Research Article

SOHAG JOURNAL OF SCIENCES

https://doi.org/10.21608/sjsci.2023.184999.1051

Bacteriological Studies on Urine and Blood of Chronic Kidney Disease Patients

Mohamed S. Youssef [1,*], Rehab M. Mohamed[1], Emad A. Mohamed[2], Samar A. Hassan[3]

[1] *Botany and Microbiology Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[2] *Internal Medicine, Nephrology Department, Faculty of Medicine, Sohag University, Sohag 82524 Egypt.*
[3] *Oncology Cancer Institute, Sohag, Egypt.*
[*] *E-mail: youssefm2006@yahoo.com*

Received: 1st January 2023, **Revised:** 30th January 2023, **Accepted:** 31st January 2023.
Published online: 25th March 2023

Abstract: In this study, we evaluated the bacterial infections in 120 samples of each urine and blood of chronic kidney disease patients collected from Sohag University Hospital and Sohag Oncology Cancer Centerat Sohag governorate, Egypt. Twenty-two pathogenic bacterial species belonging to 13 genera were isolated and identified from urine and blood samples examined. Twelve of the isolated bacterial species were Gam-positive bacteria and the other nine species were Gram-negative, in addition to one Gram-variable species. Urine **b**acterial infection (22 species, 13 genera &35836CFU) is higher than blood (10 species, 8 genera &2808CFU). Urine bacterial infection with Gram-negative species was represented by 17007CFUmatching (47.46%) the total count, Gram-positive bacteria represented by 18829CFU(42.82%) and Gram-variable **(*Lysinibacillus pakistanensis*OQ299572)** represented by 3484CFU, accounting for (9.78%)of the total count. On the other hand, in blood Gram-negative bacteria was represented by 1676 CFU(59.69%) whereas, Gram-positive bacteria was represented by 850 CFU (30.27%). Gram-variable species **(***Lysinibacillus pakistanensis* OQ299572**)** represented by 282 CFU as 10.04% of total count.To the best of our knowledge, this is the first report of isolation of *Lysinibacillus pakistanensis* OQ299572 from human clinical specimens in the world.
Keywords: Chronic kidney disease- bacterial infection-urine- blood-*Lysinibacillus pakistanensis*OQ299572- Egypt.

1. Introduction

Chronic kidney disease (CKD) is one of the most common chronic diseases with the worldwide prevalence estimated to be approximately 13.4% and projected to continue to rise annually, especially in developing countries where renal healthcare is limited [**1, 2, 3**]. CKD is emerging in the 21st century as a global public health issue, affecting more than 750 million people worldwide [**4**].The prevalence of CKD is increasing worldwide, and the mortality rate continues to be unacceptably high [**5**]. CKD is a common problem among males and females due to stress, alcoholism, hypertension and diabetes mellitus One million patients visit the emergency department, and 100,000 hospital stays every year in the United States are due to Urinary Tract Infections (UTIs). Approximately 10% of humans will have UTI at some time during their lives. Of note, UTIs are also the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections [**6**].Chronic renal failure (CRF) is a deterioration in renal function correlates with disturbances of various specific and nonspecific host defense reactions. In renal diseases, a change in the composition of urine in oliguria, anuria, albuminuria and hematuria is observed. The resultant changes in pH, osmolarity and urinary urea have their own effects in urinary tract infection (UTI). An accumulation of various uremic toxins inhibits the antimicrobial activity of granulocytes, macrophages and other defense reaction. These conditions may support the development of UTI in patients with renal disease. CRF is defined as either a level of glomerular filtration rate (GFR) less than 15 ml/min/1.73 m2, which is accompanied in most cases by signs and symptoms of uremia, or a need for initiation of renal replacement therapy **[7, 8]**. A urinary tract infection (UTI) is a bacterial infection that affects part of the urinary tract. When it affects the lower urinary tract, it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). UTIs are among the most common bacterial infections that lead patients to seek medical care [**9, 10**]. Therefore, this research was designed to study the bacterial infections in 120 urine and blood samples of chronic kidney disease patients that collected from Sohag governorate, Egypt.

2. Patient and Methods

This study was conducted in the period from 1st September 2019 to 30th August 2021. A total of 120 samples of each of urine and blood from different patient cases were collected.

2.1 Urine and blood samples collection

One hundred and twenty samples of each of urine and blood were collected from 120 patients of chronic kidney diseases in sterile cups from Sohag University Hospital and Sohag Oncology Cancer Center. The patientsincluded in this study suffering from various types of kidney failure diseases

under supervision of specific physician.Urine and blood specimens were collected in early morning from all patients. The samples were kept in ice boxes and transported to the bacteriology in Botany and Microbiology department, Faculty of Science, Sohag University for bacterial cultivation and different assays [11].

2.2. Bacterial Cultivation and Isolation

Each sample of urine and blood of every patient was cultivated on nutrient agar medium (PH 7.2) for bacterial culturing [12], the medium composed of

i. Nutrient broth: peptone, 10g; Lab-Lemco meat extract (Oxoid), 10g; sodium chloride, 5g (per 1L distilled water).
ii. Agar: 15-20 g/l

2.3. Identification of Bacterial Isolates

Identification of bacterial isolates depends on colony morphological characteristics after growth on different media such as nutrient agar, selective and differential media. Selective media allow certain types of organisms to grow and inhibit the growth of others. The selectivity is accomplished in several ways; for example, organisms that can utilize a given sugar are easily screened by making that sugar the only carbon source in the medium. On the other hand, selective inhibition of some types of microorganisms can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affects the metabolism or enzyme systems of the organisms [13-15].

