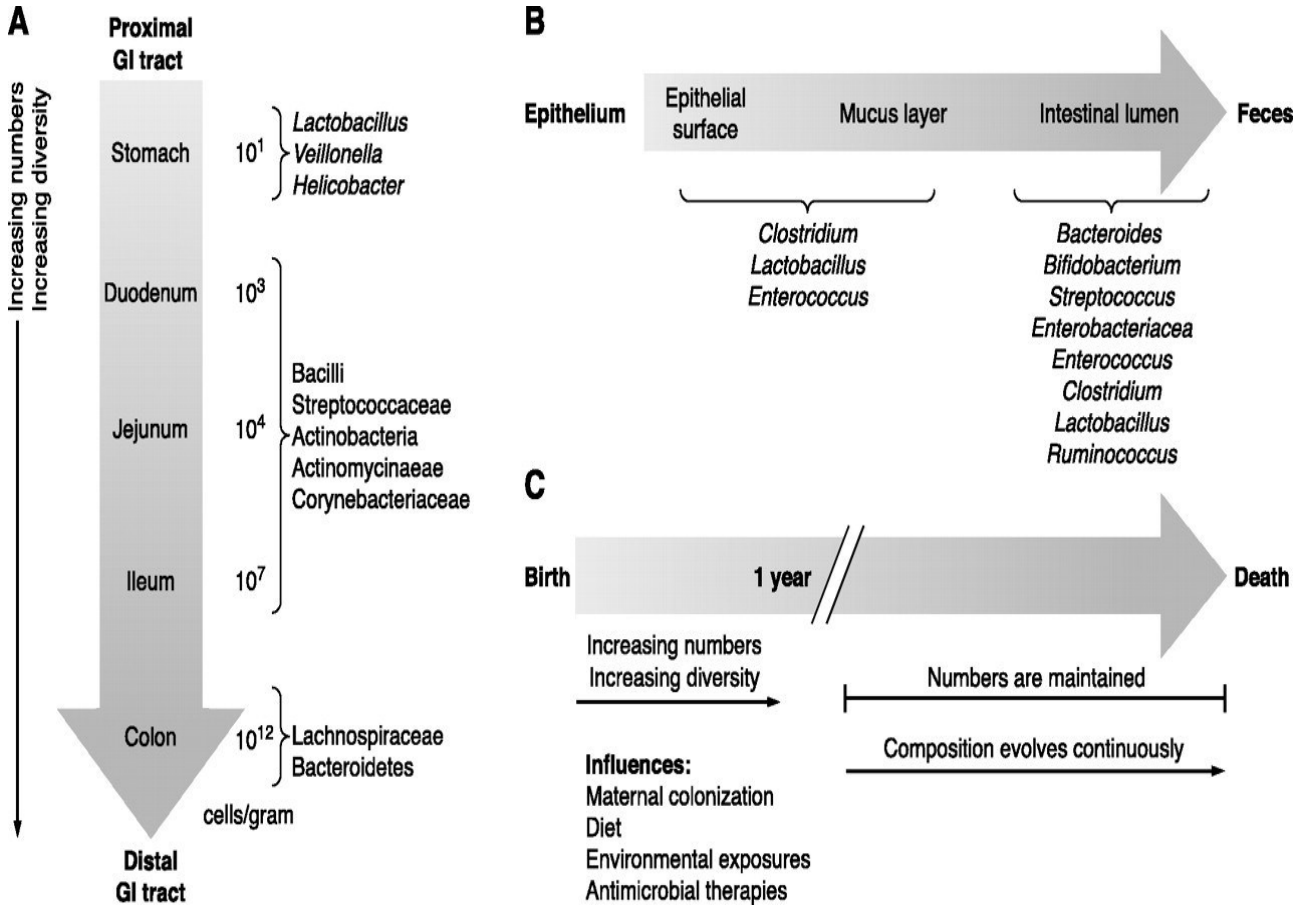

Figure 8: Spatial and temporal aspects of intestinal microbiota composition [34]

II.1.3.2 Composition and Structure

It is composed of aerobes, facultative and strict anaerobes with a predominance of strict anaerobes. There are 2 main phyla represented in the gut microbiota namely: Bacteriodetes and Firmicutes. Others such as Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria are less represented. It is generally accepted that the gut microflora contains 500 – 1000 species, though a recent study involving multiple subjects suggest that over 35000 bacterial species make up the collective microflora [34].

The number and type of bacteria vary depending on the location in the GIT. There is an increase in bacterial number from the stomach towards the colon (the colon alone harbors over 70% of the bacteria population) from 10^1 to 10^3 bacteria per gram of contents in the stomach and duodenum, progressing to 10^4 to 10^7 bacteria per gram in the jejunum and ileum and culminating in 10^{11} to 10^{12} cells per gram in the colon [34].

II.1.3.3 Functions of the Gut Microflora

These microorganisms have evolved to cohabitate and adapt to their host in a symbiotic relationship. This adaptation is displayed in the implication of various bacterial species in the development of the GIT and physiology of the host (**fig. 9**) [34].

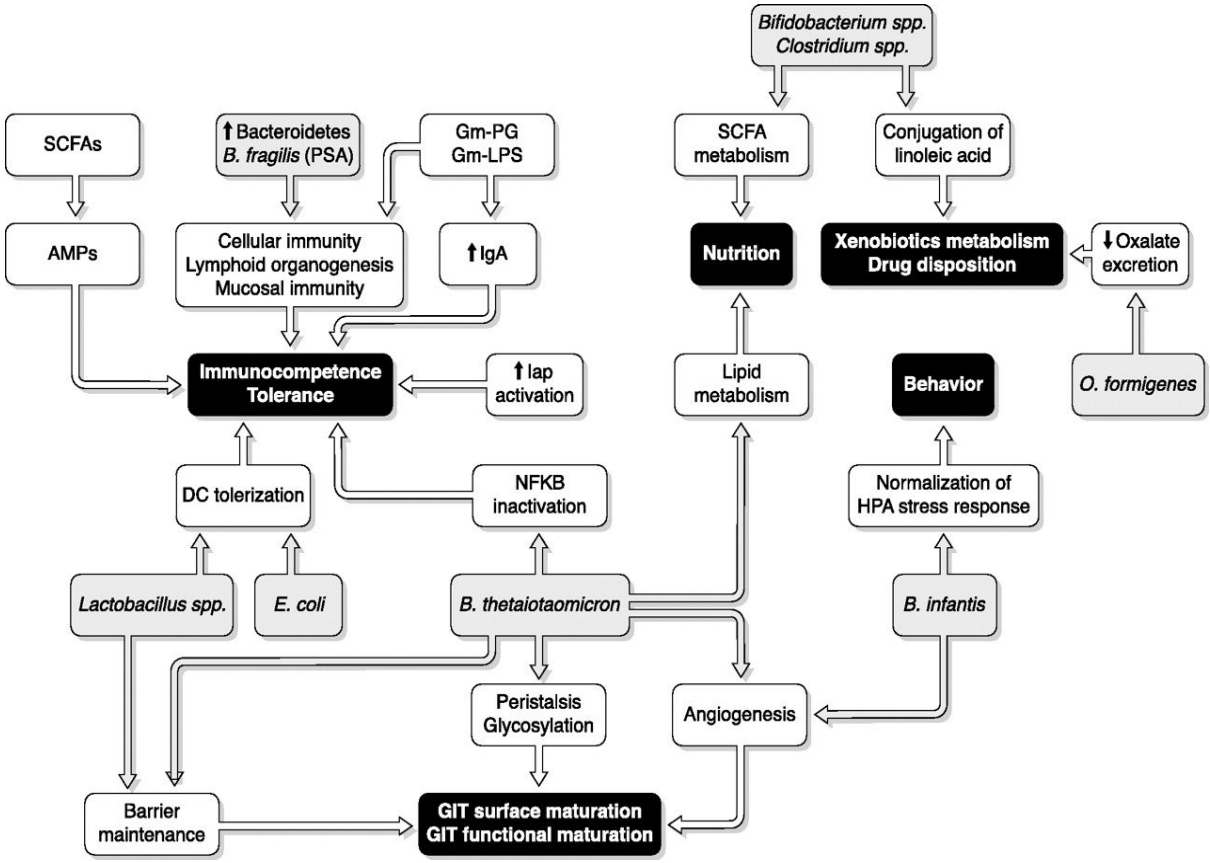

Figure 9: The complex web of gut microbiota contributions to host physiology [34]

a) Immunomodulation

It has been established that the gut microflora plays an important role in the development of GIT and systemic immune responses. The intestinal mucosa represents the largest surface area in contact with antigens from the external environment. It is composed of the gut-associated lymphoid tissue (GALT) such as the payer patches (PP) and small intestinal lymphoid tissue (SILT) in the small intestines, lymphoid aggregates in the large intestine and diffused immune cells in the lamina propria of the GIT. The gut mucosal immune system needs

to tolerate the microbiota to avoid an excessive systemic response and control microflora growth to prevent systemic translocation. The microflora helps it to perform this function [34].

b) Protection

The normal flora equally serves as a physical barrier against pathogens from the external environment via competitive exclusion by preventing them from obtaining nutrients, producing antimicrobial substances and stimulating host defense response [34].

c) Structure and Function of GIT

The microbial community equally plays a role in the development of the immature neonatal GIT among other factors. The microflora participates in the structural and functional maturation of the GIT in the following ways: involvement in the development of peristaltic motility and surface area for the acquisition of nutrients, barrier fortification and regeneration following injury hypothalamic-pituitary-adrenal [34].

d) Extraintestinal function

The gut microbiota plays a role out of the GIT. Evidence of its implication in cardiovascular and neurologic physiology has been shown through studies on germ free (GF) animals with reduced cardiac output (CO), hypotonic mesenteric vasculature, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and the brain-gut-enteric microbiota axis (causing irritable bowel disease and depression)[34].

e) Nutrition and Metabolism

Gut microbiota contribute to provide additional energy sources by extracting calories from indigestible oligosaccharides. It promotes nutrient uptake and utilization through the regulation of the intestinal absorptive capacity. Many bacterial species have been implicated in the metabolism of dietary fiber to short chain fatty acids (SCFA), accounting for a significant part of energy source. SCFA equally enhance anti-oxidant activity and reduce kidney fibrosis [34,36].

II.1.3.4 Factors that influence homeostasis of the microbiota

The composition, diversity and function of the microflora can be influenced by certain factors.

1) Age and mode of delivery

The microbial colonization of the gut begins in utero by microbiota in the amniotic fluid and placenta then continues in the postnatal life. Newborns delivered vaginally have primary gut microbiota dominated by *Lactobacillus* and *Prevotella* derived from the mother's vaginal microflora while those born through caesarean section have gut microbiota dominated by *Streptococcus, Corynebacterium* and *Propionibacterium* derived from the skin [35,37].

2) Diet

It is the most important **determinant** of microbial homeostasis. In breast feeding infants, the dominant species of the gut microbiota are *Lactobacillus* and *Bifidobacterium* which are able to break oligosaccharides in breast milk producing SCFA that direct the immune system to increase the expression of immunoglobulin G while in formula-fed infants, the dominant species are *Enterococcus, Enterobacteria, Bacteroides, Clostridia*, and *Streptococcus*. The primary microbiota acquired during infancy may play an important role in initial immunity during the growth of babies [37]. Diet continues to be an important determinant in shaping the normal flora richness even in adulthood (**fig. 10**). Intake of diets rich in fibre such vegetables, fruits and whole grains is associated with a higher richness and diversity of the gut microbiota with a higher abundance of the insoluble carbohydrate metabolizing-organisms of the Firmicutes phylum [38].

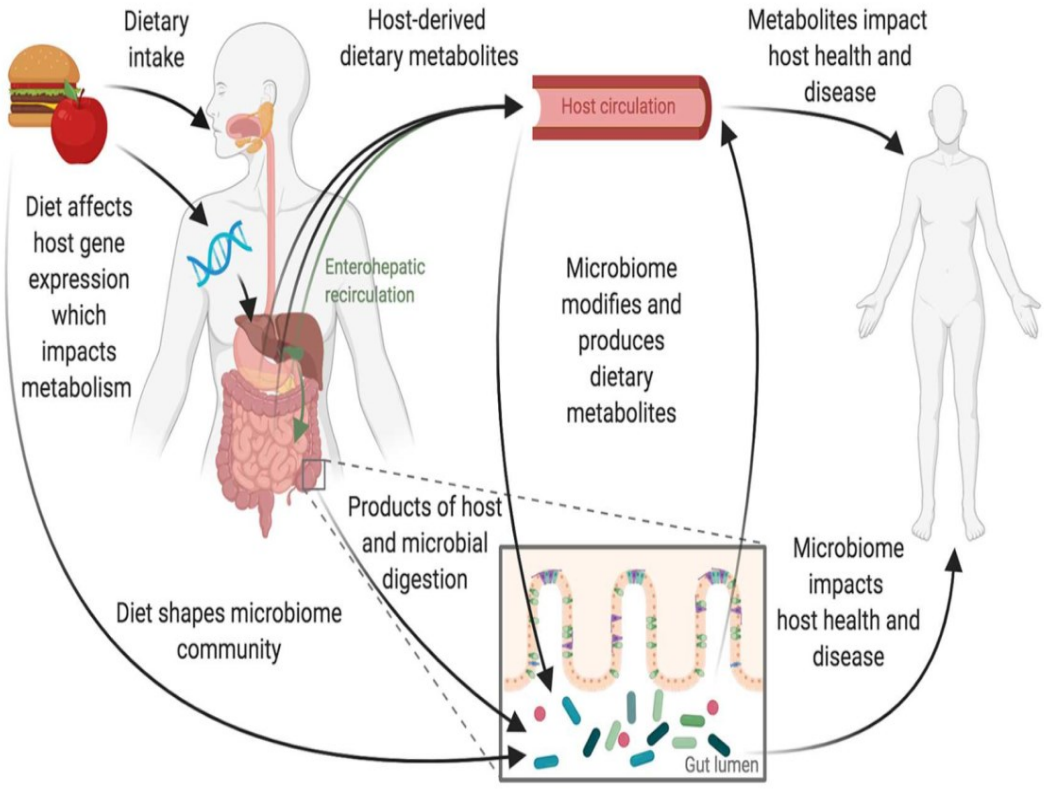

Figure 10: interaction between diet and gut microbiome[39]

3) Drugs

The microflora has the ability to modify drug chemical structure and alter drug efficacy. Also, most drugs affect microbial metabolism by altering the gut microbiota composition and function. Metformin and cholecalciferol were reported to have a beneficial effect on the gut microbiota unlike proton pump inhibitors (PPI) and laxatives [40].

Antibiotics

Despite their therapeutic role, antibiotics destroy both beneficial and pathogenic microbes indiscriminately, allowing loss of normal flora and growth of pathogenic microbes. They disrupt the competitive exclusion mechanism, a property which enables the microbiota to eliminate pathogenic microbes. This disruption promotes growth of pathogens such as *Clostridium difficile* [35,37]

4) Probiotics, Prebiotics and Synbiotics [41,42]

They modulate the gut microbiota through interactions with native microorganisms.

Probiotics are defined as live microorganisms that can provide benefits to human health when administered in adequate amounts. *Lactobacillus casei, Lactobacillus planatarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium infantis, Streptococcus thermophilus,* among others have been shown to improve immunomodulatory and gut barrier functions.

Prebiotics are defined as food ingredients that contain non-digestible oligosaccharides (e.g., inulin and galactooligosaccharides) providing energy sources for gut bugs [43].

A **synbiotic** is the combination of a probiotic and prebiotic [38].

II.1.3.5 Gut microbiota and Disease

Dysregulation of the intestinal mucosa is associated with many pathologies and may play a role in the pathogenesis of some conditions such as obesity, type 2 diabetes mellitus, irritable bowel syndrome (IBS), allergy and kidney disease [44].

Diet-induced changes in the intestinal microbiota have been described in patients with obesity and type II diabetes mellitus with a high-fat diet and food overconsumption. These changes are associated with a greater prevalence of these conditions due to altered host metabolism and altered immune homeostasis. The risk of metabolic disease is influenced by environmental changes which include the role of the microbiota in metabolism [45].

One proposed pathway involved in IBS is through a microbiota-gut-brain axis, associating changes in the gut to symptoms in the central nervous system. A recent report demonstrated that the intake of a probiotic-rich milk product resulted in alterations in brain activity in response to visual emotional stimuli as measured by functional magnetic resonance imaging as compared to the intake of a control product [45].

Some reports describe the depletion in gut microbiota alpha and beta diversity associated with infectious diseases such as human immunodeficiency virus (HIV) infection in adults as well as children [46–48].

A bi-directional functional relationship exists between the colon and the kidney. Uremia influences the colonic microbial metabolism whereas microbial-related metabolites are

involved in the progression of kidney disease. Uremic toxins such as p-cresyl sulphate and indoxyl sulphate are derived from bacterial fermentation of the aromatic amino acids tyrosine and tryptophan respectively, followed by sulphation in the colonic mucosa or the liver. Within the plasma, they are highly protein-bound and accumulate when kidney function fails constituting a negative cycle [45]. Some reports investigate the relationship between the microflora and kidney disease [7,49–52].

