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FEATURE ARTICLE:

Phytochemical, Antioxidant and Antimicrobial Activity of Ethanol and Aqueous Stem Extracts of *Pseudocedrela kotschyis*

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The Pharmaceutical Society of Kenya (PSK) is a representative organization that was formed enabling Pharmacists' to employ their professional expertise in the care of patients.

Established in 1964, PSK has its roots in the Pharmaceutical Society of East Africa, which was registered in 1950. Since its formation, PSK continues to promote a common standard for professional conduct and code of ethics for its members, as well as advocate for the welfare of Pharmacists.

EDITORIAL

KNOWLEDGE MANAGEMENT AS A BASIS FOR EFFECTIVE TREATMENT OUTCOMES

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Knowledge Management (KM) is essentially about getting the right knowledge to the right person at the right time when needed. In healthcare practice, the right information can quite literally save lives, but only if professionals have the ability to quickly access it from anywhere, at any time. However, healthcare practitioners still largely base their decisions on personal knowledge and experiences, as well as the limited patient information available to them. The medical field constantly evolves as new research is released and new treatments are discovered. Scientific evidence is increasingly being produced but the gap between evidence and decision making still needs to be filled for effective health outcomes. It is important to translate research evidence, package and communicate to the different consumers of research outcome to facilitate uptake. This is achieved through knowledge translation, which is basically the synthesis, dissemination, exchange, and sound application of knowledge to improve health. To care for patients effectively, pharmacists must be able to share pharmaceutical knowledge and current drug and treatment information with patients.

Chronic illnesses like diabetes are increasingly affecting populations in the developing world, diseases that were previously associated with the developed world. Patients with chronic diseases require different sets of knowledge needs. Patient-centered health-care practice promote knowledge creation and utilization by chronic patients through the introduction of disease-specific patient community, a forum supporting the integration of knowledge gained from the experiences of living with chronic disease in their self-management. Patients provide untapped resource of knowledge that can be employed by health-care providers to inform advances in service delivery and improve health outcomes. Formally introducing disease-specific patient community operationalizes the principles of patient-centered care by validating the needs, preferences, contributions and experiences of chronic patients as high quality sources of knowledge.

Studies have demonstrated that pharmacist-provided medication therapy management (MTM) in acute care or outpatient clinic settings, as well as in chronic care management have made positive contributions to patient care quality and safe medication use. Overuse, underuse and misuse of therapies results in reduced efficiencies and quality of life. The know-do-gap in healthcare practice and health management systems creates the need for Knowledge Translation to optimize the results in investment on research. Pharmacists have a crucial role in evaluating the evidence to choose the appropriate treatment for the patient, personalization of the therapy for the specific patient in regard to the experiences and patient preferences, and decision-making to initiate the treatment because they are the drug experts who are academically trained for this purpose. This will promote "rational use of medicines," thus improving the outcome of pharmacotherapy as well as decreasing health costs.

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Phytochemical, Antioxidant and Antimicrobial Activity of Ethanol and Aqueous Stem Extracts of *Pseudocedrela kotschyi*

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Abstract

Introduction: Secondary plant metabolites found in medicinal plants have been known to have antimicrobial activity against a wide range of microorganisms including orodental, respiratory and urinary pathogens.

Aim: The aim of this research was to evaluate the phytochemical, antioxidant and antimicrobial activity of aqueous and ethanol stem extracts of *Pseudocedrela kotschyi* (Pk), a plant of use in folklore medicine, against dental and other community acquired infections caused by pathogens, so as to validate some of its therapeutic claims.

Methodology: The plant metabolites were extracted (cold maceration method) using distilled water and 70 % ethanol for aqueous and ethanol extract respectively. Preliminary phytochemical screening was carried out on the extracts to determine various secondary metabolites. Total flavonoid and phenolic contents were determined using Folin-Ciocalteu's reagent and colorimetric aluminium chloride method respectively. The antimicrobial activity was investigated using agar well diffusion method. The antioxidant activity was assayed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^{+}$) and ferric reducing antioxidant power (FRAP).