2.4. Gram's staining

For this stainning the following reagents were used, crystal violet (2gm in 100 ml ethanol), Lugol's iodine (1gm iodine + 2gm KI in 300 ml H_2O), safranin (1gm + 10 ml ethanol + 90 ml H2O as counter staining), smears of tested cultures were prepared and heat-fixed. The heat fixed films were stained with crystal violet for about one minute. and then rinsed with tap water. These films were covered with Lugol's iodine for about 2 minutes, and then rinsed with water. The films were decolorized with 95% ethyl alcohol. Counter staining was performed with safranin about 20-30 seconds and finally these films were rinsed with water, blotted to dry and microscopically examined.

2.5. Biochemical identification of bacterial isolates

The isolates identification was confirmed using biochemical tests according to classification schemes published in Bergey's Manual [13-15].

2.6 Molecular identification of bacterial isolate

The identification of *Lysinibacillus pakistanensis* OQ299572 isolates was confirmed by Intron Biotechnology Company, Korea [16, 17].

3. Results and Discussion

This research was designed to study bacterial infections associated with urine and blood of 100 patient cases of chronic kidney diseases collected from Sohag Oncology Center and Sohag University Hospital in addition to 20 random cases of healthy peoples as control from 1st September 2019 to 30th August 2021.

3.1. Patient diagnosis

Data in Tables (1, 2 & 3**)** showed urine and blood patient samples collected from different patient cases according to gender, age, chronic diseases, blood and urine analysis. All patient were suffering from kidney failure diseases associated with troubles in kidney blood function and urine analysis. Some of them suffering from other chronic diseases such as diabetes mellitus and blood pressure. So, data divided into 3 grades:

Grade III: Fifty patient were suffering from kidney cancer or another cancer diseases as stated in Tables 1a & 1b and Fig.1

Grade II: Fifty patient were suffering from high kidney function as shown in Tables 2a & 2b and Fig.2

Grade I: Twenty cases of healthy people were as control as listed in Table 3.

Data in Tables 1, 2 & 3 and Figs.1 & 2 reflects the important cofactors for causing of chronic kidney diseases (CKD) in different grades. The increase in prevalence of CKD is a result to a real increase in its frequency, due to the increase in the average age of people surviving (2 - 88 years) and the increasing incidence of diabetes and hypertension among people. These factors together with increasing diabetes prevalence and an aging population, will result in significant increasing in chronic kidney diseases. There is now convincing evidence that CKD can be detected using simple laboratory tests such as blood kidney function and urine analysis.

Table 1a showed that 50 patient cases were collected from Oncology Sohag Cancer Center, with their different ages, gender (male, female and children), and analyses of kidney blood functions (urea and creatinine) and urine examinations. Different medical analyses and examinations of different patients werecarried out in clinical pathology laboratory of Sohag Oncology Cancer Center. All tested patient cases were suffering from cancer diseases (bladder, breast, kidney and urothelial cancer) in addition to chronic kidney diseases. Most of them have chronic diseases such as blood pressure and diabetes, or both. Some women cases had kidney cancer due to their breast cancer incidence origin. The age periods of 41-60 years and 61-88 years are more liable and susceptible to different cancer types infections followed by 21-40 years as shown in Table 1b & Fig. 1.

Table 2a showed that 50 patient cases were collected from Sohag University Hospital from Internal Medicine, Nephrology Department, with their different ages, gender (male, female and children), and analyses of kidney blood functions (urea and creatinine) and urine examinations were performed. Different medical analyses and examinations of different patients were carried out in clinical pathology laboratory of Sohag Oncology Cancer Center. All tested patient cases were suffering from hyper blood kidney function in addition to chronic kidney diseases, many of them have chronic diseases such as blood pressure and diabetes, or both, and some of them do not have chronic diseases as shown in Table 2b and Fig. 2.

Table 1a: Age and gender of patients (50) suffering from kidney cancer or other cancers affected on kidney which admitted into Sohag Oncology Cancer Center Hospital (Grade III).

no	Age	Gender	Diagnosis	Kidney function	Urine analysis	Chronic D
1	30	M	K. Cancer	High	Abnormal	Diabetes
2	50	F	Urothelial can	High	Abnormal	Nil
3	60	M	Bladder can	High	Abnormal	HTN+D
4	75	M	K. Cancer	High	Normal	Diabetes
5	44	M	Bladder can	High	Normal	Nil
6	60	F	Bladder can	High	Abnormal	Nil
7	36	M	K. Cancer	High	Abnormal	Nil
8	57	M	Bladder can	High	Abnormal	Diabetes
9	60	M	K. Cancer	High	Normal	Diabetes
10	35	F	Breast can	High	Abnormal	Diabetes
11	47	F	K. Cancer	High	Abnormal	HTN+D
12	43	M	Urothelial can	High	Normal	HTN
13	27	M	K. Cancer	High	Abnormal	Diabetes
14	39	F	Bladder can	High	Abnormal	Diabetes
15	42	F	Breast can	High	Normal	HTN+D
16	41	F	Urothelial can	High	Abnormal	HTN+D
17	33	F	Urothelial can	High	Abnormal	Nil
18	70	M	K. Cancer	High	Abnormal	Nil
19	61	F	Breast can	High	Normal	Nil
20	28	F	K. Cancer	High	Normal	Diabetes
21	34	M	Urothelial can	High	Abnormal	Diabetes
22	66	F	Urothelial can	High	Abnormal	Nil
23	50	F	K. Cancer	High	Abnormal	Nil
24	49	F	Breast can	High	Abnormal	HTN+D
25	44	M	K. Cancer	High	Abnormal	Diabetes
26	53	F	K. Cancer	High	Abnormal	HTN
27	48	M	Urothelial can	High	Abnormal	Nil
28	40	M	Urothelial can	High	Abnormal	Diabetes
29	32	M	Bladder can	High	Abnormal	HTN
30	47	F	Bladder can	High	Abnormal	Nil
31	17	F	K. Cancer	High	Abnormal	Nil
32	2	M	K. Cancer	High	Abnormal	Nil
33	10	F	Urothelial can	High	Abnormal	Nil
34	6	M	K. Cancer	High	Abnormal	Nil
35	4	M	K. Cancer	High	Abnormal	Nil
36	51	F	Bladder can	High	Abnormal	Diabetes
37	6	F	K. Cancer	High	Normal	Nil
38	43	F	urothelial can	High	Normal	Nil
39	18	F	K. Cancer	High	Abnormal	Nil
40	54	F	K. Cancer	High	Abnormal	HTN
41	58	M	K. Cancer	High	Abnormal	Nil
42	45	F	Bladder C	High	Normal	Nil
43	50	F	urothelial can	High	Normal	Nil
44	74	M	K. Cancer	High	Abnormal	Diabetes
45	79	M	Bladder can	High	Abnormal	HTN
46	70	F	K. Cancer	High	Abnormal	Diabetes
47	59	M	Bladder can	High	Abnormal	Nil
48	66	F	Bladder can	High	Abnormal	HTN+D
49	65	M	K. Cancer	High	Normal	Diabetes
50	61	M	Urothelial can	High	Abnormal	Nil