II.1.3.6 Techniques of analysis [34]

i. Culture-Based Analysis

The gut microflora has classically been studied using culture techniques that use selective and differential media to select specific bacteria groups based on their metabolic requirements. Culture-based techniques of bacterial enumeration are cost-effective and reproducible. However, they are limited in their ability to distinguish between different bacterial groups due to the complexity of the gut microbial community with the majority of these bacteria being strict anaerobes (hence difficult to culture). It is estimated that >80% of the gut microbiota cannot be cultured under standard laboratory conditions.

ii. Culture-Independent Techniques

These are molecular-based techniques that have replaced conventional culture-based methods. It uses bacterial 16S ribosomal RNA (rRNA) gene as a marker of genetic diversity. The 16S rRNA gene was chosen because of its relatively small size (~1.5 kb) and its ability to strike a balance of conservation and variability with enough variation present to distinguish between different species, yet enough similarity to identify members belonging to the same larger phylogenetic group [34]. Some of these techniques are described below.

a. Sequencing methods

Full-length 16S rRNA sequencing determines the extent of the bacterial diversity, this technique binds 16S rRNA sequences into operational taxonomic units (OTUs) based on their percent sequence identity while pyrosequencing amplifies and select variable regions within the 16S rRNA gene [53].

b. Fingerprinting methods

DNA fingerprinting is a community analysis tool that generates a DNA profile of the microbial community in each sample and thus allows comparison between samples based on the differences observed between their genetic "fingerprints."

c. Fluorescence in situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR)

These techniques utilize fewer probes to target distinct groups of bacteria and are useful when specific bacterial phylogenetic groups are being targeted.

d. The "Meta" Family of Function-Focused Analyses

They permit analysis of the functional contributions of gut microbial communities to their host which is essential to generate a complete view of the ecology and functional capacity of the gut microbiome. Metagenomics involves shotgun sequencing, where reads of large cloned fragments isolated from total community DNA (a shotgun read is equivalent to the size of ~1 gene) are stitched together into contigs (>1,000 bp of contiguous sequence) that are then assembled into scaffolds (contigs + gaps) that approximate whole genomes. Other techniques in this group include metaproteomics, metabolomics and metatranscriptomics.

II.1.3.7 Real-time polymerase chain reaction (real-time PCR)

DNAs are long molecules with double helix structure containing well-defined sequences of the 4 principal nucleotide bases adenine, thymine, guanine and cytosine. The sequence of DNA bases provides a template for precise replication, transcription and translation of genetic information. DNA stores the entire genetic information necessary to determine the structure of proteins and RNAs, to program the synthesis of cell and tissue components and their activity and set the individuality of an organism [54]. **Genes** are pieces of DNA that code for polypeptide chains and RNAs.

PCR enables the exponential amplification of DNA by doubling the number of target molecules with each amplification cycle [55,56]. It amplifies specific DNA sequences by folds of thousands to millions in a thermocycler using sequence specific oligonucleotides and heat stable DNA polymerase [57]. In conventional PCR also known as end-point PCR, detection and quantification of amplified sequences is done at the end of the reaction by post-PCR analysis methods such as gel electrophoresis. On the other hand, in real-time PCR, the amplification product is measured at each cycle. The initial quantity of the target sequence can

be determined by monitoring reactions during the exponential phase [55,58]. There are three main steps that make up each cycle in a real-time PCR reaction: denaturation, annealing and extension.

During denaturation, a high temperature usually 95°C is used to melt DNA into 2 single-stranded DNA. During annealing, an appropriate temperature based on the melting point of the primers is used to enable the attachment of primers to template DNA to form complementary sequences. Extension, usually at a temperature between 70-72°C permits optimal activity of DNA polymerase for primer extension through the addition of nucleotides. These steps are summarized on **fig. 11**.

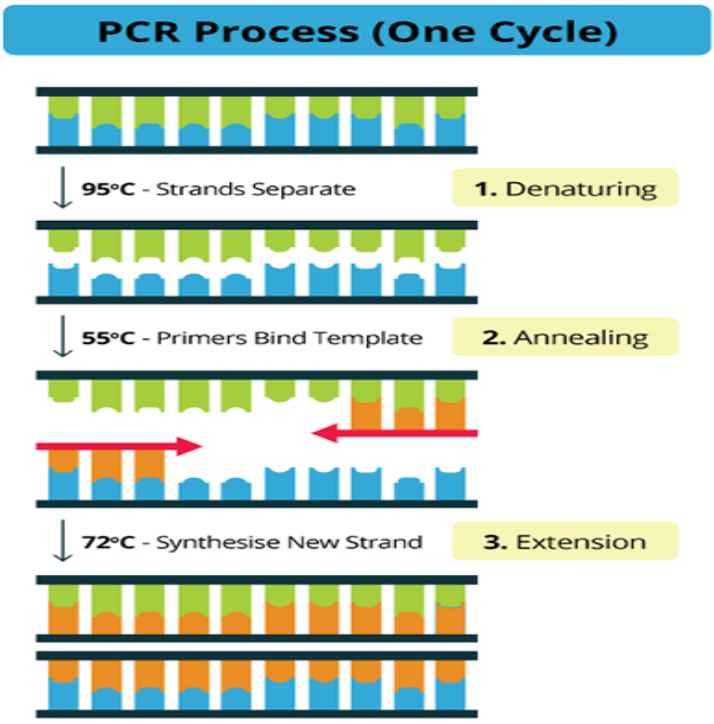

Figure 11: Summary of PCR steps

II.1.4 Gut – Kidney Axis

The association between the intestine and kidney is termed the gut–kidney axis. Gut dysbiosis is defined as an imbalance in the gut microbial community that is associated with disease which is in contrast with eubiosis the healthy state of the gut microflora. Gut dysbiosis is characterized by an abnormal intestinal microbiota composition, which causes metabolic dysfunction, immune disorders and endocrine abnormalities, all of which can cause or worsen CKD [1]. There exists a reciprocal relationship between gut dysbiosis and CKD. Chronic

kidney disease is defined as a reduction in kidney function, an estimated glomerular filtration rate (eGFR) of less than 60 mL/min per 1·73 m², or markers of kidney damage, such as albuminuria, haematuria or abnormalities detected through laboratory testing or imaging and that are present for at least 3 months [6,59].

ADPKD is one of the major causes of CKD. The enlargement of bilateral cysts in ADPKD entail a progression of structural and functional kidney damage. Kidney failure ensues only when the compensatory mechanisms notably overactivity of functional nephrons is overwhelmed. A decrease in kidney function leads to an accumulation of nitrogenous waste compounds (**fig. 12**). This causes an increase in blood urea nitrogen (BUN) and consequently an increase in intestinal urea by unclear mechanisms. The accumulation of intestinal urea favours the growth of proteolytic bacteria (some in the family Enterobacteriaceae, *Staphylococcus, Clostridium, Pseudomonas*) at the detriment of normal flora saccharolytic bacteria (*Bifidobacterium, Ruminococcus, Eubacterium, Lactobacillus, Prevotella*, *Roseburia*) hence causing gut dysbiosis [51]. Saccharolytic bacteria produce short chain fatty acids (SCFA) through fermentation of complex carbohydrate. SCFA have anti-inflammatory, immunomodulatory as well as anti-fibrosis properties which make them renoprotective. Their anti-fibrotic property is through the prevention of transforming growth factor (TGF) b1 signaling pathway and the reduction of extracellular signal-regulated kinases (ERK) phosphorylation [36].

Proteolytic bacteria produce precursors of uremic toxins which are converted to uremic toxins such as p-cresyl sulphate and indoxyl sulphate (**fig. 13**). The microflora produces indole during the metabolism of tryptophan and it is then metabolized by the liver to produce indoxyl sulfate. p-Cresol is a metabolite of tyrosine and phenylalanine and is excreted by the kidney mainly through proximal tubular secretion [60,61]. These toxins are renally excreted and accumulate in the plasma when kidney function fails. They lead to enhanced oxidative stress, inflammation and/or fibrosis which may have a negative effect on the gut microbiota and other host organs like the heart creating a vicious cycle [62].

The study of microbiota changes due to CKD in patients with ADPKD reduces the influence of confounding factors observed in other causes of CKD such as diabetic nephropathy, glomerulonephritis and hypertensive nephropathy [9].

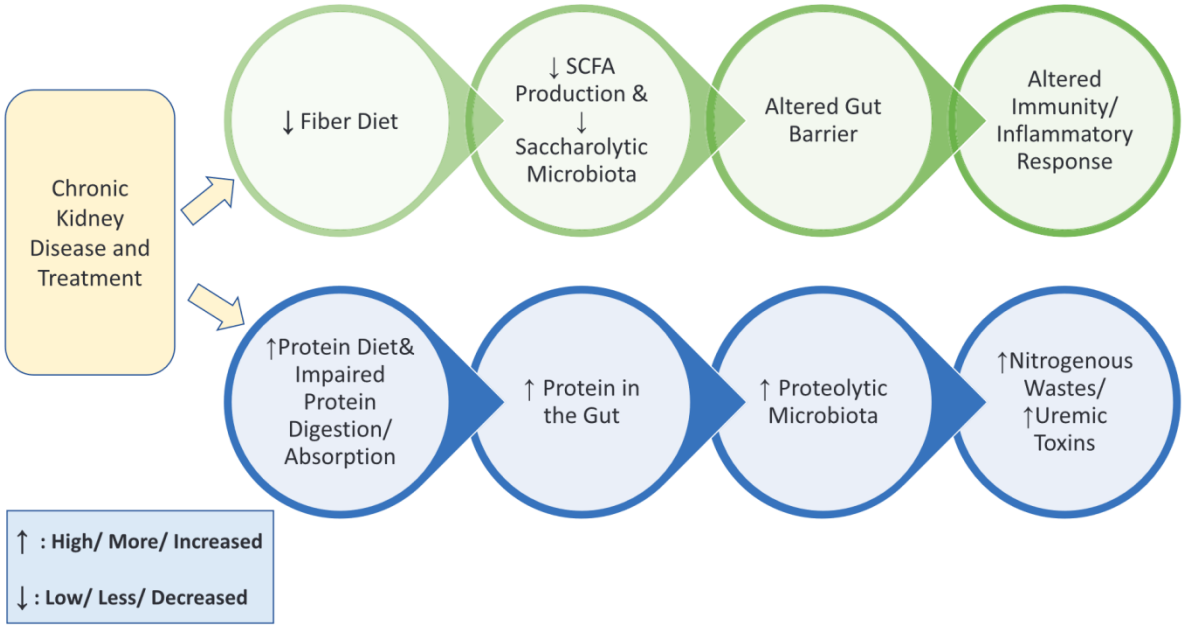

Figure 12: Proposed mechanism of gut dysbiosis in chronic kidney disease [2]

Moreover, therapeutic interventions including the use of antibiotics and diet modification in patients with an impaired kidney function may contribute to gut dysbiosis. A diet low in sodium, potassium, and phosphate impairs the absorption of essential nutrients from food including dietary fibres which are essential for the gut microflora [1,63].

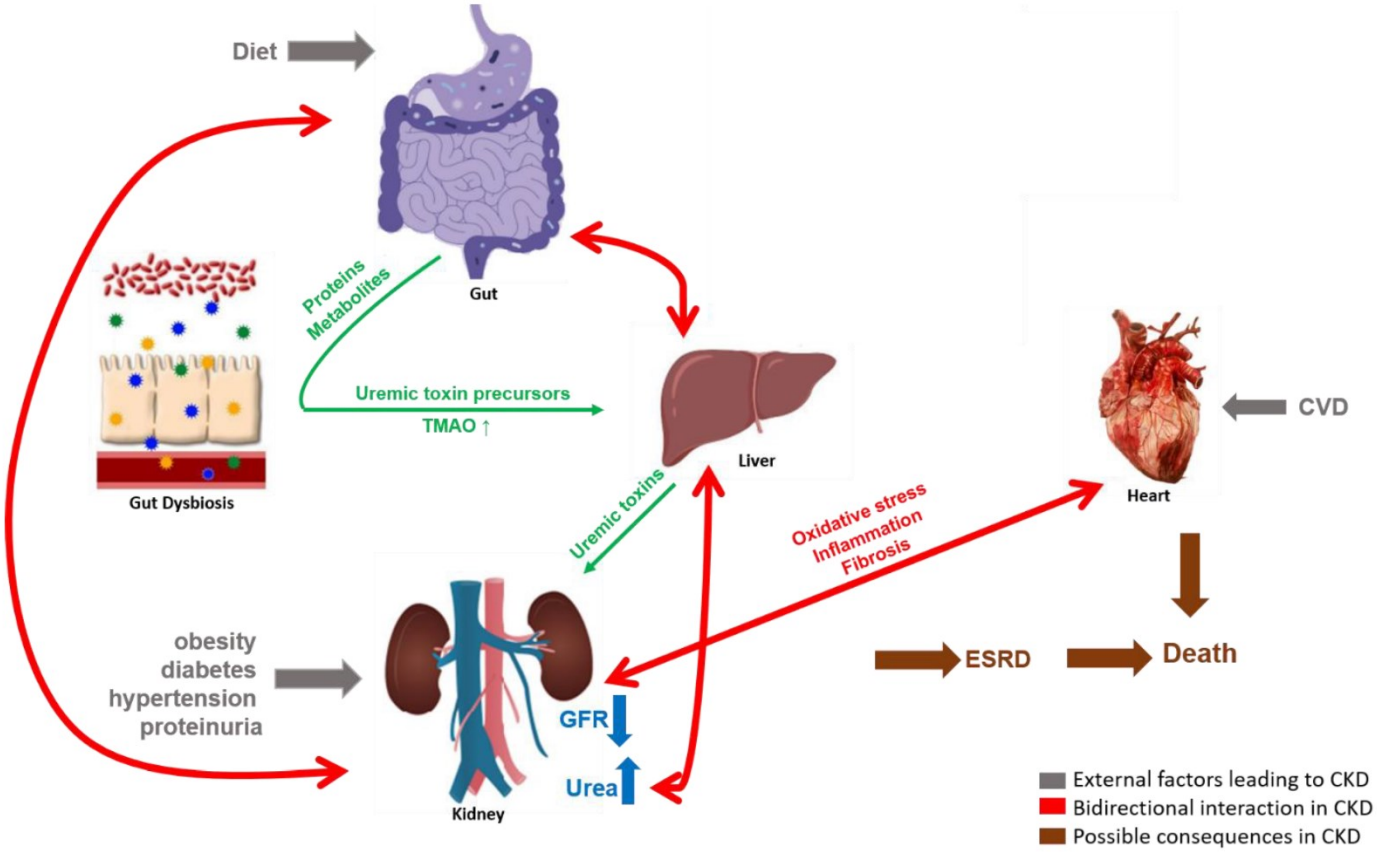

Figure 13: gut-kidney axis [8]

II.2 The gut microbiota and kidney diseases

In 2019, Yacoub *et al.* [9] carried out a cross-sectional pilot study at Buffalo university to evaluate the differences in gut microbiota composition in patients with PKD with varying degrees of kidney function. The classified their study population into three groups: eGFR > 60 mL/min (group 1), eGFR between 15-60 mL/min (group 2) and eGFR < 15 mL/min or on hemodialysis (group 3). They excluded patients with advanced liver, cardiac, autoimmune disease or those with history of intra-abdominal surgery, small or large intestine resection, small bowel obstruction, colon cancer or gastrointestinal bleeding and analyzed the microbiota by 16S sequencing. They found that patients with eGFR < 45 mL/min showed a significantly lower relative abundance of the Bacteria Tenericutes phylum, *Prevotella stercorea*, *Ruminococcus callidus, Eubacterium biforme* species.

Chung *et al.* in 2019 [2] at the Illinois state university did a systematic review on the association between gastrointestinal microbiota and CKD following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. One included study, by Wang and colleagues found in adult patients on peritoneal dialysis *Bifidobacteria, B. catenulatum, B. longum, B. bifidum, Lactobacillus plantarum, L. paracasei* and *Klebsiella pneumonia* in a lower percentage of samples and in lower mean colony counts for all identified bacteria, compared to those from healthy controls. The conclusion made by Chung *et al.* was that currently available data suggest that the microbiota shift from normal populations to an environment that is depleted in SCFA-producing bacteria.

FengXia *et al.* [64] in China studied the correlation between inflammatory factors in CKD and alterations to the gut microbiota in 2019. They conducted 16S ribosomal DNA pyrosequencing using fecal microbiota samples and analyzed the production of serum inflammatory factors in 50 patients with CKD and 22 healthy control (HC) subjects. The results revealed that compared to the HC subjects, patients with CKD exhibited a significant reduction in the richness and structure of their fecal microbiota. At the phylum level, compared to the HC group, patients with CKD also presented reduced abundance of Actinobacteria but increased abundance of Verrucomicrobia. Moreover, the genera *Lactobacillus, Clostridium IV, Paraprevotella, Clostridium sensu stricto, Desulfovibrio* and *Alloprevotella* were enriched in the fecal samples of patients with CKD, while *Akkermansia* and *Parasutterella* were enriched in those of the HC subjects.

Jiang *et al.* [50] in 2016 carried out a study on 65 CKD patients and 20 healthy controls in China to evaluate the difference in butyrate-producing species *Roseburia* spp. and *Faecalibacterium prausnitzii* in faecal samples of both groups using quantitative real-time polymerase chain reaction They observed that these species were significantly different in CKD patients and controls ($p = 0.001$; $p = 0.025$ respectively) and reduced more markedly in end stage kidney disease ($p = 0.000$; $p = 0.003$ respectively). Jiang *et al.* concluded that the negative correlation between *Roseburia spp., F. prausnitzii* and renal function suggests that the depletion of butyrate producing bacteria may contribute to CKD progression and that these species may serve as 'microbiomarkers'.

Stanford *et al.*in 2020 [51] in Australia performed a systematic review of literature on the gut microbiota profile of adults with kidney disease and kidney stones. This review included twenty-five articles with data of 892 adults with kidney disease or kidney stones and 1400 controls. As outcome, compared to controls, adults with kidney disease had an increased abundance of several microbes including Enterobacteriaceae, Streptococcaceae, *Streptococcus* and decreased abundance of Prevotellaceae, *Prevotella* and *Roseburia* among other taxa.

Zarina *et al*. in South Africa last year carried out a randomized controlled trial in which they investigated the effect of ß-glucan prebiotic on kidney function, uremic toxins and gut microbiome in Stage 3 to 5 CKD predialysis participants. Thirty participants joined the intervention group while twenty-nine joined the control group. The primary outcomes were to assess kidney function (urea, creatinine and glomerular filtration rate), plasma levels of total and free levels of uremic toxins (p-cresyl sulfate (pCS), indoxyl-sulfate (IxS), p-cresyl glucuronide (pCG) and indoxyl 3-acetic acid (IAA) and gut microbiota using 16S rRNA sequencing at baseline, week 8 and week 14. There were no differences in relative abundances of genera between groups. There was a significant reduction in uremic toxin levels at different time points, in free IxS at 8 weeks ($p = 0.003$) and 14 weeks ($p < 0.001$), free pCS ($p = 0.006$) at 14 weeks and total and free pCG ($p < 0.001$, $p < 0.001$, respectively) at 14 weeks. They concluded that the ß-glucan prebiotic significantly altered uremic toxin levels of intestinal origin and favorably affected the gut microbiota.