Results: Both the aqueous and ethanol extract were found to contain alkaloids, flavonoids, saponin, cardiac glycosides, steroids, terpenoids, carbohydrates, phenols and tannins. The flavonoid and phenolic contents in both aqueous and ethanol stem extracts of Pk was found to be 0.099 ± 0.01 and 0.399±0.0 mg/mL quercetin equivalent and 0.48±0.00 and 1.38±0.01mg/g gallic acid equivalent respectively. The zones of inhibition were found to be from 3-20mm and 4-18mm for the ethanol and aqueous extracts respectively. The standards cultures were highly susceptible to the two extracts while the clinical isolates were mostly resistant. The DPPH and percentage $ABTS^{\bullet+}$ scavenging activity was found to be highest at 0.0312 and 0.0625 mg/mL, 0.5 and 0.25 mg/mL for the aqueous and ethanol extract respectively. The percentage FRAP activity was highest at 0.50 mg/mL for both extracts.

Conclusion: This research confirms the presence of phytochemicals responsible for antioxidant and antimicrobial activity against orodental, respiratory and urinary pathogens. It was found that ethanol extract of *Pk* inhibited 80% of the pure isolates of bacterial strains under investigation while the aqueous extract inhibited less than 70% of the same organisms.

Keywords: *Pseudocedrela kotschyi, antimicrobial, antioxidant, chewing stick, ethanol and aqueous extract.*

Introduction

For several decades, infections by bacteria have been treated with a group of drugs called antibiotics or antimicrobials. An antibiotic is a chemical agent that works by inhibiting the replication (bacteriostatic) or killing the bacterium (bactericidal). The efficacy of these chemical agents against the replication of bacteria is as a result of their ability to block/inhibit vital bacterial cellular activities [1]. Nigeria is among several countries confirmed to have serious widespread bacterial infection caused by gram positive *Staphylococcus aureus*, gram-negative *Escherichia coli*, and some other enteric gram-negative bacteria [2].

Orthodox medical procedures now depend on diverse antibiotics to prevent or treat infections in neonates, surgery and organ transplants. From ancient times, plant secondary metabolites have provided important source of bactericidal agents that are currently employed as pharmaceutical drugs [3].

The management of microbial infections has been disrupted by resistance to antimicrobial agents and this constitutes a serious threat to global disease control [4]. Antibiotic resistance is a serious threat that is no longer predictable; it is occurring in every part of the world and is capable of affecting anyone, of any age, in any country [5]. The chemical metabolites produced by plants are meant to shield them against biotic and abiotic stresses but have been discovered

to be useful medicine for humans to cure various diseases [6, 7].

The phenolic antioxidants from plants became more important as a result of their potential health benefits. Several studies have proved that consumption of plant antioxidants is of help to humans because it down-regulates some degenerative activities and could also help to reduce the incidence of cardiovascular diseases and cancer [8].

Pseudocedrela kotschyi (P. kotschyi) belongs to the family Meliaceae. It is commonly found in the savannah region of West Africa [9]. It is a small, deciduous tree having an oblong to pyramid-shaped, usually dense crown; it can grow up to 12 – 20 metres tall. It is known as emi gbegi among Yoruba tribe and tuna among Hausas in Nigeria. For several decades, *P. kotschyi* has been a valuable herbal medicine because, it has been used traditionally for the management of various diseases such as anemia, helminthic infestation, sexual dysfunction, internal bleeding, rheumatism, fever and menstruation disorder [9, 10, 11].