Gender: F: Female and **M:** Male **Diagnosis:** K Cancer: Kidney cancer.

Blood kidney function: *High means: Creatinine is more than 3.0 mg/dland urea is more than 50.0 mg/dl).**Chronic diseases: HTN: Hypertension** and **D:** Diabetes M.

Table 1b: Age categories of chronic kidney diseases patients and different cancer types in grade III.

no. of cases of cancer diseases	age categories (year)			
	(2-20)	(21-40)	(41-60)	(61-88)
Kidney cancer	6	4	7	5
Bladder cancer	0	2	7	3
Urothelial cancer	1	3	6	2
Breast cancer	0	4	1	2

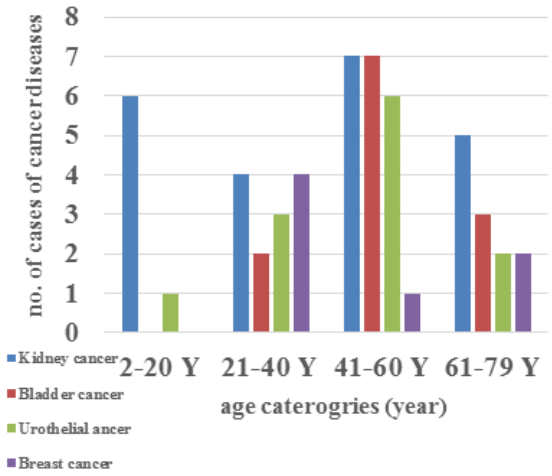

Fig. 1: Relationship between age categories of chronic kidney diseases patients and different cancer types in grade III.

Twenty random samples of both urine and blood were collected from different twenty healthy people, 8 males and 12 females, from different social environments. Their ages ranged from 14 to 60 years old, and different medical examinations (urine analyses and blood kidney function) were carried out for them in clinical pathology laboratory in Sohag Oncology Cancer Center. The results recorded inTable, 3 revealed that all examinations of tested patient cases were negative and normal except 3 cases out of them were suffering from chronic disease such as, blood pressure (one case) and Mellitus diabetes (2 cases).

3.2 Bacteria recovered in the present investigation.

Twenty-two pathogenic bacterial species belonging to 13 genera were isolated and identified from 100 samples of each of urine and blood from chronic kidney diseases patient cases in addition to 20 random cases of healthy peoples collected from Sohag Oncology Center and Sohag University Hospital. Twelve of isolated bacterial species were Gam-positive bacteria and the other nine species were Gram-negative bacteria, in addition to one Gram-varaiablespecies (*Lysinibacillus pakestinensis* OQ299572) as shown in Table 4. The bacterial infection in urine is higher than blood infection that 22 pathogenic bacterial species belonging to 13genera with (35836CFU total counts) were isolated from urine, while 10 pathogenic bacterial species belong to 8 genera with (2808CFU total counts) were recovered from blood samples on nutrient agar medium at 37°C.

3.2 Bacteria recovered in the present investigation

Twenty-two pathogenic bacterial species belonging to 13 genera were isolated and identified from 100 samples of each of urine and blood from chronic kidney diseases patient cases in addition to 20 random cases of healthy peoples collected from Sohag Oncology Center and Sohag University Hospital. Twelve of isolated bacterial species were Gam-positive bacteria and the other nine species were Gram-negative bacteria, in addition to one Gram-varaiablespecies (*Lysinibacillus pakestinensis* OQ299572) as shown in Table 4.

The bacterial infection in urine is higher than blood infection that 22 pathogenic bacterial species belonging to

13genera with (35836CFU total counts) were isolated from urine, while 10 pathogenic bacterial species belong to 8 genera with (2808CFU total counts) were recovered from blood samples on nutrient agar medium at 37°C.

Bacterial infection in urine with Gram-negative bacteria was represented by 17007 CFU (47.46%), while, Gram-positive bacteria represented by 15345 CFU (42.82%) and Gram-variable species represented by 3484 CFU (9.78 %). On the other hand, in blood Gram-negative was represented by 1676 CFU (59.69%) whereas, Gram-positive bacteria represented by 850 CFU (30.27%), whilst, Lysinibacillus pakistanensis as Gram-variable species was represented by 282 CFU (10.04 %).