Vaziri *et al.*in 2013 in the USA Vaziri studied the alteration of CKD on the intestinal microbial flora and found a significant difference in the abundance of 190 operational taxonomic units between end-stage kidney disease (ESKD) and healthy controls. They showed that saccharolytic microorganisms such as *Lactobacillus* and *Bifidobacterium* were less present in ESKD compared to healthy controls, whereas proteolytic microorganisms such as *Clostridium* and *Bacteroides* displayed an opposite pattern [7].

Kim *et al*. reviewed studies investigating the gut microflora and acute kidney injury (AKI)**.** One of the studies described gut dysbiosis characterized by a decrease in Lactobacilli and Ruminococcaceae and an increase in Enterobacteriaceae on day 1 in a mouse ischemia/reperfusion injury (IRI) model. Their results show that abrupt changes in kidney function are sufficient to provoke dysbiosis in a short time period. The same study showed that kidney IRI-induced dysbiosis is associated with leaky gut, bacterial translocation, and reduced fecal SCFA levels [65]. Equally, this review discussed gut microbiota relation and kidney stones. A review presented as findings the increase in Enterobacteriaceae and Streptococcaceae and the decrease in Prevotellaceae, *Prevotella* and *Roseburia* in the microbiota of patients with stone formation. *Oxalobacter formigenes* a gram-negative, anaerobic bacterium that degrades oxalate and hence reduces the absorption and urinary excretion of oxalate, may lead to an eventual protection against calcium oxalate stone formation. A study demonstrated that intestinal colonization with *Oxalobacter formigenes* reduced the risk of recurrent calcium oxalate stone formation [65]**.** Similar findings were obtained in another study [49]**.**

Gratiela et al. in Romania in 2018 looked into gut microbiota characteristics of persons with type 2 diabetes and CKD. They studied the gut microbiota of 9 type 2 diabetes patients with diabetic nephropathy and 5 healthy controls using 16 rDNA real-time PCR. They observed no significant differences in *Bacteroides, Prevotella* and *Porphyromonas* between type 2 diabetes patients with diabetic nephropathy and healthy controls [66].

CHAPTER III: METHODOLOGY

III.1 Type of study

A historical cohort study was conducted.

III.2 Site of study

Participants were recruited at the Yaoundé University Teaching Hospital (YUTH) and the Yaoundé General Hospital (YGH). The YUTH is a reference hospital, located in the capital city of Cameroon in Melen. It has an internal medicine department, a dialysis unit and a biochemistry laboratory equipped with a semi-automatic biochemistry analyzer. Three nephrologists, a nursing superintendent and several nurses provide specialized care for kidney diseases.

The YGH is a reference hospital located in Ngousso, a quarter of the capital city of Cameroon. It has several departments and units similarly to the YUTH. The dialysis unit is located on the ground floor of the hospital. Specialized care for kidney disease is provided by a team 4 nephrologists, a nursing superintendent and several nurses.

The Yaoundé National Veterinary Laboratory (LANAVET) is a specialized state facility in charge of research, diagnosis, vaccine production and quality control. LANAVET is based in Garoua, with a branch in Yaoundé. It collaborates with research institutions and analyses biological samples. It trains laboratory managers, technicians and interns. The Yaoundé branch has a laboratory divided into 6 subunits. The staff includes a laboratory manager, veterinary scientists and support personnel.

III.3 Study duration and period

The study ran from November 2022 to May 2023 and the recruitment phase lasted 6 weeks.

III.4 Target population

Exposed:

The source population was ADPKD patients who had consulted at the YUTH and the YGH. The study population was comprised mainly of ADPKD patients followed up at the YUTH and YGH and whose contacts were retrieved from the archives.

Inclusion criteria: we included ADPKD patients > 18 years who gave their consent to participate in the study.

Exclusion criteria: we excluded ADPKD patients > 18 years with diabetes, acute kidney injury, chronic diarrhea, chronic infections (HIV, Hepatitis B and C), on dialysis or who did not provide all specimens.

Unexposed:

Inclusion criteria: we included persons > 18 years without kidney cyst on abdominal ultrasound with a normal kidney function who gave their consent to participate in the study.

Exclusion criteria: we excluded persons > 18 years with diabetes, acute kidney injury, CKD, chronic diarrhea, chronic infections (HIV, Hepatitis B and C) or who did not provide all specimens.

III.5 Sampling

We used a non-probabilistic consecutive and non-exhaustive sampling technique. We recruited 2 ADPKD participants for every control.

III.6 Data collection

Material for collection of baseline information

- Pre-established information and consent forms
- Pre-established questionnaires
- Pens
- Medical records
- Hospital registers and archived files

Material for physical examination

- Weight scale and stadiometre
- BP machine
- Gloves
- Sterile urine containers
- Urine dipsticks

Material for specimen collection and transport

- Blood collection tubes, needles, gloves
- Tourniquet, coton, alcohol, plaster, safety box

- Sterile stool containers
- Cooler and ice pack

Material for specimen analysis (blood)

- Semi-automatic biochemistry analyzer
- Creatinine kit, gloves
- Pipette, pipette tips
- Test tubes, vortex mixer

Material for specimen analysis (stool)

- Biosafety cabinet, gloves, antiseptic solution
- Microcentrifuge tubes and racks
- Pipette, pipette tips, vortex mixer, thermal incubator
- DNA extraction kit
- Thermocycler
- Target-specific primers
- Nuclease-free water
- qPCR mastermix
- qPCR plates and seals

Material for data management and analysis

- Portable computer
- Printer
- USB flash drive
- Data entry form
- Statistical analysis software

Human Resources

- Principal investigator
- Supervisors
- Hospital staff
- Laboratory staff

III.7 Procedure

III.7.1 Administrative Procedure

Ethical clearance was obtained from the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. Administrative authorizations were obtained from the Yaoundé General Hospital and Yaoundé University Teaching Hospital.

III.7.2 Recruitment and data collection of participants

ADPKD group:

The contacts of patients who had an established diagnosis of ADPKD and who were followed up at the YGH and YUTH were obtained from archived lists. These persons were contacted and invited for the study. We met them on the days of consultation. At the out-patient department, prospective participants were welcomed and given detailed information concerning the study in their language of choice. The information was transmitted with the use of an information sheet which was duly explained. All those who met our inclusion criteria were interviewed individually and their medical records were reviewed for exclusion criteria. Thereafter, we attributed codes to retained patients, administered a pre-established questionnaire and collected urine, blood and stool specimens which were used for urine dipstick, serum creatinine tests and gut microbiota analysis.

Healthy control (HC) group:

We approached and invited prospective participants who potentially matched to the ADPKD group for sex and age ± 5 years [67]. They ranged from hospital staff, relatives of ADPKD patients to volunteers. At the out-patient department, prospective participants were welcomed and given detailed information concerning the study in their language of choice. The information was transmitted with the use of an information sheet which was duly explained. All those who met our inclusion criteria were interviewed individually. Thereafter, we attributed codes to retained persons and performed renal ultrasounds to confirm the absence of kidney cysts. We administered a pre-established questionnaire and collected urine and stool specimens which were used for urine dipstick and gut microbiota analysis.

Summary of Procedure

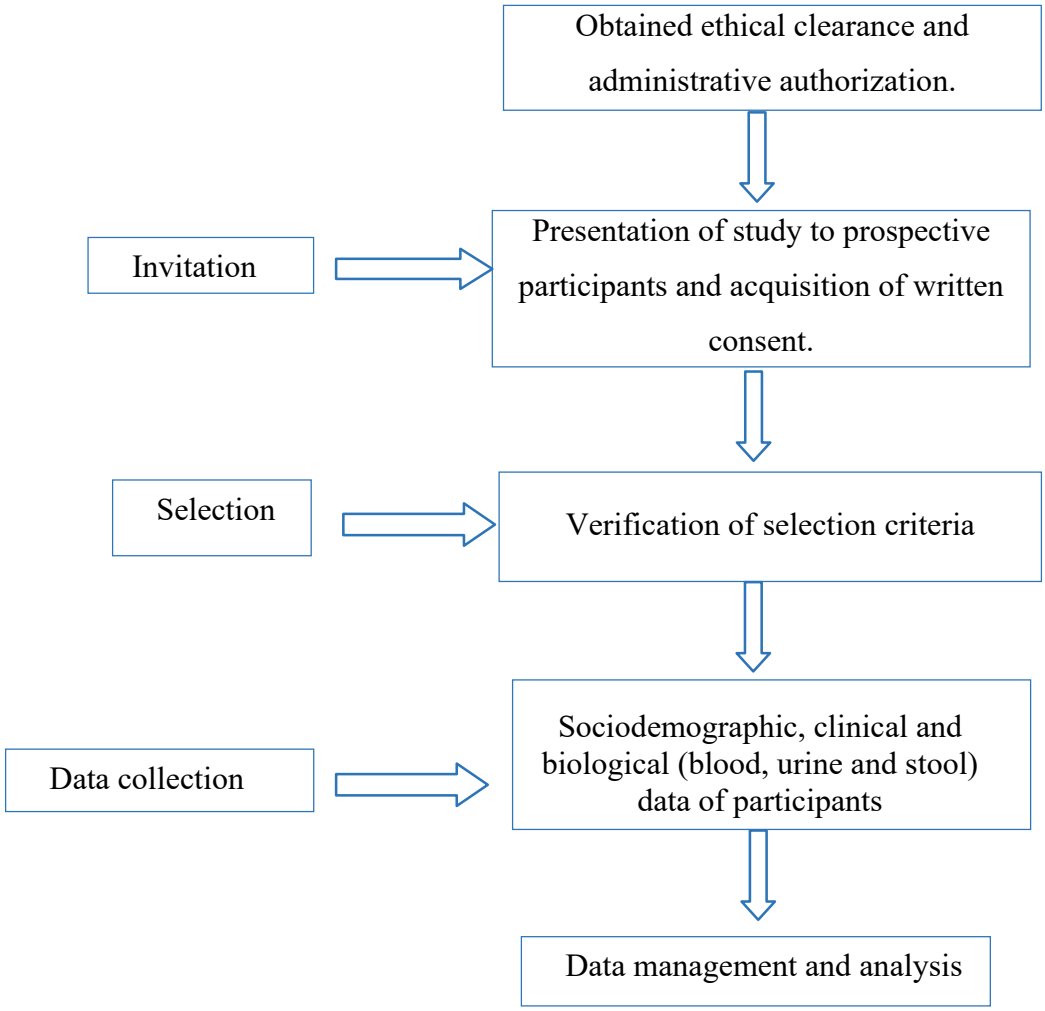

Figure 14: Procedure flow chart

III.7.3 Variables

III.7.3.1 Sociodemographic variables

- **Age**: the age of the participants was expressed in years. We noted their date of birth according to their national identification cards and calculated their age from the date of interview.
- **Sex**: the sex of the participants either male or female was obtained from their national identification cards.

III.7.3.2 Clinical variables

- **Medical history:** we obtained from participants and their medical records history of; ADPKD, hypertension, urinary tract infection (UTI) and kidney stone.
- **Drug history:** we obtained from participants and their medical records history of intake of; vitamin D supplementation, proton pump inhibitors and antihypertensives.
- **Bristol stool scale (BSS):** participants self-classified stool consistency into one of seven categories [68].
- **Dietary assessment:**

  We obtained information on dietary intake by means of a 24h diet recall and a 58-item food frequency questionnaire. The food frequency questionnaire (FFQ) was used to assess the frequency of consumption of certain foods over the last 3 months. The FFQ was used to elaborate scores: score I – microbiota protective pattern and score II- microbiota non-protective pattern. Every participant obtained a score at the end of the assessment [69–71].

- **Clinical parametres**
  - **Weight measurement**

Participants' weight was measured in a standing position after belongings had been kept aside. Before the measurement, the scale was adjusted to 0Kg. Weight was expressed to the nearest Kg. The same electronic device was used for all participants in the study.

  - **Height measurement**

Participant height was measured using a standard graduated stadiometer recorded in metres. After the removal of shoes, participants stood in an erect position with the occiput, shoulders,

buttocks and calf in contact with the vertical portion of the stadiometer and arms freely hanging on either side of the body and facing the thighs. The heels were placed together, the tips of both feet were slightly apart to permit equal weight distribution on both legs. The sliding plate of the stadiometer was lowered to the top of the participant's head and the height was measured.

- **Body Mass Index(BMI/Kg/m^2):**

BMI was calculated using Quetelet's formula and expressed in Kg/m^2 to the nearest one decimal place.

- **Blood pressure (BP/mmHg):**

The BP was assessed using a BP-103H digital blood pressure monitor (Aspen Diagnostics Pvt. Ltd., India). Before assessment, the participant was asked to relax and sit on a chair with their feet on the floor, legs uncrossed, back supported, for at least 5 minutes. The participant was asked not to talk, read or use electronic devices during the rest period. An adapted cuff-for-arm was snugly fitted with the distal part of the cuff placed 1–2 cm above the cubital fossa. The patient's arm was supported so that the middle of the cuff was at heart level. BP was measured in both arms. The measurement was repeated about 2minutes after in the arm with a higher reading and the average of the last two readings was recorded [72].

- **Urine dipstick:**

Pre – analytic phase

This was done using URS-11 (Teco Diagnostics, California, USA) urinalysis strip test. Participants had to avoid all intense exercise 72hours prior to urine collection. Urine was collected 1 week after the end of menstruation. We noted results relating to the presence of proteins, leucocytes and blood in urine.

The urine dipstick test was done by analyzing midstream urine in a container. The test strip was immersed in the urine container for less than a second. The strip was taken out and excess urine removed by means of a paper towel. Leukocytes were read at 120 seconds by comparison to the color chart [73].

Analytic phase

The principle behind this test is the reaction between urine and dry reagents on the strip.

1. **Proteins**: The strip test is based on the color change of the indicator tetrabromophenol

blue in the presence of proteins. A positive test was considered when the color change corresponded to a value of at least 0.3g/l.

2. **Blood**: The strip test is based on haemoglobin pseudoperoxidase activity which catalyzes thereaction of 3,3',5,5'tetramethylbenzidine with buffered organic hydrogen peroxide. A positive test was considered when the color change corresponded to a value of at least 10 RBC/µL

3. **Leukocytes**: The strip test reveals the presence of leucocyte esterase. The esterase cleaves a derivatized pyrazole amino acid ester to release. The cleaved molecule reacts with dizonium salt to produce a purple color. A positive test was considered when the color change corresponded to a value of at least 70 WBC/µL.

III.7.3.3 Laboratory variables

III.7.3.3.1 Kidney Function Test

Serum Creatinine [74]

Pre- analytic phase

Specimen collection

A 5ml specimen of venous blood from the antecubital vein of the arm was collected by means of a needle and a collection tube. The participant was seated and calm. With the elbow extended, we ensured asepsis by means of alcohol at 70º and absorbent cotton. We drew 5 ml of blood with a suitable needle. After removal of the needle, the puncture site was compressed for one minute and a pressure dressing was placed. The blood was collected into a sterile, pre-labelled plain tube. The specimen was transported in a box at room temperature about 3hours after collection to the biochemistry laboratory.

Analytic phase

Principle: it is based on the reaction between creatinine and sodium picrate according to Jaffe's reaction. Serum creatinine reacts with picric acid at alkaline temperature to form a yellow orange complex. The rate of change of absorbance at 490nm (490-510nm) is proportional to the creatinine concentration in the sample.

Apparatus: pipettes, pipette tips, test tubes, centrifuge, reagents from BIOLABO® Creatinine kit (BIOLABO SA, France), BIOLABO® KENZA MAX photometer (appendix) were used.

Reagents

Table IV: Serum creatinine test reagents

Product	Quantity
R1	
Sodium hydroxide	150mmol/L
Disodium phosphate	6.4mmol/L
R2	
Picric acid	4.0 mmol/L
Sodium decyl sulphate	0.75mmol/L
R3	
Etalon	117mmol/L

Procedure: the specimen was centrifuged at 5000 revolutions/ minute for five minutes. Afterwards, 500µl of reagent 1(sodium hydroxide and disodium phosphate) and 500µl of reagent 2 (picric acid and sodium dodecyl sulphate) provided in the kit were aspirated with a pipette and placed in three test tubes.

- 100 µl of the calibrator (R3), 100 µl of serum control and 100 µl of the specimen's serum was added to the three test tubes containing reagent 1 and reagent 2 solutions.
- The test tubes were placed in the biochemical analyzer and creatinine values read and recorded about two minutes later. The order was: the calibrator, the serum control and the specimen.

Reference values: The normal values of creatinine according to the reagent used were 9-13g/L for adult males and 6-11mg/L for adult females.

The serum creatinine was used to estimate glomerular filtration rate (eGFR/in ml/min/1.73m^2**).**

III.7.3.3.2 Gut Microbiota analysis

- **Pre-analytic phase**

Specimen collection

Participants were given a sterile stool collection container with instructions. They were instructed to avoid urine contamination by voiding bladder first and to collect the specimen by means of the spatula embedded in the container. The containers were labelled and transported to LANAVET by means of a cooler and ice packs about 4 hours following collection. Once there, they were immediately stored in a -80°C freezer [47,58,75].

- **Analytic phase**

DNA extraction

Principle: The principle of the extraction of DNA is summarized by three main steps. First, the disruption of cytoplasmic and nuclear membranes. Secondly, the separation and purification of DNA from other components of the cell lysate such as proteins, lipids and other nucleic acids and thirdly the concentration and purification of DNA [76]**.**

Apparatus: pipettes, pipette tips, microcentrifuge tubes, vortex mixer, centrifuge, thermal incubator, DNeasy Blood & Tissue Kit (Qiagen, Dusseldorf, Germany) were used.

Procedure:

Specimens were removed from the -80°C freezer and thawed on ice in a biosafety cabinet.