In this study, a number of phytochemicals were qualitatively and quantitatively analysed. These include: alkaloids, terpenes, saponins, flavonoids, tannins, glycosides, carbohydrates etc. [12]. The aqueous and ethanol extracts of *P. kotschyi* stem were evaluated for activity against orodental, respiratory and urinary pathogens so as to validate some of its therapeutic claims

Methodology

Chemicals and Reagents

Ethanol (JHD Chemicals, China), Aluminium chloride ($AlCl_3$) (BDH Chemicals Ltd., Poole England), Gallic acid (BDH Laboratory supplies, Poole England), Folin-Ciocalteu's phenol reagent (JHD Chemicals, China), Sodium carbonate (Na_2CO_3) (BDH Chemicals Ltd., Poole England), Sodium chloride (NaCl) (Qualikems chemicals, India), 1,1-Diphenyl-2-picrylhydrazyl (DPPH),2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), Iron III chloride ($FeCl_3$) (BDH Chemicals Ltd., Poole England), acetic anhydride (BDH Chemicals Ltd., Poole England), concentrated sulphuric acid (BDH Chemicals Ltd., Poole England), distilled water, aqueous sodium hydroxide (BDH Chemicals Ltd., Poole England), magnesium ribbon, concentrated hydrochloric acid (BDH Chemicals Ltd., Poole England), glacial acetic acid (JHD Chemicals, China), quercetin (BDH Chemicals Ltd., Poole England), chloroform (JHD Chemicals, China), Mayer's and Wagner's reagents, All chemicals and solvents employed in this research were of analytical grade.

Plant material

The stem of *P. kotschyi* was purchased from a local herbal market, located in Abeokuta, South west Nigeria. The plant was identified at the herbarium unit, University of Ilorin, Nigeria. The stem was air-dried and powdered.

Aqueous extract: Dry powder of the stem of *P. kotschyi* (150g) was soaked in 740mL distilled water in a reagent bottle for 24hours. The solution was filtered through a Whatman No. 1 filter paper. The filtrate was freeze-dried to obtain a solid mass (extract) which was kept in a glass container with airtight cap.

Ethanol extract: Dry stem powder of *P. kotschyi* (167g) was soaked in740mL of 70% ethanol in a reagent bottle for 24hours with intermittent shaking. The solution was filtered through a Whatman No. 1 filter paper. The filtrate was air-dried and the solid mass (extract) was stored in a glass container with airtight cap.

The percentage yield of aqueous and ethanol stem extracts were calculated using the formula below:

$$\%\ Yield = \frac{Weight\ of\ the\ extract\ obtained}{Total\ weight\ of\ the\ sample\ loaded} * 100$$

Phytochemical Analysis

Qualitative Phytochemical Analysis of *P. kotschyi*

Phytochemical screening to determine the presence of alkaloids, flavonoids, saponins, steroids, terpenes, carbohydrates, glycosides, phenols and tannins was carried out on the extracts using standard methods [13, 14].

Quantitative Phytochemical Analysis

Total phenolic contents (TPC) were evaluated for each extract using Folin-Ciocalteu method [15-17]. A 50 mg/mL stock solution of each extract was prepared. 0.125 mL of stock solution was withdrawn and diluted to 6.25 mL with methanol to give a concentration of 1 mg/mL. Thereafter, 200 µL of these crude extracts (1 mg/mL) were made up to 1 mL with distilled water, mixed thoroughly with 1 mL of Folin–Ciocalteu reagent. After 5 minutes, 0.8 mL of 7% Na_2CO_3 solution was added with mixing. The resultant mixture was shaken for 5 seconds and left to stand for colour change. The absorbance was determined at 765 nm using UV-vis spectrophotometer. The prepared extracts were evaluated at a concentration of 0.1 mg/mL, while gallic acid (a phenolic acid) at concentration range 0.1 to 0.7 mg/ml was used as standard. All tests were performed in duplicates. Total phenolic content was expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve:

$$y=1.6232x$$
$$R^2 = 0.6658$$

Where y was the absorbance and x was the concentration.

Determination of Total Flavonoid Content

Colorimetric aluminium chloride method was used for flavonoid determination of each extract [16, 17]. 0.3 mL was taken out of the previously prepared crude extracts of 1 mg/mL of each fraction and diluted to 1.5 mL with methanol. 1.5 mL of 2% aluminium chloride was added to each of these solutions. The mixture was allowed to stand for one hour at room temperature and then absorbance was determined at 420 nm. Samples of the extract were evaluated at a concentration of 0.1 mg/mL. A stock solution of quercetin was prepared by dissolving 5.0 mg in 1.0 mL methanol. Then,

standard solutions of quercetin (0.06 to 0.18 mg/mL) were prepared by serial dilution using methanol. All determinations were performed in duplicates. The concentration of total flavonoid content in the test samples was calculated from the calibration curve (y=0.23x, R^2=0.942) and expressed as mg/g quercetin equivalent of dried plant material.