Table 2a: Age and gender of patients (50)suffering from chronic kidney diseases (High kidney function) which admitted into Sohag University Hospital, Grade II.

no	age	Gender	Diagnosis	Kidney function	Urine analysis	Chronic D
1	55	F	HKF	High	Abnormal	NIL
2	49	F	K Failure	High	Abnormal	HTN
3	50	F	HKF	High	Abnormal	HTN + D
4	42	F	K Failure	High	Abnormal	D
5	70	F	K Failure	High	Abnormal	D
6	64	F	HKF	High	Abnormal	D
7	53	M	KC+HKF	High	Abnormal	HTN + D
8	22	F	HKF	High	Abnormal	NIL
9	56	M	K Failure	High	Abnormal	NIL
10	33	M	HKF	High	Abnormal	NIL
11	30	M	K Failure	High	Abnormal	D
12	17	F	HKF	High	Normal	NIL
13	22	F	HKF	High	Normal	NIL
14	10	M	HKF	Moderate	Normal	NIL
15	75	M	KC+HKF	High	Abnormal	HTN + D
16	13	M	HKF	Moderate	Normal	NIL
17	88	F	KC+HKF	High	Abnormal	D
18	79	M	HKF	High	Normal	D
19	64	M	K Failure	High	Abnormal	NIL
20	18	F	K Failure	High	Abnormal	NIL
21	33	F	K Failure	High	Abnormal	HTN +D
22	80	F	K Failure	High	Abnormal	HTN +D
23	46	F	HKF	High	Abnormal	HTN +D
24	75	F	K Failure	High	Abnormal	HTN +D
25	28	M	K Failure	High	Abnormal	NIL
26	22	F	HKF	High	Normal	NIL
27	60	M	HKF	High	Abnormal	NIL
28	55	M	HKF	High	Abnormal	D
29	44	M	HKF	High	Abnormal	NIL
30	43	F	HKF	High	Normal	HTN +D
31	39	M	HKF	High	Normal	NIL
32	71	M	HKF	High	Normal	D
33	66	M	HKF	High	Abnormal	D
34	63	M	HKF	High	Abnormal	D
35	50	F	HKF	High	Abnormal	HTN
36	34	F	KC+HKF	High	Normal	D
37	41	M	HKF	High	Abnormal	D
38	35	M	HKF	High	Abnormal	D
39	56	M	HKF	High	Abnormal	NIL
40	47	M	K Failure	High	Abnormal	NIL
41	53	M	HKF	High	Abnormal	NIL
42	36	F	HKF	High	Normal	NIL
43	70	M	HKF	High	Abnormal	D
44	69	F	HKF	High	Abnormal	D
45	41	F	HKF	High	Normal	NIL
46	68	F	HKF	High	Abnormal	D
47	20	M	HKF	High	Abnormal	NIL
48	81	M	HKF	High	Abnormal	D
49	29	F	HKF	High	Abnormal	NIL
50	22	F	HKF	High	Abnormal	NIL

Gender: F: Female and M: Male, Diagnosis: HKF: high kidney function, KF: kidneyfailure
Blood kidney function:
***High means:** Creatinine is more than 3.0 mg/dl and Urea is more than 50.0 (mg/dl).
Chronic diseases: HTN: Hypertension and **D:** Diabetes Mellites, **NIL**: negative results

Table 2b: Relationship between age categories of patients and chronic kidney diseases in grade II.

no. of cases of cancer diseases	no. of patients age categories (years)			
Chronic kidney diseases	10-20	21-40	41-60	61-88
High kidney function	4	10	12	11
Kidney failure	1	4	4	4

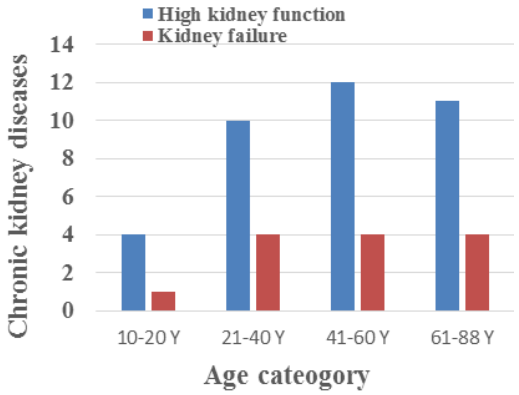

Fig. 2 Relationship between age categories of patients and chronic kidney disease in grade II.

Also, isolated genera in urine were higher than those in blood (13 and 8 genera). On the other hand, isolated species in urine (22 species) were more than twice times in blood (10 species). Total count of bacteria in urine (35836 CFU) was more twelve times than bacterial blood total count (2808 CFU). As well as, Gram-positive and Gram-negative bacterial genera in urine (6 & 6 genera) were more than that isolated from blood (3 & 4 genera), in addition one common genus represented by one species isolated in both cases (Lysinibacillus pakistanensis OQ299572).

Five genera *(Micrococcus*, *Proteus*, *Pseudomonads*, *Enterococcus* and *Streptomyces*) and twelve species (*Bacillus subtilis*, *Clostridium perfringens*, *C. welchii*, *Enterococcus faecalis*, *Klebsiella oxytoca*, *Micrococcus luteus*, M. *roseus*, *Proteus vulgaris*, *Pseudomonads aeruginosa*, *P. florescence*, *P. pyocyanea* and *Streptomyces albus* were recorded in urine and completely absent in blood specimens, while Clostridium difficile only was appeared in blood and disappeared in urine as shown in Table 4.