- 1.5mL microcentrifuge tubes were labelled and 400µL of phosphate-buffered saline (PBS) was transferred in each tube.
- About 0.2g of each specimen was transferred into each tube containing PBS. The tubes were vortexed and centrifuged at 14000 revolutions/min(rpm) for one minute.
- 200µL of the supernatant was transferred to new labelled microcentrifuge tubes.
- 180 µl Buffer ATL and 20 µl Proteinase K was added to each tube. The tubes were vortexed thoroughly and incubated at 56°C for 10 minutes.
- 200µL of 70° alcohol was added to the mixture and the tubes were vortexed.
- 700µL of the new mixture (lysis product) was placed in a spin column in a 2mL collection tube. The tubes were centrifuged at 8000rpm for 1minute.
- The collection tubes were discarded, replaced and 500µL of AW1 buffer was added to each filter and centrifugation followed at 8000rpm for 1minute.

- The collection tubes were discarded, replaced and 500µL of AW2 buffer was added to each filter and centrifugation followed at 13000rpm for 3minutes. The filters were transferred to 1.5mL microcentrifuge tubes with lids.
- 200µLof elution buffer was transferred to the centre of the filter and each tube was centrifuged at 8000rpm for 1minute. The filters were discarded, the tubes were closed, DNA quantity and quality were evaluated using a spectrophotometer and the tubes were stored at -80°C.

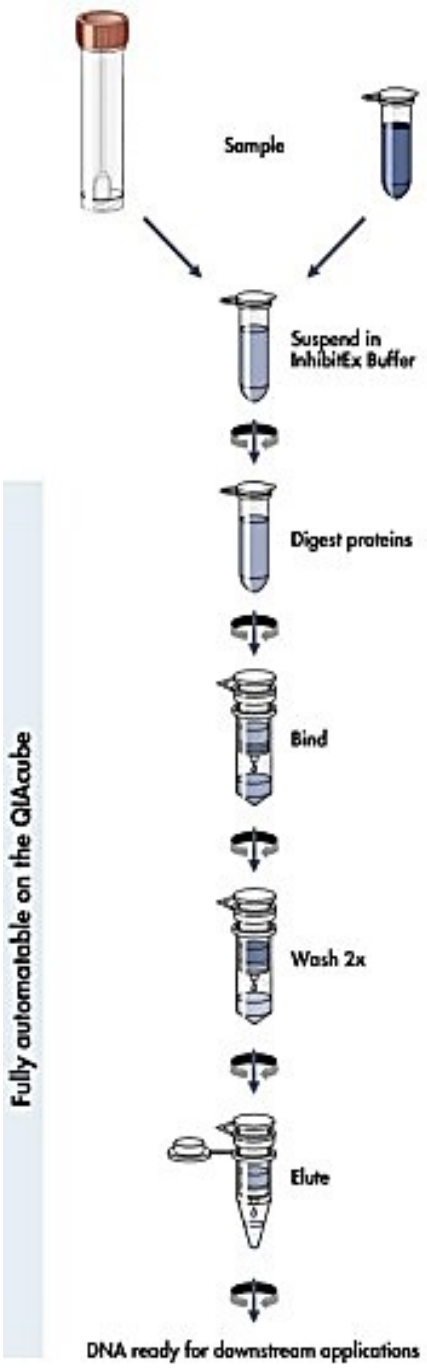

Figure 15: Summarized steps of DNA extraction

Real-time PCR

Principle: The principle is the selective amplification of specific DNA sequences by several folds in a thermocycler using primers and heat stable DNA polymerase. This enables the quantification of the initial target sequence.

Apparatus: Biosafety cabinet, PCR tubes, pipette, pipette tips, vortex mixer, vortex shaker, PCR mastermix (Luna® universal qPCR master mix, New England BioLabs) and thermocycler CFX96 Touch Real-Time PCR Detection System by BIORAD (appendix).

Procedure:

Primer pairs for target sequences are designed, blasted synthesized and reconstituted to stock concentration. A real-time PCR mastermix is equally obtained and tested with the pairs of primers and a positive control [49,50]. In this case, the controls used were *E. coli* and *S. enterica* cultures for Enterobacteriaceae and a yoghurt DNA extract for *Lactobacillus* and *Bifidobacterium* (appendix)

Table V: The sequence and specifications of the primers used in this study

Bacteria	Sequence (5' – 3')	Product size (bp)	Reference
Lactobacillus	F: GCAGCAGTAGGGAATCTTC R: AGTTACTACCTCTATCCTTCTTC	119	[49]
Bifidobacterium	F: CGCGTCCGGTGTGAAAG R: CCCCACATCCAGCATCCA	244	[49]
Enterobacteriaceae	F: GGGGATAAC(T/C)ACTGGAAACGGT(A/G)GC R: GCATGGCTGCATCAGG(C/G)TT(G/T)C		[77]

F = forward; R = reverse

Reaction Setup:

Table VI: real-time PCR reaction set-up

Component	20µL reaction	Final concentration
Luna universal qPCR master mix	10µL	1X
Forward primer (10µM)	0.5µL	0.25µM
Reverse primer (10µM)	0.5µL	0.25µM
Template DNA	variable	< 100ng
Nuclease-free water	to 20µL	

- Luna Universal qPCR Master Mix, primers and DNA extracts are thawed at room temperature then placed on ice.
- The total volume for the appropriate number of reactions was determined, plus 10% overage and the assay mix of all components was prepared except DNA template
- . The mixture was gently vortexed and centrifuged.
- The assay mix was aliquoted into qPCR tubes, DNA templates were added to the mix and the qPCR tubes were spun.
- The real-time instrument was programmed with the indicated thermocycling protocol (**Table VII**). A plate read was included at the end of the extension step and the SYBR scan mode setting was selected on the real-time instrument.

Table VII: Real-time PCR protocol used in this study

Cycle step	Temperature	Time (in seconds)	Cycles
Initial denaturation	95°C	60	1
Denaturation	95°C	15	40-45
Extension	60°C	30 (+ plate read)	
Melt curve	60-95°C	various	1

Melt curve analysis was done to verify the specificity of amplifications and the quantification cycles (Cq) of each specimen for each target bacteria (appendix) was used to estimate initial target DNA quantity in ng/µL using the positive controls' DNA yield in ng/µL and linear equations. The standard equation used was:

Target bacteria DNA (ng/µL) = [Cq $_{(p)}$ * DNA yield$_{(p)}$ (ng/ µL)] / Cq $_{(t)}$

Where;

Cq = quantification cycle

p = positive control

t = target bacteria

We compared the estimated DNA abundance in ng/µL of three bacterial groups (one family and two genera) notably: Enterobacteriaceae, *Lactobacillus* and *Bifidobacterium.*

III.7.4 Definition of terms

Hypertension:

It was defined as BP ≥140/90 mmHg and/or the use of antihypertensive medications.

Urinary tract infection (UTI):

Patients for whom UTI was mentioned as a complication were defined as those who had a history of UTI.

Kidney stone:

Patients for whom kidney stones were mentioned as a complication were defined as those who had a history of kidney stone on ultrasound.

Family history of ADPKD:

Patients who were said to have a family history were those who had a first-degree relative with an established diagnosis of ADPKD.

Bristol stool scale (BSS):

BSS is a measure of stool consistency, a reflection of faecal water content which correlates with colon transit time. Low scores (1-3) represent firm stool and slow transit while high scores (5-7), loose stool and fast transit.

The 24h recall:

The 24h diet recall is a tool for diet assessment in which the participant is asked to report dietary intake of the last 24 hours. Total calorie, protein, carbohydrate, fat and fibre intake were obtained from this report [70,71].

Food frequency questionnaire (FFQ):

The 58-item FFQ was derived from reports of local meal consumption with an emphasis on food items known to promote gut microbiota health. The FFQ was analyzed using the Fornés scoring method [69], the food items recorded through the FFQ were classified into one of six categories of consumption frequency: f1 – never; f2 – foods consumed less than once a month;

f3 – foods consumed one to three times a month; f4 – food consumed once or twice a week; f5 – foods consumed three or four times a week; f6 – foods eaten daily. To treat each frequency of consumption as a quantitative variable, a weight was assigned to each category of frequency of consumption, based on the frequency of consumption during the last 3 months. A maximum weight of 1 was assigned for foods consumed the daily. The other weights were calculated using the formula:

$Sn = (1/90)[(a + b)/ 2]$

Where a and b are the number of days of frequency. Sn is the weight.

For each individual, the calculation of the food consumption frequency score corresponding to two groups (Group I and Group II) was obtained. Group I was formed by foods considered to be protective for the gut microbiota and Group II was formed by foods that are non-protective for the gut microbiota.

Group I: fruits, vegetables, fermented foods, yoghurt, seeds, grains (pumpkin seed, sesame), popcorn, fish, palm oil, resistant starch (cocoyam, cassava). Group II: meat (beef, pork, poultry), egg, white rice, bread, margarine, mayonnaise, ripe plantains, sugar, alcoholic beverages

Consumption frequency scores were calculated by simple summation, in which the sum of the consumption frequency weighting values for the foods corresponding to each group was calculated.

Score I was represented by the sum of the weighting values for the foods that made up Group I and score II, by the sum of the weighting values for the foods that made up Group II [78–87].

BMI:

BMI is an indicator that evaluates the health risk related to body fat in adults. It is calculated as the body mass divided by the square of the body height and expressed in Kg/m^2 and is classified into categories:

Underweight – BMI < 18.5 Kg/m^2

Normal weight – BMI between 18.5 to 24.9 Kg/m^2

Overweight – BMI between 25 to 29.9 Kg/m^2

Obesity class I – BMI between 30 to 34.9 Kg/m^2

Obesity class II – BMI between 35 to 39.9 Kg/m^2

Obesity class III – BMI greater than or equal to 40 Kg/m^2

Dipstick proteinuria:

A specimen was considered to have proteinuria if the urine strip indicated any of the following:

- 1+: >30 mg/dl
- 2+: 100 mg/dl
- 3+: 300 mg/dl
- 4+:>1000 mg/dl

Dipstick hematuria:

A specimen was considered to have hematuria if the urine strip indicated any of the following:

- 1+:10 RBC/µL
- 2+:50 RBC/µL
- 3+:250 RBC/µL

Dipstick leucocyturia:

A specimen was considered to have leucocyturia if the urine strip indicated any of the following:

- 1+:70 WBC/µL
- 2+:125 WBC/µL
- 3+:500 WBC/µL

Kidney function:

It was estimated from the glomerular filtration rate calculated by software using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2001 equation.

$GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] x 1.159 [if black] Where:

Scr is serum creatinine (mg/dL)

κ is 0.7 for females and 0.9 for males

α is -0.329 for females and -0.411 for males, Min indicates the minimum of Scr/κ or 1 Max indicates the maximum of Scr/κ or 1.

eGFR less than 60 ml/min/1.73m^2 corresponded to an impaired kidney function.

Chronic kidney disease was defined and staged according to the 2012 Kidney Disease Improving Global Outcomes (KDIGO) criteria as either;

- a reduction in kidney function, an estimated glomerular filtration rate (eGFR) of less than 60 mL/min per 1·73 m²,
- or markers of kidney damage, such as albuminuria, haematuria or abnormalities detected through laboratory testing or imaging and that are present for at least 3 months

Table VIII: Stages of Chronic Kidney Disease [6]

STAGE	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal or high GFR	≥90
2	Kidney damage with mild decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure	<15 or dialysis

III. 8 Statistical analysis

Data was analysed using the software IBM-SPSS (Statistical Package for Social Siences) version 26. The threshold of statistical significance was set at $p < 0.05$. All obtained data was summarized and presented on tables, histograms and boxplots. Results were expressed as proportions for categorical variables and mean ± standard deviation or median [interquartile range] for continuous variables. The chi-squared test, the Student's t test and non-parametric tests were used to compare qualitative and quantitative variables.

III.9 Ethical considerations

III.9.1 Ethical clearance

Ethical clearance was obtained from the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences to carry out the study. Equally, research authorizations were gotten from the YUTH and YGH. A written informed consent was obtained from each participant after a detailed explanation in his language of choice and we ensured that participants understood well all the sections.

III.9.2 Ethical issues

The data collected throughout the study was made confidential. Confidentiality was assured by assigning codes to every study participant and the codes were used as labels for all participant document and specimen. Results of all analysis were shared with the participants. Any abnormal result led to an immediate consultation by the nephrologist for better assessment and treatment.

CHAPTER IV: RESULTS

In total, all the ADPKD participants whose eligibility was verified accepted to participate in this study. We recruited both the ADPKD and the healthy control group over a period of six weeks. Twenty-nine ADPDK participants were included alongside a group of 15 matched controls. **Fig. 17** summarizes the number of participants at major stages.

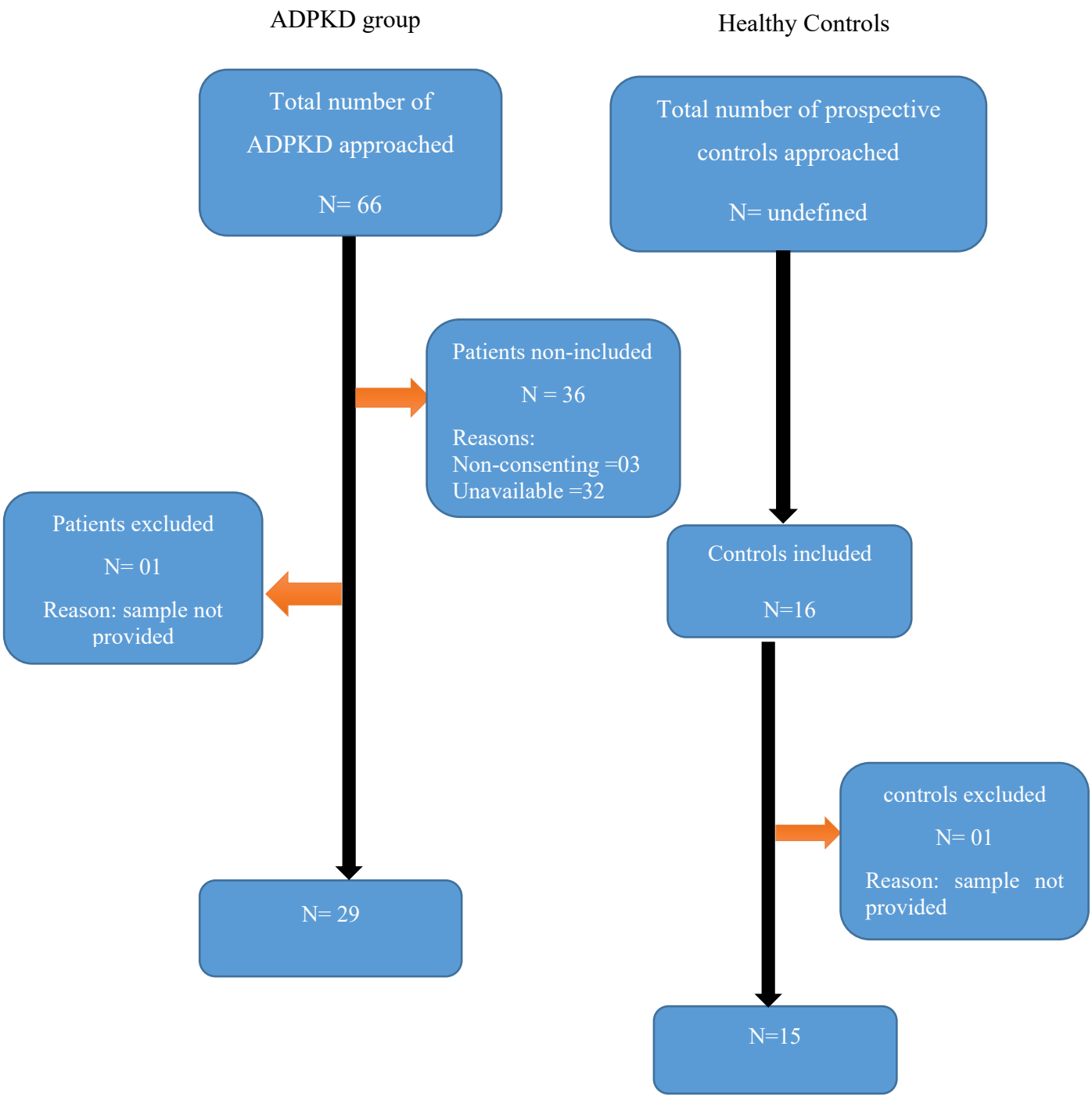

Figure 16: Participant enrolment flow chart

IV.1 Characteristics of study population

IV.1.1 Demographic characteristics of study population

Of the 44 participants enrolled, 65.9% were females. The age of the participants ranged from 20 years to 65 years with a mean age of 40.6±11.9 years. There were 29 (65.9%) ADPKD patients and 15 (34.1%) controls.

IV.1.2 Demographic, clinical and dietary characteristics of participants

The demographic, clinical and dietary characteristics of the 2 groups were comparable except for the serum creatinine and eGFR which were significantly different between both groups. Equally, the gut microbiota-protective diet pattern was significantly different between both groups.