Where y was the absorbance and x was the concentration.

Antimicrobial activity

Standard and clinical isolates were used in this research. The clinical isolates used included *Klebsiella ozaenae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Yersinia enterocolitica, Serratia liquefaciens, Citrobacter diversus, Staphylococcus aureus* and *Citrobacter freundii*. The standard cultures used included *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Salmonella typhi* ATCC 14028, *Salmonella typhi* ATCC 700729 and *Citrobacter freundii* ATCC 8090.

Agar well diffusion method

The antimicrobial activity of ethanol and aqueous stem extracts were evaluated by agar well diffusion method [18]. Nutrient agar was prepared, sterilized and employed as the growth medium for the test microorganisms. Sterile 0.9 % sodium chloride solution employed as solvent to prepare the suspension of microorganisms and which was diluted to the McFarland's scale of 1.5×10^8 cfu/mL [19].

The inoculum of the test organisms was spread on the prepared Muller-Hinton Agar plates and was allowed to dry for 5 minutes. A sterile cork-borer was employed to bore standard wells on the medium and the extracts were applied into the wells.

The ethanol extract and the aqueous extract were dispersed in fresh 70% ethanol and sterile water respectively to final concentrations of 1000mg/mL. A 100 µL aliquot was withdrawn from the solution of each plant extract and transferred into each well on the medium seeded with the test microorganism. The plates were allowed to stand for one hour for proper diffusion, after which they were incubated at 37°C for 24h and observed for zones of inhibition of growth, measured in millimeter (mm) around the wells from the edge of each disc after the incubation period.

Antioxidant assay

Assay of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH radical-scavenging activities of the stem extracts were determined according to standard method [17]. A standard sample solution (1.0 mg/mL) was prepared and diluted to the following concentrations: 250, 125, 50, 25, 10 and 5 µg/mL in methanol and distilled water. 1.5 mL of each concentration was added to 1.5 mL of methanolic solution of DDPH (100µM). The mixture was allowed to stand at 27°C for 30 minutes in the dark. Ascorbic acid was used as positive control. All determinations were carried out in triplicates. The absorbance was measured at 518 nm. Percent radical scavenging activity was calculated as follows:

$$\%\ DPPH\ scarvenging\ activity = \frac{Absorbance\ (DPPH) - Absorbance\ (Extract)}{Absorbance\ (DPPH)} \quad X\ 100$$

ABTS·$^+$radical cation-based radical scavenging assay

Free radical scavenging activity of plant samples was determined by ABTS·$^+$ radical cation decolorization assay [20]. ABTS·$^+$ cation radical was produced by the reaction between 7 mM ABTS·$^+$ in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·$^+$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µL of plant extract to 3.995 m of diluted ABTS·$^+$ solution, the absorbance was measured 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out three times. Percent inhibition of absorbance at 734 nm was calculated using the formula,

$$\%\ ABTS^{\cdot+}\ scavenging\ effect = \frac{(AB-AA)}{AB} \quad X\ 100$$

Where AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS·$^+$ radical + sample extract/standard. Trolox was used as standard substance.

Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of the medicinal plants was estimated spectrophotometrically following the procedure of Benzie and Strain [21]. The method is based on the reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) colourless complex to Fe^{2+} -tripyridyltriazine blue colored complex formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl and 20 mM $FeCl_3$.6H2O in the proportion of 10:1:1 at 37°C. 3.995 mL of freshly prepared working FRAP reagent was pipetted using a 1-5 mL variable micropipette and mixed with 5 µL of the appropriately diluted extract and mixed thoroughly. An intense blue color complex was formed when Fe^{3+} TPTZ complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 mL FRAP reagent+5 µL distilled water) after 30 min incubation at 37°C. All the determinations were performed in triplicates. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mg of Trolox equivalent per gram of sample.