It is worth to mention that the urine twenty specimens of random healthy people (as a control), eleven of them Nos. (23, 38, 48, 55, 56, 58, 95, 96, 98, 114 & 120) have not any bacterial colonies, while the other nine specimens Nos. (9, 29, 36, 42, 54, 70, 82, 88 & 101) collectively were contaminated by 376 CFU (1.05%) of urine total bacterial count of examined specimens, and their counts ranged between 13 - 65 CFU in comparison to the 100 urine specimens of chronic kidney diseases patients have contaminated with 35460 CFU without control as represented in Table, 8 and Fig. [7, 8]. While the blood twenty specimens of random healthy people (as a control),

have not any bacterial infection, in comparison to the 100 blood specimens of chronic kidney diseases patients have been contaminated with 2808.

Table 4: Comparison between bacterial infections in urine and blood samples of chronic kidney patients.
H: High occurrence (more than 60 cases out of 120 patient samples tested)

Bacteria and species	Urine				Blood			
	TC CUF	TC (%)	NCI	OR	TC CUF	TC (%)	NCI	OR
Gross total count	35836	100	120		2808	100%	120	
Bacillus	2347	6.55	37	M	49	1.75	4	R
B. cereus	1635	4.56	25	M	49	1.75	4	R
B. subtilis	712	1.99	12	L	-	-	-	-
Clostridium	2567	7.16	49	M	454	16.17	12	L
C. tetani	1212	3.38	30	M	182	10.90	6	
C. difficile	-	-	-	-	272	9.69	6	
C. perfringens	914	2.55	12	R	-	-	-	-
C. welchii	441	1.23	7	R	-	-	-	-
Enterobacter aerogenes	3099	8.65	43	M	503	17.91	13	R
Enterococcus faecalis	2806	7.83	48	M	-	-	-	-
Escherichia coli	7099	19.81	78	H	820	29.20	22	L
Klebsiella	3095	8.64	51	M	259	9.22	11	L
K. pneumoniae	1904	5.31	30	M	259	9.22	11	L
K. oxytoca	1191	3.32	21	L	-	-	-	-
Lsinibacillus pakistanensis SA33	3484	9.72	62	H	282	10.04	9	R
Micrococcus	1521	4.24	36	M	-	-	-	-
M. roseus	531	1.50	14	R	-	-	-	-
M. luteus	990	2.76	25	L	-	-	-	-
Proteus vulgaris	948	2.65	30	M	-	-	-	-
Pseudomonas	995	2.78	43	M	-	-	-	-
P. aeruginosa	434	1.21	18	L	-	-	-	-
P. fluorescence	340	0.95	18	L	-	-	-	-
P. pyocyanea	221	0.62	11	R	-	-	-	-
Staphylococcus	5568	15.54	79	H	347	12.36	16	L
S. aureus	1990	5.55	36	M	245	8.73	12	R
genera *S. epidermidis*	3578	9.98	55	M	102	3.63	4	R
Streptomyces albus	138	0.39	11	R	-	-	-	-
Serratia marcescens	1771	4.93	31	M	94	3.35	4	R

M: Moderate occurrence (between 30 – 60 cases).
L: Low occurrence (between 15 – 29 cases)
R: Rare occurrence (less than 15 cases).
NCI: Number of cases of isolation of bacteria.

3.3 Biochemical tests of isolated pathogens

Biochemical examinations carried out according to classification schemes published in Bergey's Manual (1984; 1994;2005). Tables (14 & 15) showed the results of biochemical tests for Gram- negative and Gram-positive bacterial isolates. In comparing obtained results of biochemical tests with those in Bergey's manual [13, 14, 15], the Gram-positive bacterial isolates were identified as *Bacillus cereus, B. subtilis, Clostridium tetani, C. difficile, C. perfringens, C. welchii, Enterococcus faecalis* (*Streptococcus faecalis*), *Micrococcus luteus, M. roseus, Staphylococcus aureus, S. epidermidis* and *Streptomyces albus.* While, Gram-negative bacterial isolates were identified as *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxycata*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. pyocyanea* and *Serratia marcescens*.

4. Conclusion

Chronic kidney disease (CKD) is one of the most common chronic diseases with the worldwide prevalence estimated to be approximately 13.4% and projected to continue to rise annually, especially in developing countries where renal healthcare is limited [1, 2, 3]. CKD is emerging in the 21st century as a global public health issue, affecting more than 750 million people worldwide [4]. The prevalence of CKD is increasing worldwide, and the mortality rate continues to be unacceptably high [5]. CKD is a common problem among males and females due to stress, alcoholism, hypertension and diabetes mellitus. One million patients visit the emergency department, and 100,000 hospital stays every year in the United States are due to Urinary Tract Infections (UTIs). Approximately 10% of humans will have UTI at some time during their lives. Of note, UTIs are also the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections [6].

The kidneys play a vital role in the body by filtering blood and metabolic waste, while also modifying the content and composition of fluids in the body. They facilitate waste excretion, nutrient reabsorption, secrete hormones, regulate osmolarity, regulate blood pressure and maintain systemic acid base homeostasis [18].

In the present study, the obtained results showed that all patient cases tested (100 cases) were suffering from kidney failure diseases associated with troubles in kidney blood function and urine analysis. Some of them suffering from other chronic diseases such as diabetes mellitus and hypertension. So, patient cases were divided into 3 grades:

Grade III: fifty patient cases were suffering from kidney cancer or another cancer diseases, grade II: fifty patient cases were suffering from high kidney function and grade I: twenty cases as healthy people used as control for the study.

Also, the obtained data reflects the important cofactors for causing of chronic kidney diseases (CKD) in different grades. The increase in prevalence of CKD is a result to a real increase in its frequency, due to the increase in the average age of people surviving (2 - 88 years), better detection of CKD, the increasing incidence of diabetes and hypertension among people. The factors, together with increasing diabetes prevalence and an aging population, will result in significant increasing in chronic kidney diseases (CKD). There is now convincing evidence that CKD can be detected using simple laboratory tests such as blood kidney function and urine analysis.