Table IX: Demographic and clinical characteristics of study participant groups (n=44)

Characteristic	ADPKD (n=29)	Control (n=15)	p value
Age [I]	40.2±11.2	41.3±13.4	0.795
Sex: female (n)	20	9	
BMI (kg/m^2) [I]	21.6±3.4	23.3±3.5	0.136
Serum creatinine(mg/L)*	11.6[8.7-16.3]	8.9[8.0-10.6]	**0.022***
eGFR(ml/min/1.73m^2)*	74.4[51.2-94.6]	94.5[77.3-111.7]	**0.022***
Blood pressure (mm/Hg)			
SBP [I]	128.9±22.7	127.7±16.2	0.709
DBP [I]	83.8±13.7	78.9±13.3	0.241
Urine dipstick			
Leukocytes	11	0	0.314
Proteins	3	0	0.540
Blood	3	0	0.540
Nitrites	2	0	0.169
BSS			0.615
Type 2	1	1	
Type 3	5	1	
Type 4	20	10	
Type 5	2	2	
Type 6	0	1	
Type 7	1	0	

SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, BSS = Bristol stool scale; eGFR = estimated glomerular filtration rate; * median [interquartile range], [I] mean ± standard deviation

Table X: dietary characteristics of study population (n = 44)

	ADPKD (n=29)	Control (n=15)	p value
	$Q_2[Q_1 - Q_3]$		
Nutrient intake (24h recall)			
Energy (Kcal)	1544.1 [1014.5-1517.9]	1520.0 [1015.9-1822.8]	0.757
Proteins (g)	40.3 [24.9-56.5]	38.9 [32.8-50.6]	0.853
Carbohydrates(g)	114.6 [68.1-163.4]	114.1 [100.2-159.4]	0.421
Fibres(g)	19.4 [4.8-20.4]	16.9 [14.9-27.7]	0.052
Fats (g)	69.1 [36.2-127.1]	64.2 [44.8-131.9]	0.738
Dietary pattern (FFQ)			
Score I	5.2 [4.4-6.0]	4.1 [3.2-5.1]	**0.041***
Score II	2.8 [2.0-3.4]	3.7 [2.7-3.9]	0.134

Score 1: « Protective » of gut microbiota, Score II: « Non - protective » of gut microbiota; Q2[Q1-Q3] = median [interquartile range]

IV.1.3 Clinical characteristics of participants with Autosomal dominant polycystic kidney disease.

There were 18 (62.1%) ADPKD patients with flank pain, 14(48.3%) with hypertension, 17 (58.8%) with a family history of ADPKD. Eleven (38%) of ADPKD participants had CKD stages G3-5.

Table XI: Clinical features of participants with Autosomal Dominant Polycystic Kidney Disease (n=29)

Clinical feature	Frequency	Percentage (%)
Complications		
Flank pain	18	62.1
Hypertension	14	48.3
Gross haematuria	7	24.1
Urinary tract infection	5	17.2
Kidney stone	5	17.2
Family History		
Yes	17	58.8
Unknown	12	41.2
Extra-renal cysts		
Yes	11	37.9
Liver	10	
Pancreas	1	

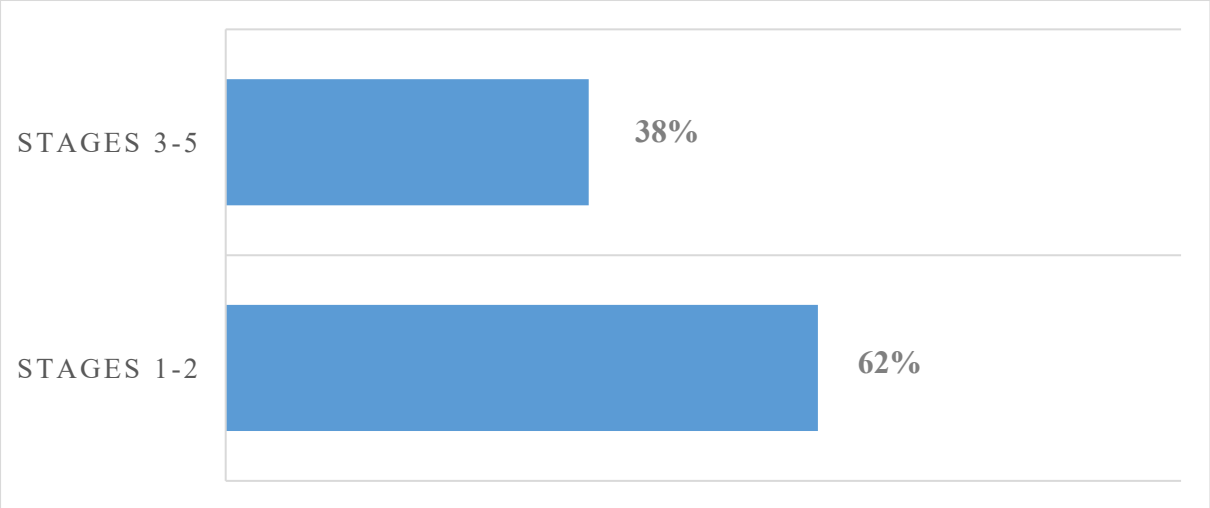

Figure 17: CKD staging of ADPKD patients

IV.2 Quantification of target gut bacteria of study population

Bifidobacterium had a median value of 0.72 [0.64- 0.80]ng/µL in the ADPKD group and 0.74 [0.71 – 0.89]ng/µL in the HC group. *Lactobacillus* had a median value of 0.58 [0.53 – 0.65]ng/µL in ADPKD group and 0.56 [0.23 – 0.65]ng/µL in HC group. All stool samples had Enterobacteriaceae while *Lactobacillus* and *Bifidobacterium* were absent in 2 (100% ADPKD) and 10 (60% ADPKD) specimens respectively**.** There was no statistically significant difference in the abundance of target bacteria between the 2 groups.

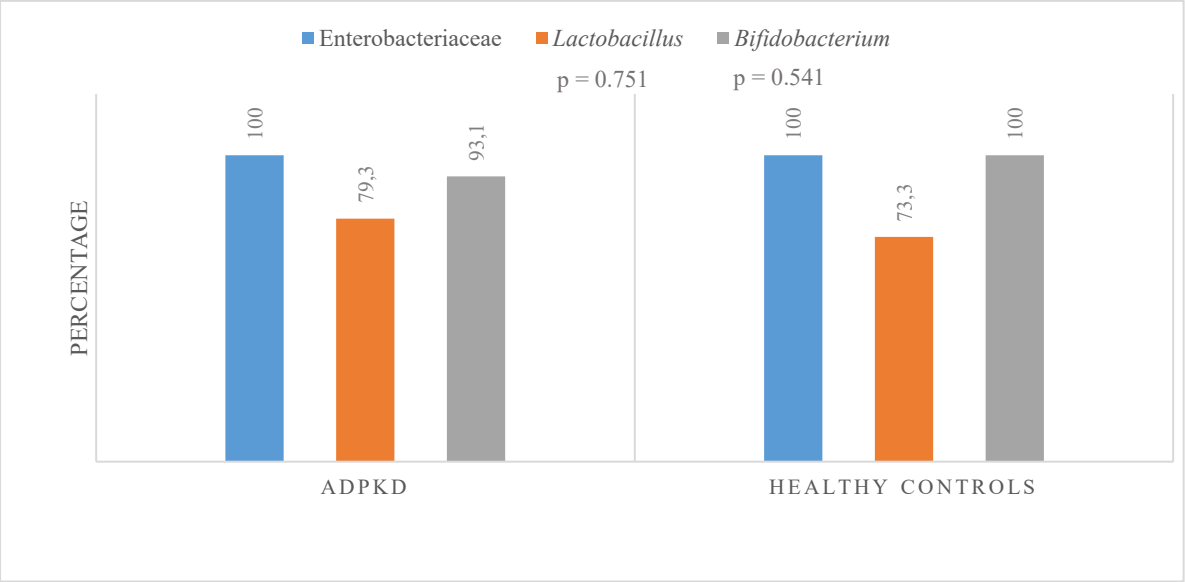

Figure 18: frequency of the presence of each bacteria in Autosomal Dominant Polycystic Kidney Disease and Healthy control groups

Table XII: Target bacterial abundance of study population groups

Bacteria(ng/µL)	**ADPKD (n=29)**	**Control (n=15)**	**p value**
	$Q_2[Q_1 - Q_3]$		
Bifidobacterium	0.72 [0.64-0.80]	0.74 [0.71-0.89]	0.322
Lactobacillus	0.58 [0.53-0.65]	0.56 [0.23-0.65]	0.542
Enterobacteriaceae	72.26 [68.84-77.85]	72.71 [65.96-73.73]	0.235

Q2[Q1-Q3] = median [interquartile range]

IV.3 Association between gut microbiota and kidney function of study population

IV.3.1 Enterobacteriaceae between population sub-groups

There was no difference in DNA abundance between ADPKD and HC groups (p=0.235). It was significantly more abundant in ADPKD with CKD G3-5 compared to CKD G1-2 (p=0.048) and HC (p=0.045)

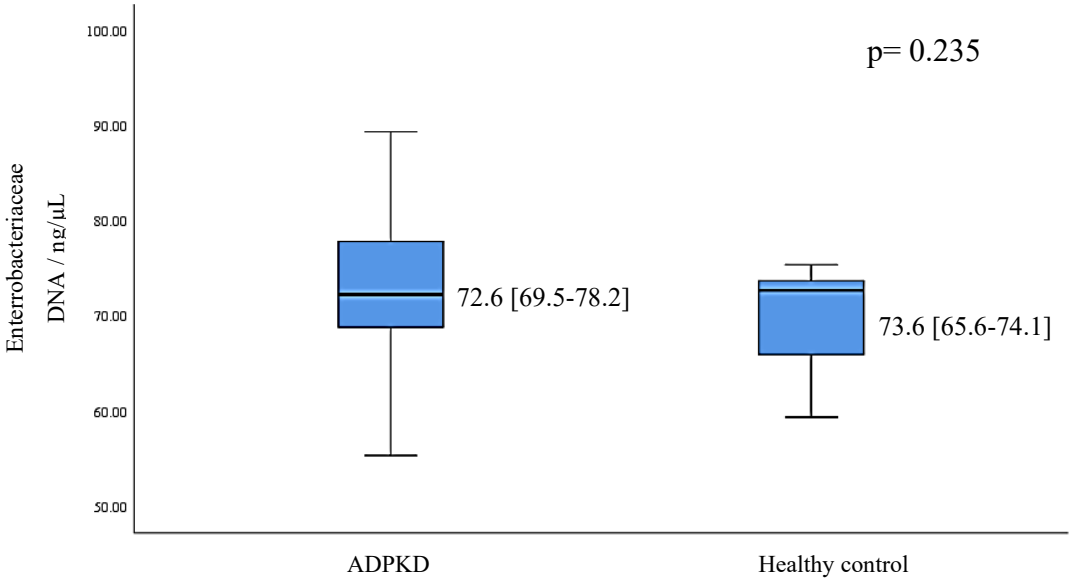

Figure 19: Comparison of Enterobacteriaceae DNA abundance in the study population

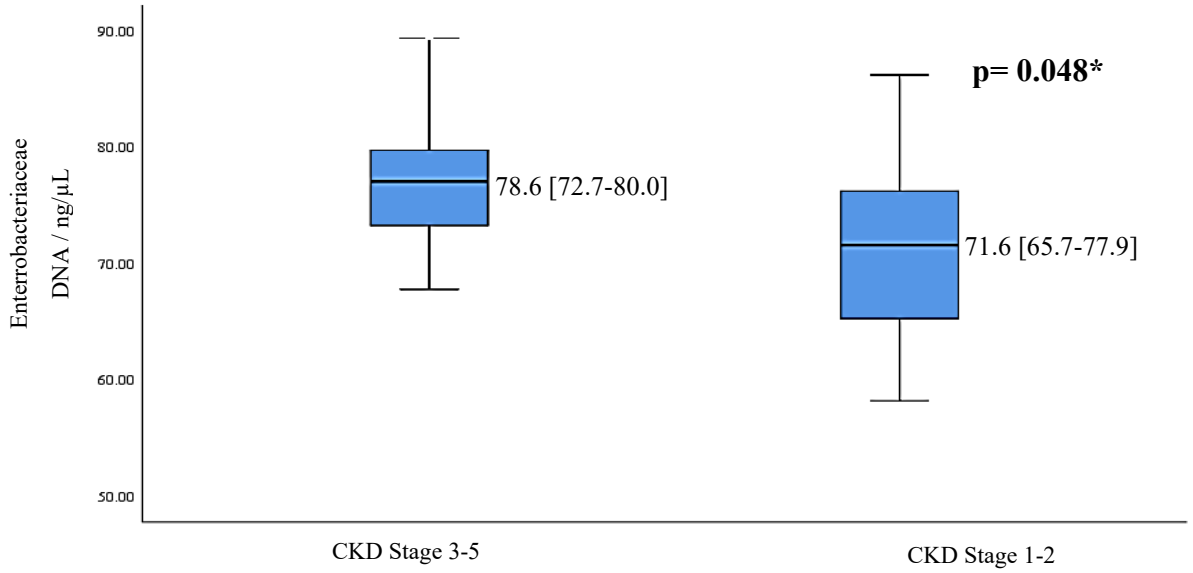

Figure 20: Comparison of Enterobacteriaceae DNA abundance within Autosomal Dominant Polycystic Kidney Disease group

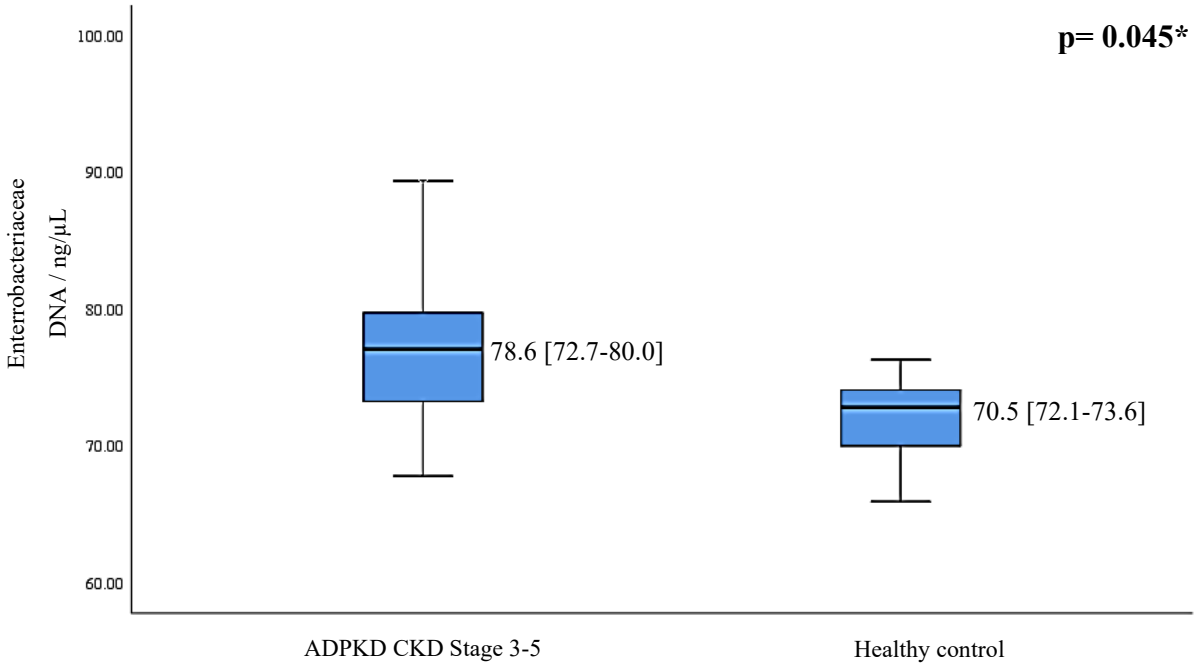

Figure 21: Comparison of Enterobacteriaceae DNA abundance between ADPKD group with CKD 3-5 and HC

IV.3.2 *Lactobacillus* between population sub-groups

There was no difference in DNA abundance between ADPKD and HC groups (p=0.542). The abundance of *Lactobacilllus* was significantly lower in ADPKD with CKD G3-5 compared to CKD G1-2 (p=0.047) and HC (p=0.043).