The obtained results were in full harmony with those obtained by different investigatories [19, 20, 21], who reported that diabetes, hypertension (HTN), and kidney diseases are closely interlinked diseases. HTN may be due to multiple factors in the setting of renal dysfunction. The increase in prevalence of CKD is partly due to a real increase in its frequency (due to the increase in the average age of people surviving), better detection of CKD, but also to the increasing incidence of diabetes and hypertension among people today, not only within the developed world, but also increasingly within the emerging world. Furthermore, hypertension, smoking, hypercholesterolemia, and obesity, currently among the World Health Organization's (WHO's) top 10 global health risks, are strongly associated with CKD. The factors, together with increasing diabetes prevalence and an aging population, will result in significant global increases in chronic kidney diseases (CKD) end stage renal disease (ESRD) patients. Graciano Coutinho *et al.*, [4] on their study on urinary tract infection in patients of chronic kidney diseases in São Paulo, Barazil reported that the risk factor Diabetes Mellites, hypertension, heart disease, neoplasms and thyroid and

autoimmune disease stand out in the infected group ($p< 0.001$).

A urinary tract infection (UTI) is a bacterial infection that affects part of the urinary tract. When it affects the lower urinary tract, it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). UTIs are among the most common bacterial infections that lead patients to seek medical care [9, 10]. Twenty-two pathogenic bacterial species belonging to 13 genera were isolated and identified from 100 samples of each of urine and blood from chronic kidney diseases patient cases in addition to 20 random cases of healthy peoples collected from Sohag Oncology Center and Sohag University Hospital. Twelve of isolated bacterial species were Gam-positive bacteria, the other nine species were Gram-negative bacteria, in addition to one Gram-variable species (*Lysinibacillus pakistanensis* OQ299572).

The bacterial infection in urine is higher than blood infection that 22 pathogenic bacterial species belonging to 13 genera with total count (35836 CFU) were isolated from urine, while 10 pathogenic bacterial species belong to 8 genera with total count (2808 CFU) were recovered from blood samples on nutrient agar medium at 37ºC. Five genera (*Micrococcus*, *Proteus*, *Pseudomonas*, *Enterococcus* and *Streptomyces*) and twelvespecies (*Bacillus subtilis, Clostridium difficile, C. welchii, Enterococcus faecalis, Klebsiella oxytoca, Micrococcus luteus, M. roseus, Proteus vulgaris, Pseudomonas aeruginosa, P. fluorecens, P. pyocyanea and Streptomyces albus* were recorded in urine and completely absent in blood samples, while *Clostridium perfringens* only was appeared in blood and disappeared in urine cases examined.

Bacterial infection in urine with Gram-negative bacteria was represented by 17007 CFU (47.46%), Gram-positive bacteria represented by 15345 CFU (42.82 %) and Gram-variable species represented by 3484 CFU (9.78 %). On the other hand, in blood Gram-negative was represented by 1676 CFU (59.69%) whereas, Gram-positive bacteria represented by 850 CFU (30.27%), whilst, *Lysinibacillus pakistanensis* SA33 as Gram-variable species was represented by 282 CFU (10.04 %).

The most dominant pathogenic bacterial genera isolated in high frequencies of occurrence from urine patient cases tested were *Staphylococcus* spp. represented by 2 species (*S. aureus* and *S. epidermis*) followed by *Escherichia* which represented by one species *E. coli* and *Lysinibacillus* spp. represented by one species only *Lysinibacillus pakistanensis* AS33 in 79, 78, 62 cases out of 120 cases tested, respectively. The previous dominant pathogenic bacterial species were isolated from patient cases suffering from kidney cancer disease in addition to other types of cancer diseases such as bladder, urothelial and breast which play an important role in patient immunosuppressive or immunodeficiency. While in blood samples investigated, the most prevalent pathogenic bacterial genera and species were isolated in low frequencies of occurrence. These were *Escherichia* sp. which represented by one species *E. coli*, 22 cases out of 120 cases tested, *Staphylococcu*s sp. (16 cases), represented by 2 species (*S. aureus* (12 cases) and *S. epidermis* (4 cases), *Enterobacter* sp., represented by one species *E. aerogenes* (13 cases) and *Klebsiella* sp., represented by *K. pneumoniae* (11cases).

Whilst four species were isolated in rare frequencies of occurrence (less than 15 cases out of 120 tested) and these were *Clostridium* sp., represented by two species *C. tetani, C. perfringens*, (12 cases), *Lysinibacillus* represented by one species *Lysinibacillus pakistanensis*, (9 cases), *Bacillus cereus* (4 cases) and *Serratia marcescens* (4 cases). The obtained results of bacterial infection of urine and blood of chronic kidney patients were in full harmony with results of different researchers in different regions around the world. Graciano Coutinho *et al*., [4] on their study on urinary in São Paulo, Brazil reported that the prevalence of UTI is 22% of tested specimens and women are often more susceptible to UTI than men because of their anatomy. Most of the microorganisms found in urine cultures (87.9%) were Gram-negative bacteria, being *Escherichia coli* (50.70%), followed by *Klebsiella pneumoniae* (23.1%) and *Enterococcus* spp. (9.7%). Also, they isolated *Citrobacter* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Morangella morangii* and *Klebsiella oxytoca* as Gram-negative bacteriain low occurrence. While Gram-positivespecies were *Staphylococcus aureus* and *Enterococcus* spp. Ariza-Heredia *et al*., [22] and Fiorentino *et al.*, [23] reported that 90% of bacterial infection of kidney is Gram-negative bacteria including *E. coli, Enterobacter cloacae, Pseudomonas aeruginosa* and *Klebsiella* (*K. pneumoniae, K. oxytoca*), while Gram-positive bacteria including *Enterococcus* species which are more frequently beside *Staphylococcus* species, *Streptococcus* species, *Corynebacterium urealyticum* which are rarely recorded., as well as,,in Kaski, Nepal, Jaiswal*et al.,* 2013 recorded that bacterial species causing UTI were *Escherichia coli, Staphylococcus aureus, Streptococcus* spp., *Klebsiella* spp., *Micrococcus* spp., *Enterococcus* spp., *Proteus* spp. Also, the bacterial spp. in diagnosis of UTI was 33.33%. *E. coli* which was predominating bacteria isolated followed by *Staphylococcus aureus, Klebsiella* spp., *Salmonella* spp., respectively. These obtained results of bacterial infection in urine were in full harmony with those obtained by different researchers in different regions around the world [24, 25, 26, 27, 28].

On the other hand, the recorded results of bacterial infection in blood of chronic kidney diseases patients in the present investigation were in full agreement with that reported by Jamil *et al*. [29] who studied bacterial infection in blood of chronic kidney diseases and renal transplant patients and isolated *Escherichia coli, Klebsiella pneumonia, Enterobacter cloacae, E. aerogenes, Staphylococcus aureus, Acinetobacter baumannii, Burkloderia cepacian, Enterococcus faecalis, E. faecium, Pseudomonas aeruginosa* and *Proteus mirabilis*. Also, Muhsin and Nasreen Kamel [30] in their research on bacteriological and physiological study of bacterial infection of the blood stream in Iraq, they recorded positive bacterial growth at 16 blood samples out of 100 tested. They isolated 11 isolates as Gram-positive, ten of them are *Staphylococcus* species and one isolate is *Clostridium perfringens.* Also, five isolates were Gram-negative bacteria *Burkloderia cepacian, Pseudomonas alcaligenese, Stenotrophomonas maltophilia, Klebsiella pneumonia* and *E. coli.* Also, Matthew *et al.*, [31] on their study on risk of blood stream infection in patients with

chronic kidney disease not treated with dialysis, they reported that most microbial infections with blood stream caused by *E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* as Gram-negative bacteriaand *Staphylococcus aureus* and *Streptococcus* species as Gram-positive bacteria.

To the best of our knowledge, this is the first report of isolation of *Lysinibacillus pakistanensis* OQ299572 from human clinical specimens as pathogenic bacteria in the world. Hayat *et al*., [32] and Ahmed *et al*., [33] for the first time reported in their research on a moderately boron-tolerant candidatus novel soil bacterium *Lysinibacillus pakistanensis* sp. nov. cand., isolated from soybean (Glycine max L), that *Lysinibacillus pakistanensis* DSM 24784 is an-aerobe, spore-forming, mesophilic bacterium that was isolated from rhizospheric soil of soybean (Glycine max L.) at Rawalpindi, research farm of PirMehr Ali shah, Arid Agriculture University, Pakistan, Asia. As well as, previously, some species of *Lysinibacillus* were isolated from different sources in different places around the world. Usta**, [34]** isolated *Lysinibacillus macrolides, L. fusiformis* and *L. varians* from Varroa destructor insect. El-Sayed *et al*., **[35]** isolated *L. sphaericus* from agricultural soil in Egypt. Also, Eeyildiz *et al*., **[36]** isolated *L. massiliensis* from the synovial fluid. Eman Desouky *et al*., **[37]** isolated *L. sphaericus* from intestine of broiler chickens in Egypt. As well as, Wenzler *et al*., **[38]** isolated *L. fusiformis* and *L. sphaericus* from severe sepsis human disease.

5. References

[1] R.A. Hamer, A.M. EL Nahas, *BMJ*, 2006, 332, 563.

[2] W.G. Couser, G. Remuzzi, S. Mendis, M. Tonelli, *Kidney International*, 80*(* 2011) 1258-1270.

[3] J. Coresh, *Journal of the American Society of Nephrology*, 2017, 28, 1020-1022.

[4] M.M.G. Coutinho, E.C. Silva, C.R.V. Campanharo, A.G.S. Belasco, C.D. Fonseca, D.A. Barbosa, *Rev Bras Enferm*., 75 (2022) 1-7.

[5] I. Lousa, F. Reis, I. Beirão, R. Alves, L Belo,. A. Santos-Silva, *Int. J. Mol. Sci.*, 2021, 22, 43. P 2-40.

[6] A. Nial Hickey, A. Liliana Shalamanova, A. Kathryn Whitehead, D. Nina Hibbert, *Critical Reviews in Microbiology* (2020) 1-17. ISSN 0045-6454 (P1-27)

[7] S.K. Jadav, S.M. Sant, V.N. Acharya, *Bombay*, India, 1977, 23(1):10–18p.

[8] N.D. Vaziri, T. Cesario, K. Mootoo, L. Zeien, S. Gordon, C. Byrne, *Arch Intern Med*., 142 (1982 1273–1276.

9] K. Anding, P. Gross, J.M. Rost, D. Allgaier, E. Jacobs, *Nephrol Dial Transplant*, 18 (2003) 2067–2073p.

[10] C.O. Alebiosu, O.O. Ayodele, A. Abbas, A. Ina Olutoyin, *African Health Science*, 6 (2010) 132- 138.

[11] W.A. Rutala, D.J. Weber, and the Healath care Infection Control Practices Advisory Committee (HICPAC), (2019) 1-163.

[12] W.F. Harrigen, M.F. Macance, *Acad. Press*. London, (1966).

[13] N.R. Krieg, J.G. Holt, , Baltimore: Williams and Wilkins. Co., Baltimore, Maryland, 21202 (1984).