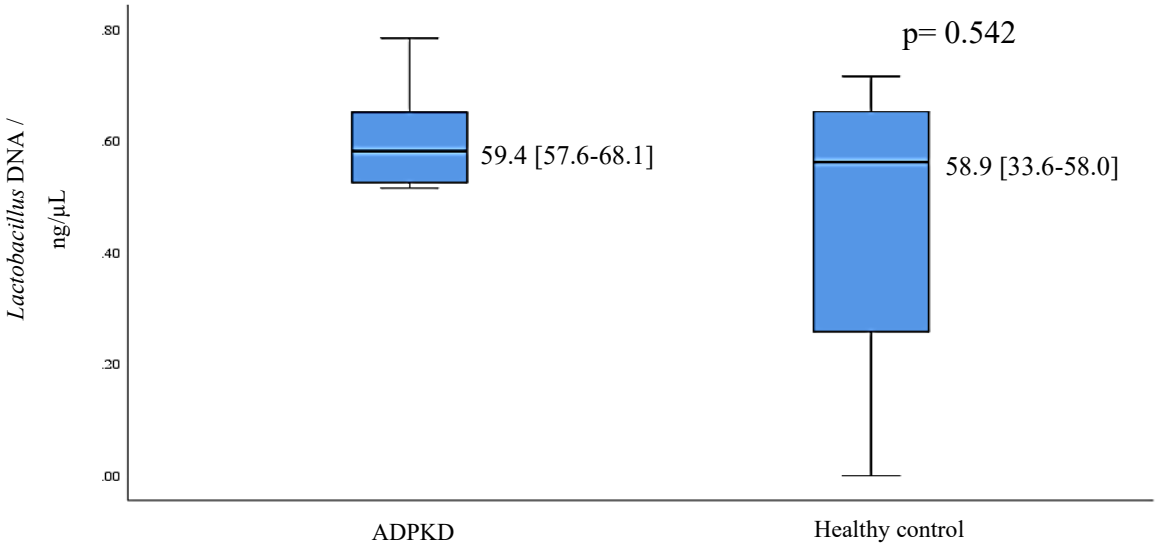

Figure 22: Comparison of *Lactobacillus* DNA abundance in the study population

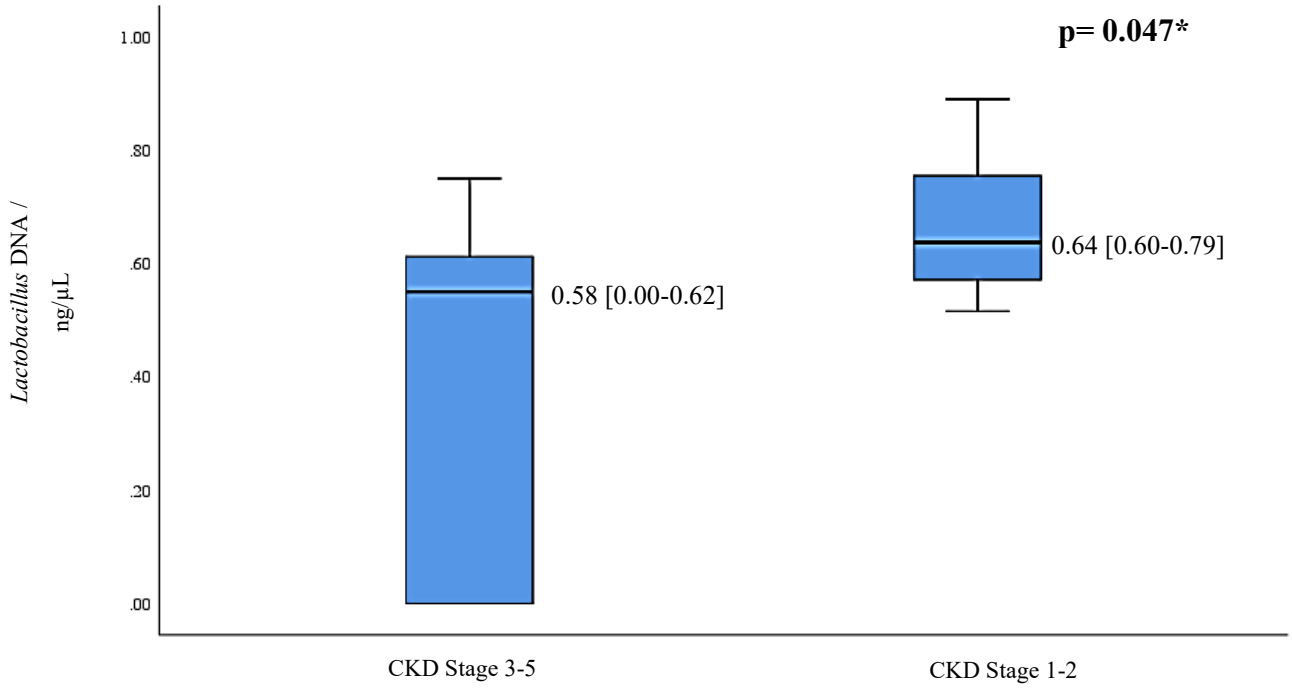

Figure 23: Comparison of *Lactobacillus* DNA abundance within Autosomal Dominant Polycystic Kidney Disease group

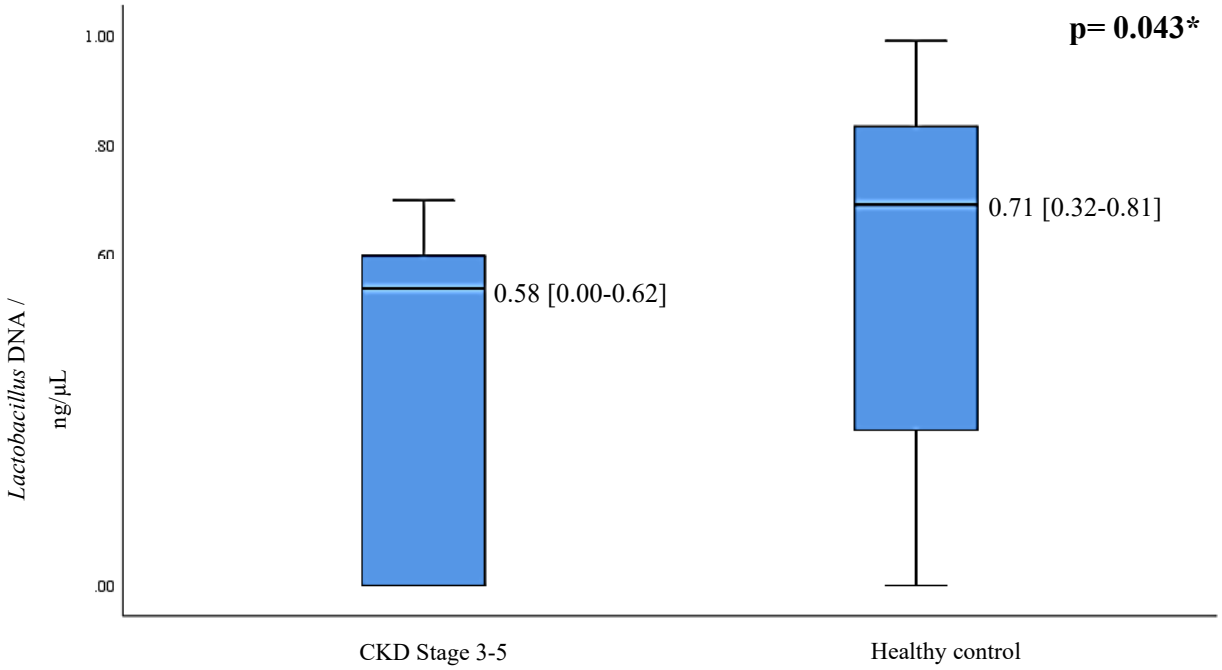

Figure 24: Comparison of *Lactobacillus* DNA abundance between Autosomal Dominant Polycystic Kidney Disease group with chronic kidney disease 3-5 and HC

IV.3.3 *Bifidobacterium* between population sub-groups

There was no difference in DNA abundance between ADPKD and HC groups (p=0.322), ADPKD with CKD G3-5 compare to CKD G1-2 (p=0.714) and HC (p=0.751).

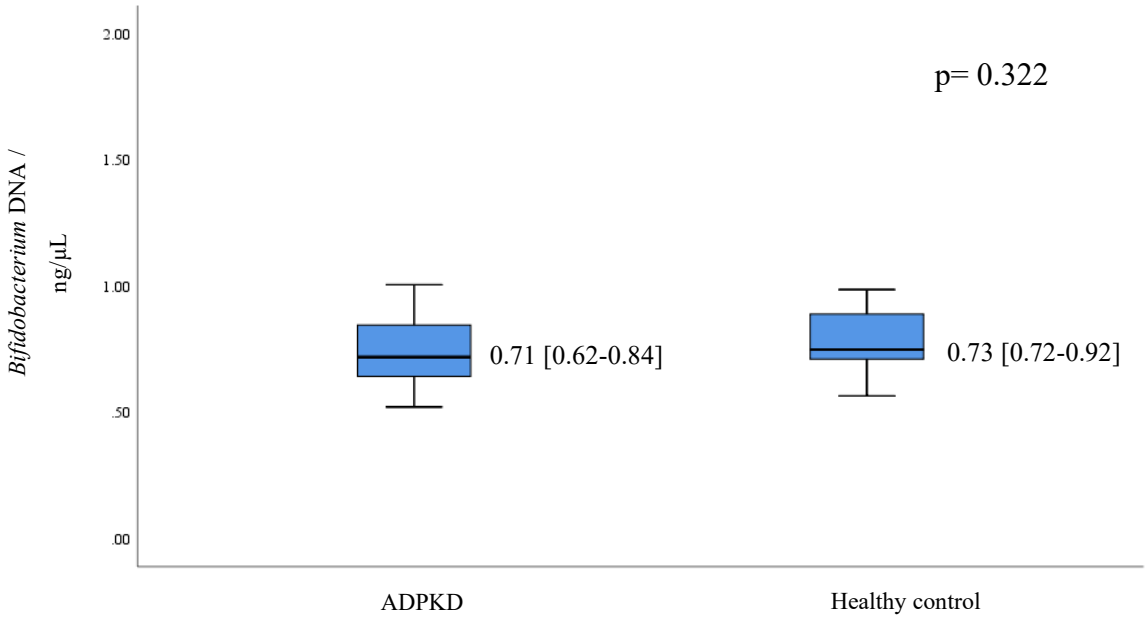

Figure 25: Comparison of *Bifidobacterium* DNA abundance in the study population

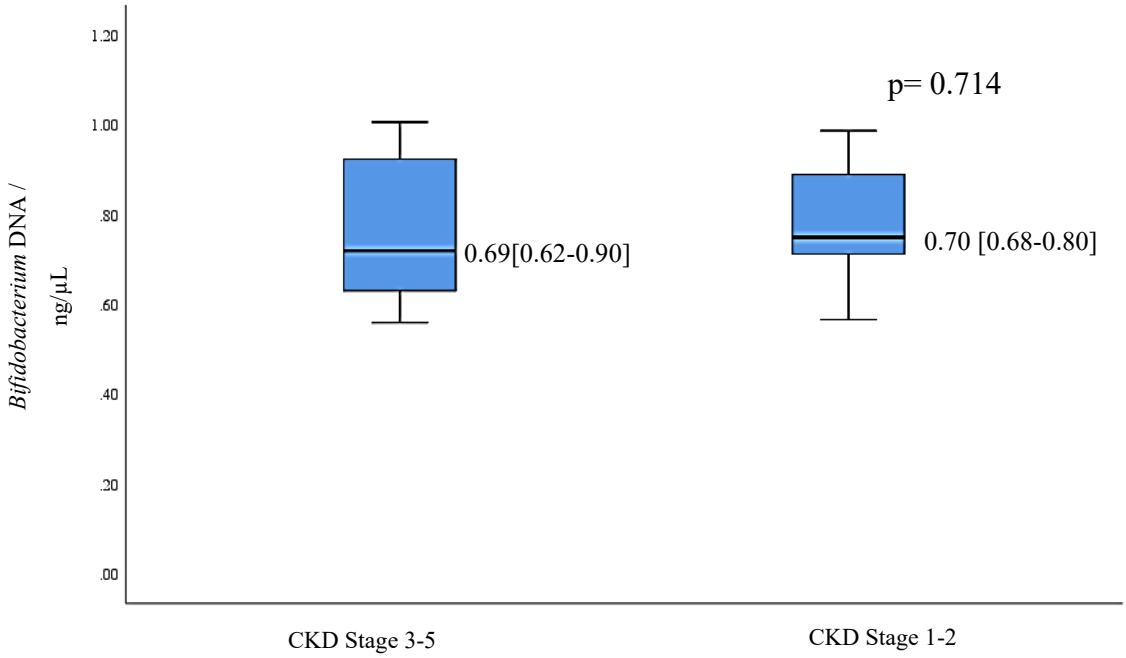

Figure 26: Comparison of *Bifidobacterium* DNA abundance within Autosomal Dominant Polycystic Kidney Disease group

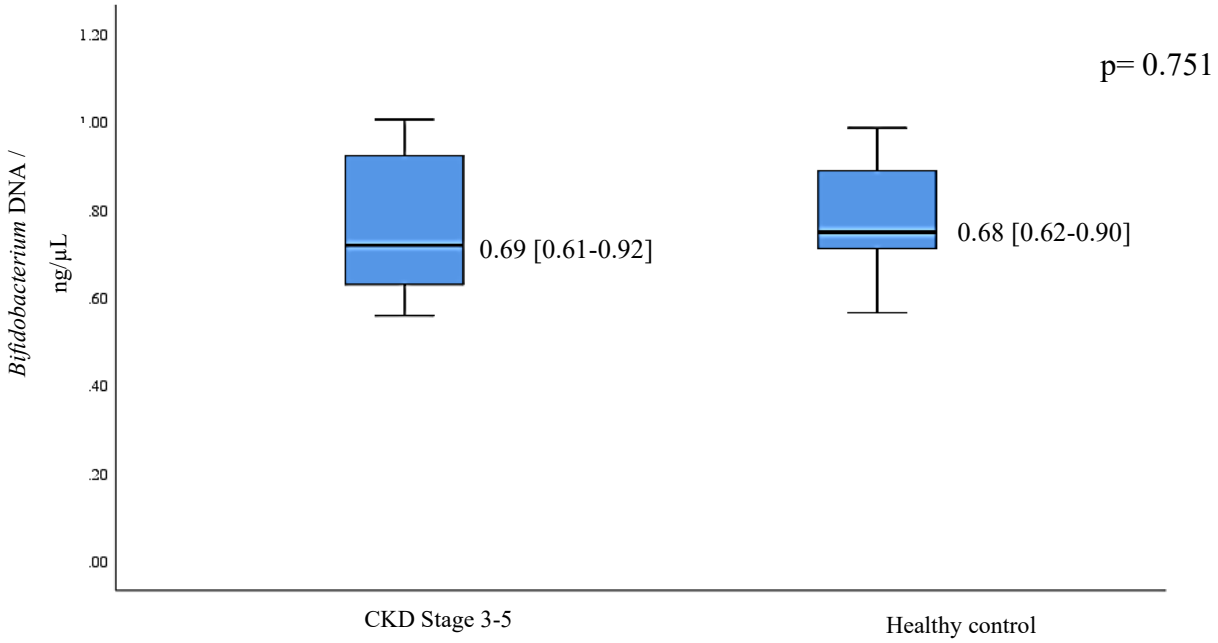

Figure 27: Comparison of *Bifidobacterium* DNA abundance between Autosomal Dominant Polycystic Kidney Disease group with chronic kidney disease 3-5 and HC

CHAPTER V: DISCUSSION

An impaired kidney function is associated with the retention of uremic toxins that promote the growth of proteolytic microbes that eventually gives rise to gut dysbiosis. A dysbiotic gut may lead to metabolic dysfunction and endocrine abnormalities, aggravating CKD as suggested by some studies [9,51]. In this historical cohort, we evaluated gut microbiota pattern in relation to kidney function in patients with ADPKD as we targeted specific bacteria taxa whose compositions have been shown to vary proportionally to CKD. We enrolled 44 participants, 29 (65.9%) of them were ADPKD patients while 15 (34.1%) were healthy controls (HC). The median value of eGFR in the ADPKD group was significantly lower compared to the HC group. *Bifidobacterium* was present in 95.45% of all specimens, owing to its absence in 2 ADPKD specimens. We observed no significant difference in the quantity of *Bifidobacterium* in the ADPKD group compared to the HC group with a median quantity of 0.72[0.64-0.80]ng/µL vs 0.74[0.71 – 0.89]ng/µL. *Lactobacillus* was present in 77.27% of all specimens, owing to its absence in 6 ADPKD and 4 HC specimens. We observed no significant difference in the quantity of *Lactobacillus* in the ADPKD group compared to the HC group with a median quantity of 0.58[0.53-0.65] ng/µL vs 0.56[0.23-0.65] ng/µL. Equally, Enterobacteriaceae was present in 100% of all specimens and there was no significant difference in its quantity in the ADPKD group compared to the HC group with a mean value of 72.26[68.84-77.85] ng/µL vs 72.71[65.96-73.73] ng/µL. Moreover, *Lactobacillus* had a lower abundance in ADPKD with CKD G3-5 compared to CKD G1-2 (p=0.047) and HC (p=0.043) while Enterobacteriaceae was significantly more abundant in ADPKD with CKD G3-5 compared to CKD G1-2 (p=0.048) and HC (p=0.045). No difference was observed among groups and subgroups for *Bifidobacterium*. These findings indicate that as kidney function declines, estimated DNA quantity of faecal Enterobacteriaceae rises while that of *Lactobacillus* falls. Enterobacteriaceae is a family with dysbiotic species while *Lactobacillus* is an eubiotic bacteria genus.

The ADPKD group had a mean age of 40.2 ±11.2 years. This finding is different from that obtained by Nzana *et al*. in 2011 in Cameroon who had a mean age of 52.2±12.3years [19] and Solazzo *et al.* in Italy in 2018 with median age of 56.1years [88]. The difference observed with the previous Cameroonian study could be explained by the increase in awareness of ADPKD over ten years and earlier screening accounting for the younger mean age. We observed a family history in 58.8% of ADPKD participants, unlike 39.0% reported by an earlier Cameroonian study [19]. This could equally be ascribed to increase awareness and screening.

Bifidobacterium was present in 93.1% of ADPKD specimens and 100% of HC specimens. The median abundance of *Bifidobacterium* in the ADPKD group was not significantly different from that of the HC group (p = 0.322). Equally, there was no significant difference when the median *Bifidobacterium* DNA abundance in ADPKD CKD 3-5 was compared to the HC group. This finding is dissimilar to those of Chung *et al.* who carried out a systematic review on the gastrointestinal microbiota in patients with CKD. They found that *Bifidobacterium* had a lower abundance in adult patients undergoing peritoneal dialysis compared to HC. Equally, Bifidobacteriaceae was lower in patients who had non-progressing Immunoglobulin A nephropathy. Moreover, they reported an experimental study that found a lower baseline abundance of *Bifidobacterium* in hemodialysis patients compared to HC [2]. Other reviews had similar findings as those of Chung *et al.* [8,51,60]. This dissimilarity could be explained by a significant difference in dietary intake between our study groups. The ADPKD group had a better protective microbiota pattern and a higher fibre intake compared to the HC group which may have promoted *Bifidobacterium* growth. This premise is supported by the finding of Joyce *et al.* [89], notably the increase in *Bifidobacterium* abundance following the intake a beta-glucan a type of dietary fibre. *Lactobacillus* was present in 79.3% of ADPKD specimens and 73% of HC specimens. The median abundance of *Lactobacillus* in the ADPKD group was not significantly different from that of the HC group (p = 0.542). However, the median *Lactobacillus* DNA abundance was lower in ADPKD CKD 3-5 compared to the HC group (p = 0.043). This result is similar to that of Hobby *et al.* [60], who described a reduced abundance of *Lactobacillus plantarum* in adults on peritoneal dialysis compared to HC. Butyrate-producing Lactobacillaceae was found to have a lower abundance in CKD patients compared to control [51] and a lower abundance in post-nephrectomy rats compared to controls [7]. There is a general fall in the abundance of symbionts in subjects with CKD due to the retention of uremic toxins with decline in kidney function which rather promote proteolytic pathobiont growth. Enterobacteriaceae was present in all specimens. The median abundance of Enterobacteriaceae in the ADPKD group was not significantly different from that of the HC group (p = 0.235). However, the median DNA abundance of Enterobacteriaceae was higher in ADPKD CKD 3-5 compared to the HC group (p = 0.045). This finding is comparable to that of Vaziri *et al.* who reported a higher abundance of Enterobacteriaceae in ESKD patients compared to controls [7]. Moreover, Chung *et al.* [2] had a similar result in patients with progressing and non-progressing immunoglobulin A nephropathy. Other studies had

comparable findings [51,60]. There is a general rise in the abundance of pathobionts in subjects with CKD due to the progressive kidney function decline.

The DNA abundance of *Bifidobacterium* was not significantly different in the ADPKD CKD 3-5 group compared to ADPKD CKD 1-3. The study that evaluated the microbiota in persons with PKD [9] did not describe *Bifidobacterium*. According to Gryp *et al.*, a decline in kidney function is negatively associated with *Bifidobacterium* among CKD patients stage 1-5 [90]. The dissimilarity between our study and that of Gryp *et al.* could be due to the fact that only 38% of our ADPKD population were CKD G3-5. *Lactobacillus* abundance was significantly lower in ADPKD CKD 3-5 compared to ADPKD CKD 1-2. Gryp *et al.* found that Lactobacillus was reduced with impaired kidney function [90]. Our result is unlike that of Yacoub *et al.*[9] who found an increase in *Lactobacillus iners* with respect to kidney function in PKD participants. This difference could be explained by the fact that our study targeted the genus while theirs a species. Enterobacteriaceae abundance was significantly higher in ADPKD CKD 3-5 compared to ADPKD CKD 1-2. The study that evaluated the microbiota in persons with PKD [9] did not describe Enterobacteriaceae. According to Gryp *et al.*, a decline in kidney function is positively associated with Enterobacteriaceae among CKD patients stage 1-5 [90]. The latter study equally reported an increased abundance in Enterobacteriaceae in hemodialysis patients compared to early stages of CKD. The Enterobacteriaceae family includes several pathobionts and their increase with the decline in kidney function is attributed to the retention of uremic toxins that promote pathobiont growth.

CONCLUSION, PERSPECTIVES AND RECOMMMENDATIONS

CONCLUSION

At the end of our study, with the aim of evaluating gut microbiota pattern and kidney function in ADPKD patients, we made the following conclusions;

- In ADPKD, the frequency of Bifidobacterium, Lactobacillus and Enterobacteriaceae were respectively 93%, 79.3% and 100% compared to 100%, 73% and 100% in HC without any significant difference in their abundance in both groups.
- The median value of eGFR in the ADPKD group was 74.4[51.2-94.6]ml/min/1.73m^2.
- There was a decrease in symbiont Lactobacillus and an increase in pathobiont Enterobactericeae with kidney function decline in the ADPKD population.

PERSPECTIVES

The limit of this study was that we weren't able to carry out PCR under optimal conditions that is by carrying out reactions in triplicates due to the shortage of PCR reagents. Moreover, performing absolute and relative quantification was a challenge. This was because a standard curve using serially diluted target gene DNA could not be done coupled to the absence of internal controls for this PCR. Concerning participants, the faint representation of predialysis ADPKD patients in stages 4-5 hindered a deeper appreciation of kidney function and gut microbiota association. Nonetheless, the findings of this study could broaden research perspectives on absolute or relative quantification of clinically relevant bacteria in ADPKD population with CKD 3-5.

Gut bacteria such as Prevotella and Roseburia could equally be identified and quantified to investigate their pattern relative to kidney function in a similar study population. Moreover, more insight could be obtained with the involvement of gut microbiota at the species level while targeting specific "biomarkers" in CKD and gut microbiota-derived metabolites such as SCFA.

The pattern of the gut microbiota could be explored in other specialties such as gastroenterology. This could enable the development of novel therapeutic targets for conditions such as irritable bowel syndrome.

RECOMMENDATIONS

To nephrologists

- Prescribe diet that promotes symbionts growth to ADPKD patients

To the scientific committee

- Carry out prospective studies on effect of modified diet on microbiota and kidney function.
- To pursue in-depth studies on gut microbiota pattern and interventions in conditions such as irritable bowel disease.

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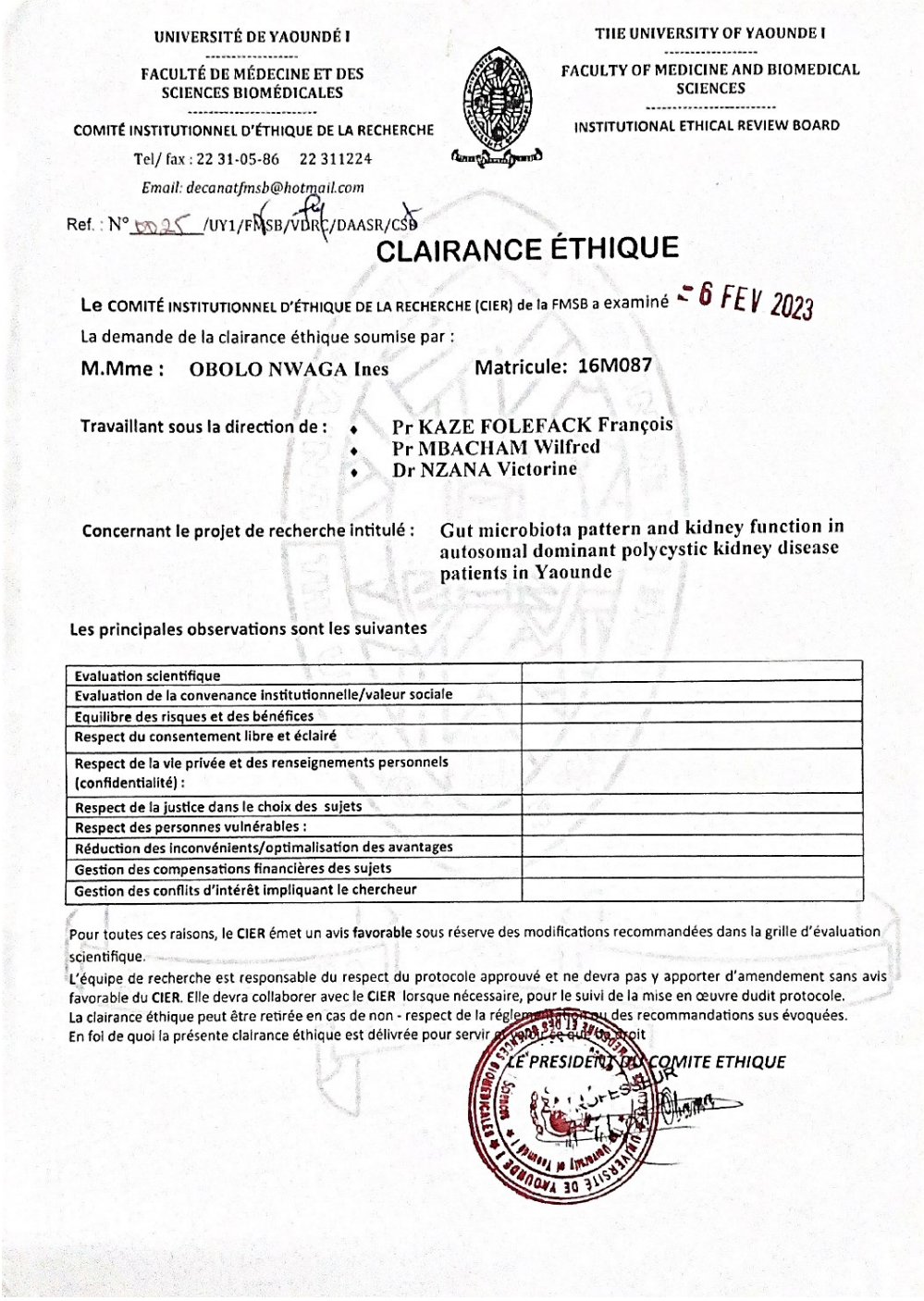
APPENDICES

APPENDIX 1: Ethical Clearance



UNIVERSITÉ DE YAOUNDÉ I
FACULTÉ DE MÉDECINE ET DES SCIENCES BIOMÉDICALES
COMITÉ INSTITUTIONNEL D'ÉTHIQUE DE LA RECHERCHE
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Email: decanatfmsb@hotmail.com

THE UNIVERSITY OF YAOUNDE I
FACULTY OF MEDICINE AND BIOMEDICAL SCIENCES
INSTITUTIONAL ETHICAL REVIEW BOARD

Ref. : N° 0025 /UY1/FMSB/VDRC/DAASR/CSD

CLAIRANCE ÉTHIQUE

Le COMITÉ INSTITUTIONNEL D'ÉTHIQUE DE LA RECHERCHE (CIER) de la FMSB a examiné - 6 FEV 2023

La demande de la clairance éthique soumise par :

M.Mme : OBOLO NWAGA Ines Matricule: 16M087

Travaillant sous la direction de :
- Pr KAZE FOLEFACK François
- Pr MBACHAM Wilfred
- Dr NZANA Victorine

Concernant le projet de recherche intitulé : Gut microbiota pattern and kidney function in autosomal dominant polycystic kidney disease patients in Yaounde

Les principales observations sont les suivantes

Evaluation scientifique	
Evaluation de la convenance institutionnelle/valeur sociale	
Equilibre des risques et des bénéfices	
Respect du consentement libre et éclairé	
Respect de la vie privée et des renseignements personnels (confidentialité) :	
Respect de la justice dans le choix des sujets	
Respect des personnes vulnérables :	
Réduction des inconvénients/optimalisation des avantages	
Gestion des compensations financières des sujets	
Gestion des conflits d'intérêt impliquant le chercheur	

Pour toutes ces raisons, le CIER émet un avis favorable sous réserve des modifications recommandées dans la grille d'évaluation scientifique.
L'équipe de recherche est responsable du respect du protocole approuvé et ne devra pas y apporter d'amendement sans avis favorable du CIER. Elle devra collaborer avec le CIER lorsque nécessaire, pour le suivi de la mise en œuvre dudit protocole.
La clairance éthique peut être retirée en cas de non - respect de la réglementation ou des recommandations sus évoquées.
En foi de quoi la présente clairance éthique est délivrée pour servir et valoir ce que de droit

LE PRESIDENT DU COMITE ETHIQUE

APPENDIX 2: Research Authorization

REPUBLIQUE DUCAMEROUN
Paix – Travail – Patrie

MINISTERE DE LA SANTE PUBLIQUE

REPUBLIC OF CAMEROON
Peace – Work – Fatherland

MINISTRY OF PUBLIC HEALTH

C
U H C
U
YAOUNDE

CENTRE HOSPITALIER ET UNIVERSITAIRE DE YAOUNDE
YAOUNDE UNIVERSITY TEACHING HOSPITAL
Tél : 22 31 25 66 Fax 22 31 25 67
Site web : www.chu-yaounde org
DIRECTION GENERALE
CELLULE D'APPUI PEDAGOGIQUE, DE LA RECHERCHE ET DE LA COOPERATION
BUREAU DE LA CAPRC

N°03 /AR/CHUY/DG/DGA/CAPRC

AUTORISATION DE RECHERCHE

Dans le cadre de la rédaction d'un mémoire de fin d'études, en vue de l'obtention de son Diplôme de Doctorat en Médecine, Madame OBOLO NWAGA Inès est autorisée à mener une recherche au CHUY sur le thème : « GUT MICROBIOTA PATTERN AND KIDNEY FUNCTION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE PATIENTS IN YAOUNDE ».

Ces travaux se dérouleront dans le service de Médecine sous la supervision du Chef de Service.

Toutefois, elle devra obligatoirement déposer un exemplaire de son mémoire au CHUY (Bureau de la CAPRC)

En foi de quoi la présente autorisation lui est délivrée pour servir et valoir ce que de droit./-

Yaoundé, le 03 JAN 2023

LE DIRECTEUR GENERAL

Pr. Arthur Essomba

COPIE :
- CAPRC
- BSF
- SUPERVISEUR
- CHRONO

APPENDIX 3: Research Authorization

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

MINISTERE DE LA SANTE PUBLIQUE

HOPITAL GENERAL DE YAOUNDE

DIRECTION GENERALE

BP 5408 YAOUNDÉ - CAMEROUN
TÉL (237) 22 21 31 81 FAX (237) 22 21 20 15

REPUBLIC OF CAMEROON
Peace - Work - Fatherland

MINISTRY OF PUBLIC HEALTH

YAOUNDE GENERAL HOSPITAL

GENERAL MANAGEMENT DEPARTMENT

N/Réf. : 022-23/HGY/DG/DPM/APM-TR

Yaoundé, le 18 JAN 2023

Le Directeur Général

A/TO

Madame OBOLO NWAGA Inès
Etudiante en 7ème année Médecine Générale
Tél : (237) 695 044 892 – Mle 16M087
e-mail : bekines3@gmail.com
FMSB – UNIVERSITE DE YDE I

Objet/subject :
v/demande d'autorisation de recherches.

Madame,

Nous accusons réception de votre courrier du 20 décembre 2022 dont l'objet est repris en marge.

Y faisant suite, nous marquons un avis favorable pour effectuer des recherches au SERVICE NEPHROLOGIE de l'Hôpital Général de Yaoundé dans le cadre de votre étude dont le thème porte sur : « **Gut Microbiota Pattern and Kidney Function in Autosomal Dominant Polycystic Kidney Disease Patients in Yaounde** ».

Cette étude sera supervisée par le Docteur MAIMOUNA, médecin néphrologue.

Vous observerez le règlement intérieur de l'établissement pendant la durée des recherches. Toutefois, les publications se rapportant à ce travail devraient inclure les médecins de l'Hôpital Général de Yaoundé.

Recevez, Madame, nos salutations distinguées./-

Copies :
- DPM
- Chef Service Néphrologie
- Docteur MAIMOUNA
- Archives/chrono

HOPITAL GENERAL YAOUNDE H.G.Y Le Directeur Général Adjoint YAOUNDE GENERAL HOSPITAL

Le Directeur Général,

Prof. EYENGA Victor

APPENDIX 4: Consent form (English)

I, the undersigned, Mr. / Mrs. / Ms. ……

(Name and surname)

Confirm that it was proposed to me to participate in the study entitled, "Gut Microbiota Pattern and Kidney Function in Autosomal Dominant Polycystic Kidney Disease patients in Yaoundé" being carried out by OBOLO N. Inès, a 7th year medical student at the Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1. This study will be supervised by Pr KAZE FOLEFACK (nephrologist), Pr MBACHAM (biotechnologist) and Dr NZANA (nephrologist) of Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1.

- I have understood the content of the information sheet provided for this study.
- I have understood the aim and objectives of this study.
- All questions I had concerning this study have been answered appropriately.
- The risks and benefits of this study have been explained to me.
- I understand that I am free to either accept or decline to be part of the study.

I freely accept too participate in his study according to the specified conditions on the information sheet, to answer truthfully to all questions asked with regard to the study and I authorize the consultation of my medical records. I freely authorize that my stool samples collected be analyzed and used in the publication of this study.

Date: ……. / ……./ ……

Investigator (Name, address and signature) Participant (Name, address and signature

Consentement éclairé (Français)

Je, soussigné, Mr. / Mme. /Mlle. ……

(Nom, prénom)

Confirme qu'il m'a été suggéré de participer à l'étude intitulée « Microbiote intestinal et fonction rénale chez les patients atteints de la polykystose rénale autosomique dominante à Yaoundé.»

Etant effectué par OBOLO N. Inès, étudiante en 7ème année en médecine à la Faculté de Médecine et des Sciences Biomédicales, de l'Université de Yaoundé 1. Cette étude sera supervisée par le Pr KAZE FOLEFACK (nephrologue), Pr MBACHAM (biotechnologiste)et le Dr NZANA (nephrologue)de la Faculté de Médecine et des Sciences Biomédicales de l'Université de Yaoundé 1.

- J'ai compris le contenu de la fiche d'information fournie pour cette étude.
- J'ai compris les buts et objectifs de cette étude.
- Toutes les questions que j'ai eus à poser concernant cette étude ont été répondues.
- Les risques et bénéfices de cette de cette étude m'ont été bien expliqués.
- Je comprends que je suis libre d'accepter ou de refuser de participer à cette étude.

J'accepte librement de participer à cette étude selon les conditions spécifiques sur la fiche d'information, à répondre honnêtement a toutes les questions posées concernant l'étude et j'autorise la consultation de mon dossier médical. J'autorise librement que mes échantillons de selles prélevés soient analysés et utilisés dans a publication de cette étude.

Date : ……/ ……/ …….

Enquêteur (Nom, adresse et signature) Participant (Nom, adresse et signature)

APPENDIX 5: Information form (English)

Title of the study: Gut Microbiota Pattern and Kidney Function in Autosomal Dominant Polycystic Kidney Disease patients in Yaoundé

Principal Investigator: OBOLO N. Inès, 7th year medical student at the Faculty of Medicine and Biomedical Sciences (FMBS) of the University of Yaoundé 1

Supervisor:
Pr KAZE FOLEFACK
Nephrologist,Faculty of Medicine and Biomedical Sciences (FMBS) of The University of Yaoundé I.

Co-supervisor:
Pr MBACHAM
Public Health Biotechnologist, Faculty of Medicine and Biomedical Sciences (FMSB) of The University of Yaoundé I.
Dr NZANA Victorine
Nephrologist, Faculty of Medicine and Biomedical Sciences (FMSB) of The University of Yaoundé I.

Purpose of the study: The study has as aims to quantify Bifidobacterium, Lactobacillus and Enterobacteriaceae in faecal samples of study participants. to compare the glomerular filtration rate between the ADPKD and healthy control groups and to assess the association between Bifidobacterium, Lactobacillus and Enterobacteriaceae and kidney function.
Procedure: If you agree to participate in the study, you will have to sign a consent form. We will ask you some questions to fill our questionnaire. Your height, weight and blood pressure shall be measured. Specimens of blood, urine and stool shall be collected and analysed.

Benefits: You will be screened for kidney function and gut microbiota freely.

Risks: The process of filling the questionnaire and stool specimen collection will take some of your time.

Confidentiality: Any information you provide will be treated as confidential. Only medical personnels will have access to your data. The information obtained will be used only for the purpose of the study.

Voluntarism: Your participation in the study is voluntary. You can change your mind and decide to decline to participate in the study without any penalty nor prejudice.