[14] J.G. Holt, P.H.A. Kreig, J.T. Sneath, J.T. Staley, S.T. Williams, 9th edn., Williams and W., Baltimore, Maryland, 21202 (1994).

[15] D.J. Brenner, N.R. Krieg, J.T. Staley, G.M. Garrity, Springer, New York, 2005.

[16] M.J. Zimbro, D.A. Power, S.M. Miller, G. E. Wilson, B.S. MBA, J.A. Johnson; Difco & BBL Manual of Microbiological Culture Media, 2nd Edition. Becton, Dickinson and Company, Maryland, USA, (2009).

[17] T.J. White, T. Bruns, S. Lee, & J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to Methods and Applications (ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White), 1990, pp. 315-322, Academic Press: San Diego, U.S.A.

[18] J.L. Jameson, J. Loscalzo,; Harrison's nephrology and acid-base disorders, 3e, McGraw-Hill Education, 2016.

[19] A.J. Collins, R. Foley, C. Herzog, B. Chavers, D. Gilbertson, A. Ishani, B. Kasiske, J. Liu, L.-Wen Mau, M. McBean, A. Murray, W. St Peter, J. Xue, Q. Fan, H. Guo, L. Qi, L. Shuling, L. Suying, Y. Peng, Y. Qiu, S. Roberts, M. Skneans, J. Snyder, C. Soild, C. Wang, , E. Weinhandl, D. Zaun, R. Zhang, C. Arko, S.-Cheng Chen, F. Dalleska, , Daniels, F.,Dunninf, S., Ebben, J., Frazier, E., C. Hanzlik, R. Johnson, D. Sheets, X. Wang, B. Forrest, E. Constantini, S. Everson, P. Eggers, L. Agodoa, *Am J Kidney Dis* (2008) 51: S1-320.

[20] D. Paikopoulou, *International Journal of Occupational Health and Public Health Nursing 6* (2020) 75-78.

[21] A. Jairam, V.K. Jha,; Hypertension, diabetes, and kidney disease, Chapter 100, 1-9, 2021. https://www.researchgate.net/publication/350441399

[22] E.J. Ariza-Heredia, A.M. Gulbis, K.R Stolar, P. Kebriaei, D.P. Shah, K.K. McConn, R.E. Champlin, R.F. Chemaly, *Transpl Infect Dis*, 16 (2014) 878–886.

[23] M. Fiorentino, F. Pesce, A. Schena, S. Simone, G. Castellano, L. Gesualdo, *J. Nephrol.*, 32 (2019) 32, 751–761. *[CrossRef] [PubMed]*

[24] S. Jaiswal, R. Das, S. Sharma, P. Paudel, S.R. Lamichhane, *Journal of Life Sciences*, 3 (2013) *2249*-8656

[25] C.Y. Hsiao, H.L. Lin, Y.K. Lin, C.W. Chen, Y.C. Cheng, W.C. Lee, and T.C. Wu, *Turk J Med Sci.*, 44 (2014) 145-149.

[26] C. Richa, C.S. Bhushan, S.P. Kumar, P.A. Dev, and P. Nabaraj, *Journal of Microbiology & Experimentation*, 3 (2016) 70–74.

[27] T. Gryp, R.B. Geert Huys, M. Joossens, W.V. Biesen Wim Van Biesen, W.V., Y. Griet Glorieux, Y. Mario Vaneechoutte, *Int. J. Mol. Sci.*, (2020) 1-19.

[28] M. Shankar, S. Narasimhappa, N.S. Madhura, *Cureus* 13 (2021) 2486. DOI 10.7759/cureus.12486.

[29] B. Jamil, M.T.M. Bokhari, A. Saeed, M.Z.M. Bokhari, Z. Hussain, T. Khalid, H. Bukhari, M. Imran, S.A. Abbasi, *Journal of the Pakistan Medical Association*, (2016) 705-709.

[30] H.E. Muhsin, K. Nasreen Kamel, Tikrit Journal of Pure Science, 23 (2018) 20-25.

[31] T. Matthew, M.D. James, B. Kevin, M.D. Laupland, D.M.D. Tonelli, J. Braden, M.D. Manns, F. Bruce, ,

M.D. Culleton, R. Brenda, M.D. Hemmelgarn, *Arch Intern Med.*, 168 (2008) 2333-2339.
[32] R. Hayat, I. Ahmed, J. Paek, M. Ehsan, M. Iqbal, Y.H. Chang, *Pak. J. Bot*., 2013, 45(SI): 41-50, January.
[33] I. Ahmed, Y. Sin, J. Paek, M. Ehsan, R. Hayat, M. Iqbal, Y. Hyo, *International journal of Agricultrue& Biology*, ISSN Print: 1560-8530; ISSN Online:1814-9596, 13-1196/2014/16-2-447-450, https://www.fspublisheres.org.
[34] M. Usta, Egypt J Biol Pest Control 31 (2021) 136 https://doi.org/10.1186/s41938-021-00482-7
[35] A.F. El-Sayeda, N.A. Abo-Sereiha, A.E. Mahmoudb, T.M. El-Kawokgya, A.A. El-Ghameryc, Egypt Pharmaceut J 18 (2019) 341–355.
[36] C. Eeyildiz, K. Tabakçioĝlu, S. Kehaya, N. Şakru, S.Ş. Gűrcan; A Case Report, FLORA 25 (2020) 595-598. doi: 10.5578/flora.70014
[37] E.M. Desouky, H.N. Deif, J.K. Eljakee, Journal of Applied Veterinary Sciences, 6 (2021) 23 -27 Journal homepage: https://javs.journals.ekb.eg
[38] E. Wenzler, K. Kamboj, J.-Miquel B.-Llasat, International Journal of Infectious Diseases 35 (2015) 93–95.