Contact Information:

If you have any questions about the study, you can contact;

OBOLO N. Inès, 695044892, bekines3@gmail.com, Faculty of Medicine and Biomedical Sciences (FMSB), The University of Yaoundé I.

Pr KAZE FOLEFACK

Nephrologist,Faculty of Medicine and Biomedical Sciences (FMBS) of The University of Yaoundé I.

Pr MBACHAM Wilfred,

Public Health Biotechnologist, Faculty of Medicine and Biomedical Sciences (FMSB) of The University of Yaoundé I.

Dr NZANA Victorine

Nephrologist, Faculty of Medicine and Biomedical Sciences (FMSB) of The University of Yaoundé I.

Fiche d'information (Français)

Titre: «Microbiote intestinal et fonction rénale chez les patients atteints de la polykystose rénale autosomique dominante à Yaoundé »

Enqueteur: OBOLO N. Inès, Etudiante 7e année, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Superviseur:

Pr KAZE FOLEFACK
Nephrologue, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Co-superviseur:

Pr MBACHAM,

Biotechnologiste en santé publique, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Dr NZANA Victorine,

Nephrologue, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université de Yaoundé I.

But de l'étude: quantifier Bifidobacterium, Lactobacillus et Enterobacteriaceae dans les échantillons fécaux des participants à l'étude, comparer le débit de filtration glomérulaire entre le groupe PKRAD et le groupe de contrôle sain et évaluer l'association entre Bifidobacterium, Lactobacillus et Enterobacteriaceae et la fonction rénale.

Procédure: Si vous acceptez de participer à l'étude vous allez signer un consentement éclairé. Nous allons vous poser certaines questions pour remplir notre questionnaire. Votre taille, poids et pression artérielle seront mesurés. Des analyses de sang, urine et selles seront faites après avoir recueillit vos echantillons.

Avantage: Les examens sont faite gratuitement.

Inconvénients: Le procédé de remplir le questionnaire, la collecte d'echantillon prendra du temps.

Confidentialité:

Toutes les informations que vous allez fournir seront confidentiels. Seuls le personnel médical aura accès à ces informations. Les informations obtenues seront utilisées uniquement dans le but de notre recherche.

Voluntarisme:

Votre participation à cette étude est volontaire. Vous pouvez décider de changer d'avis et ne plus poursuivre l'étude sans aucune pénalité ni préjugé.

Pour plus d'information:

OBOLO N. Inès, bekines3@gmail.com, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Pr KAZE FOLEFACK François,

Nephrologist, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Pr MBACHAM Wilfred,

Public Health Biotechnologist, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

Dr NZANA Victorine,

Nephrologue, Faculté de Médecine et des Sciences Biomédicales (FMSB), Université deYaoundé I.

APPENDIX 6: Questionnaire

Date of enrolment _____	Patient code…….. _____	File No. _____

N°	QUESTIONS	CODE
SECTION 1 : SOCIODEMOGRAPHIC DATA		
Respondent Type **Case: ADPKD**		
1.1	**What is your date of birth?** _____ **DD/MM/YY**	
1.2	**Sex** Male =1 Female=2	
SECTION 2: CLINICAL DATA **2A History**		
2.1	**In what year were you diagnosed ADPKD?**_____	
2.2	**Did you have flank pain?** Yes=1 No=2	
2.3	**Did you have bloody urine?** Yes=1 No=2	
2.4	**Did you have a urinary tract infection (UTI)?** Yes=1 No=2 I don't know=3	
2.5	**Did you have any kidney stone?** Yes=1 No=2 I don't know=3	
2.6	**Did you have cysts in another organ?** Yes=1 No=2 I don't know=3	
2.6.1	**If yes, which organ?**	
2.6.2	**Liver**Yes=1 No=2 I don't know=3	

2.6.3	**Pancreas**Yes=1 No=2 I don't know=3	
2.6.4	**Spleen** Yes=1 No=2 I don't know=3	
2.6.5	**Other** _____	
2.7	**Bristol stool scale** **Type 1=1 Type 2=2 Type 3=3 Type 4=4 Type 5=5 Type 6=6 Type 7=7**	
2B Comorbidities and Family History		
2.1	**Hypertension** Yes=1 No=2I don't know=3	
2.2	**Does anyone else in your family have autosomal polycystic kidney disease (ADPKD)?** Yes=1 No=2I don't know=3	
2.2.1	**Yes**	
2.2.2	**Parent** Yes=1 No=2I don't know=3	
2.2.3	**Sibling** Yes=1 No=2I don't know=3	
2.2.4	**Child** Yes=1 No=2I don't know=3	
2.2.5	**Other** _____	
2C Drug History **Do you occasionally take the following drugs ?**		
2.1	**Proton pump inhibitor** Yes= 1 No =2I don't know=3	
2.2	**Vitamin D supplements** Yes= 1 No =2I don't know=3	
2.3	**Laxatives** Yes= 1 No =2I don't know=3	
2.4	**Antihypertensives** Yes= 1 No =2I don't know=3	
SECTION 3 : PHYSICAL EXAM		
3.1	**Mean blood pressure reading** **SBP _____mmHg**	

	DBP _____mmHg	
3.2	**Weight (Kg)_____**	
3.3	**Height (cm) _____**	
3.4	**BMI _____**	
3.5	**Urine dipstick**	
SECTION 4 : PARACLINICAL DATA		
4.1	**Serum creatinine Level (mg/dL) _____**	
4.2	**eGFR (CKD-EPI) _____**	
4.3	**CKD stage _____**	
SECTION 5: GUT MICROBIOTA ANALYSIS **Bacteria identified**		
5.1	**Bifidobacterium**	
	Abundance (ng/µL)	
5.2	**Lactobacillus**	
	Abundance (ng/µL)	
5.3	**Enterobacteriaceae**	
	Abundance (ng/µL)	

Date of enrolment _____	Patient code……..……	File No. _____

N°	QUESTIONS	CODE
SECTION 1 : SOCIODEMOGRAPHIC DATA		
Respondent Type **Healthy controls**		
1.1	**What is your date of birth?** _____ **DD/MM/YY**	
1.2	**Sex** Male =1 Female=2	
SECTION 2: CLINICAL DATA **2A History**		
2.1	**Bristol stool scale** **Type 1=1 Type 2=2 Type 3=3 Type 4=4 Type 5=5 Type 6=6 Type 7=7**	
2B Comorbidities and Family History		
2.1	**Hypertension** Yes=1 No=2 I don't know=3	
2C Drug History **Do you occasionally take the following drugs ?**		
2.1	**PPI** Yes= 1 No =2I don't know=3	
2.2	**Vitamin D supplements** Yes= 1 No =2I don't know=3	
2.3	**Laxatives** Yes= 1 No =2I don't know=3	

2.4	**Antihypertensives** Yes= 1 No =2I don't know=3	
SECTION 3 : PHYSICAL EXAM		
3.1	**Mean blood pressure reading** **SBP _____mmHg** **DBP _____mmHg**	
3.2	**Weight (Kg)_____**	
3.3	**Height (cm) _____**	
3.4	**BMI _____**	
3.5	**Urine dipstick**	
SECTION 4 : PARACLINICAL DATA		
4.1	**Serum creatinine Level (mg/dL) _____**	
4.2	**eGFR (CKD-EPI) _____**	
SECTION 5: GUT MICROBIOTA ANALYSIS **Bacteria identified**		
5.1	**Bifidobacterium**	
	Abundance (ng/µL)	
5.2	**Lactobacillus**	
	Abundance (ng/µL)	
5.3	**Enterobacteriaceae**	
	Abundance (ng/µL)	

SECTION 6: DIET ASSESSMENT

24H RECALL

Instructions for filling out a 24-hour recall

- Report all the foods and beverages consumed in the preceding 24 hours along with further descriptions of the food where it is requested.
- Start with the first thing eaten in the morning until the last food item consumed before waking up the next morning, reporting for each meal the time along with the place consumed.
- Report for each meal consumed: name of food, food description, if possible household measures (e.g. slices, teaspoons, etc.), unit of measure if possible (e.g. grams, ml etc.) and finally the kind of preparation methods used and/or ingredients.

Breakfast			Time Location	
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

Snack (mid-morning) Time Location				
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

Lunch			Time Location	
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

Snack (mid-afternoon)		Time Location		
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

Dinner			Time Location	
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

Others	Time Location			
Name	**Food description**	**Household amount**	**Amount**	**Preparation**

SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Instructions for filling out the food frequency questionnaire

- Mark with an 'X' the frequency with which you consumed the food recorded (during the last 3 months): never, seldom (less than once a month), number of times per month, week or day;
- Record the amount of food consumed under the column 'Amount', using household measurements as reported (cup, teaspoon or slice, etc.) or if you have the weight or volume please specify, and include the units (e.g. grams, millilitre or litre, etc.).
- Please answer every question, do not leave ANY lines blank.

	Average use last 3 months						
Food items	Never	less than once per month	1 to 3 times per month	1 to 2 times per week	3 to 4 per week	daily	
Meat and fish							
Beef							
Pork							
Chicken							
Liver							
Snails							

Dry fish (bonga, morue,…)							
Oily fish; mackerel, sardine, catfish							
Dairy products							
Egg; boiled, fried, scrambled							
Full fat or greek yoghurt							
Full-cream milk powder							
Skimmed milk powder							
Cheese							
Starchy food							
Cassava							
Sweet potatoes							
Plantains; ripe, unripe							
Maize; fresh corn, cornfufu, koki corn, pap							
Yam							
Rice							
Cocoyams-taro							
White bread							
Sugar*							
Fruits							
Avocado							
African pear							
Banana							
Garden egg							
Coconut							

Pawpaw							
Pineapple							
Watermelon							
Vegetables, seeds, grains							
Huckleberry							
Bitter leaves							
Okro							
Gnetum; eru, okok							
Green (folong)							
Onions							
Folere leaves							
Legumes; beans, lentils, cowpea							
Pumpkin seeds (egusi)							
Sesame							
Bushmango seeds							
popcorn							
Fats							
Margarine							
Palm oil							
Groundnut oil							
Olive oil							
Sunflower oil							
Mayonnaise							
Drinks							
Fruit juice; orange, pineapple							
Baobab juice							
Hibiscus juice							
Beer							
Wine							

Spirits							
Fermented foods							
Dairy; kossam, pendidam							
Palm wine							
Cereal-based; sha'a, kounou							

Table XIII: Taxonomic units of target bacteria of our study [91]

Domain	Kingdom	Phylum	Family	Genus	Species
Eubacteria	Bacteria	Actinobacteria	Bifidobacteriaceae	Bifidobacterium	104
		Proteobacteria	Enterobacteriaceae	Escherichia (53)	170
		Firmicutes	Lactobacillaceae	Lactobacillus	261
		Bacteroidetes	Prevotellaceae	Prevotella	55
		Firmicutes	Lachnospiraceae	Roseburia	5

Figure 28: Photometer of the YUTH biochemistry laboratory(KENZA MAX BioChemisTry analyser, BIOLABO)

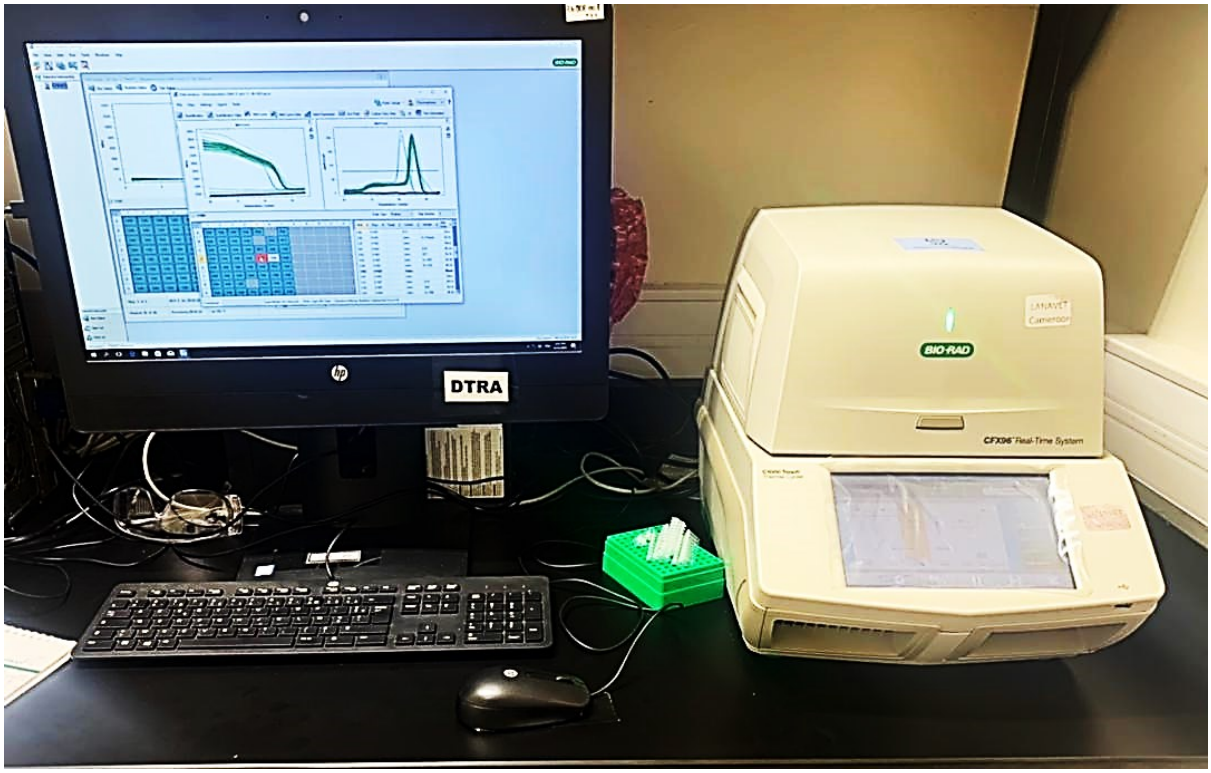

Figure 29: thermocyler (CFX96 Touch Real-Time PCR Detection System, BIORAD) and monitor

Salmonella enterica ATCC 14028

Lactobacillus casei

Escherichia coli ATCC 35218

Figure 30: bacterial cultures used as qPCR positive controls

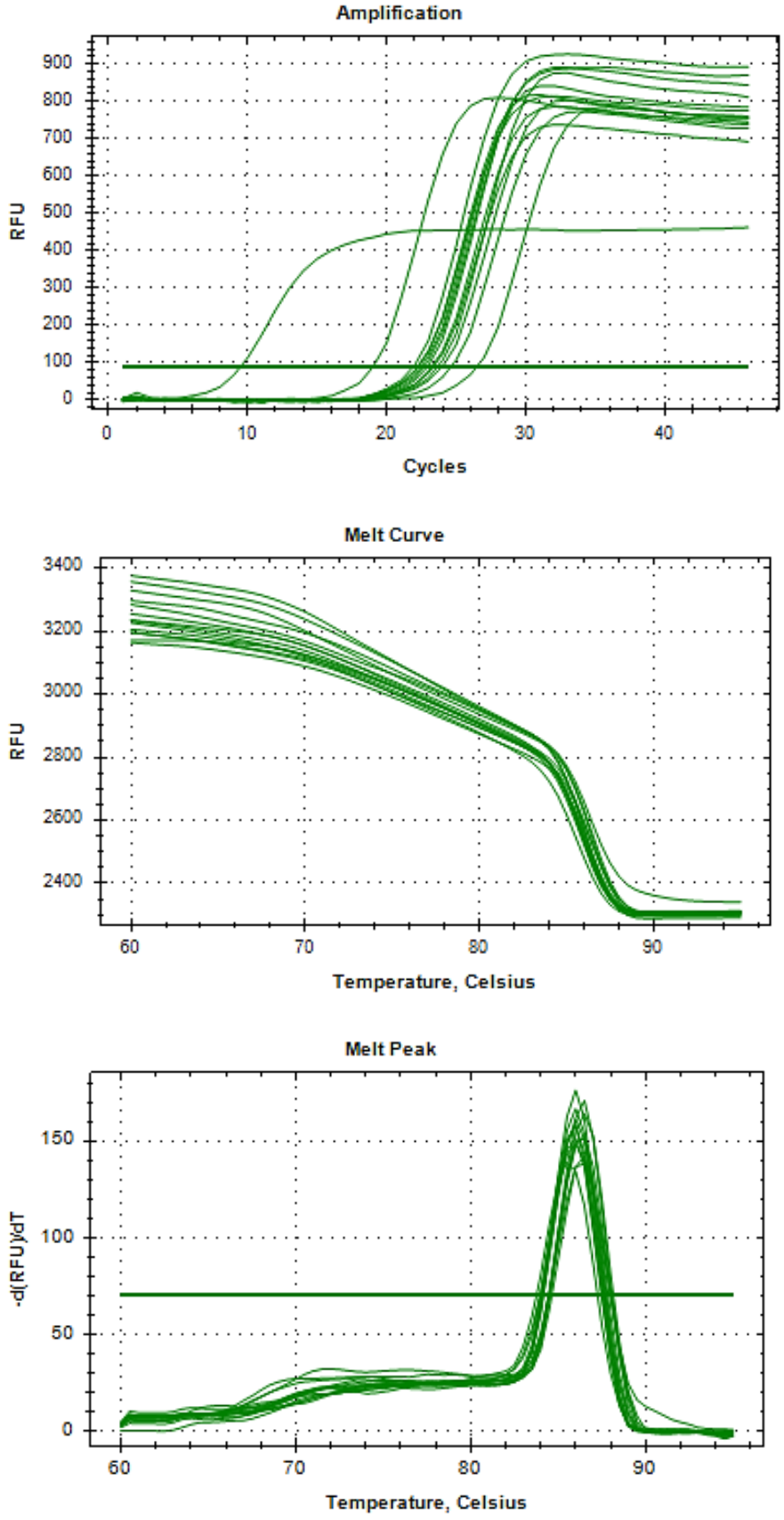

Figure 31: qPCR amplification, melt curve and melt peak plots