Statistical analysis

Results were analysed using Graph Pad prism 7 (Graph Pad prism 6 software. Inc., USA), and the results were expressed

as mean ± standard deviation. Differences of $p < 0.05$ were taken as statistically significant, others were presented as %.

Results

Percentage yield of aqueous and ethanol extracts of P. kotschyi stem

The percentage yield of aqueous and ethanol stem extracts were found to be 3.33% and 7.56% respectively as shown in **Table 1**.

Table 1. Percentage yield of aqueous and ethanol stem extracts of *P. kotschyi* stem

Solvent	Weight of plant (g)	Weight of extract (g)	Percentage yield (% w/w)
Ethanol (70%)	167	12.6	7.56
Water	150	5. 0	3.33

Qualitative phytochemistry analysis of ethanol and aqueous extracts of bark of *P. kotschyi*

The qualitative phytochemistry of the stem of *P. kotschyi* is shown in **Table 2**. The phytochemicals found to be present in the ethanol and aqueous extract of *P. kotschyi* included alkaloids, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, carbohydrates, phenols and tannins.

Table 2. Qualitative phytochemistry of ethanol and aqueous extracts of *P. kotschyi* stem

Phytochemical constituents	Test	Ethanol extract	Aqueous extract
Alkaloids	Wagner's Reagents	+	+
Flavonoids	Mayer's Reagents	+	+
Saponin	NaOH Test	+	+
Cardiac Glycosides	Frothing Test	+	+
Steroids	Keller-Kilani Test	+	+
Terpenoids	$CHCl_3$ + Conc. H_2SO_4	+	+
Carbohydrates	$CHCl_3$ + Conc. H_2SO_4	+	+
Phenols and	Fehling Test	+	+
Tannins	$AlCl_3$ Test	+	+

Key: + = present

Total flavonoid: the flavonoid content of the aqueous and ethanol extracts was found to be 0.099 ± 0.01 and 0.399±0.0 (mg/mL) quercetin equivalent respectively as shown in **Table 3.**

Table 3. Total flavonoid contents of aqueous and ethanol stem extracts of *P. Kotschyi* calculated as quercetin equivalents

Extracts	Absorbance at wavelength (420nm)	Quercetin equivalent of flavonoid (mg/mL)	Average Quercetin equivalent of flavonoid (mg/mL)
Ethanol extract	0.089 0.089 0.090	0.399 0.399 0.399	0.399±0.0
Aqueous extract	0.025 0.019 0.022	0.112 0.085 0.099	0.099 ± 0.01

Total phenolic content: the phenolic content of the aqueous and ethanol extracts of the stem of *P. kotschyi* was found to be 0.48±0.00 and 1.38±0.01mg/g gallic acid equivalent as shown in **Table 4**.

Table 4. Quantitative phytochemical analysis of *P. kotschyi* showing the total phenolic contents calculated as gallic acid equivalents

Extracts	Absorbance at wavelength (765nm)	Gallic acid equivalent of phenol (mg/g)	Average Gallic acid equivalent of phenol (mg/g)
Ethanol extract	3.011 2.965 2.988	1.387 1.364 1.376	1.380 ±0.01
Aqueous extract	1.252 1.130 1.191	0.506 0.444 0.475	0.480 ±0.001

Antimicrobial activity

Antimicrobial activity for both ethanol and aqueous extracts of stem of *P. kotschyi* is presented in **Table 5**. Both the extracts were found to inhibit the standard cultures of the test organisms more than the clinical isolates. *Salmonella typhi* ATCC 700729 was the most susceptible to both the extracts at concentration of 1000 mg/mL followed by *Escherichia coli* ATCC 25922 while *Salmonella typhi* ATCC 14028 was susceptible to only the ethanol extract.

Most of the clinical isolates used were resistant to the extracts at the same concentration having exhibited small zones of inhibition.

Table 5. The diameter of zone of inhibition (mm) of *P. kotschyi* stem extracts against test microorganisms

Extracts	Zone of inhibition (mm)	
	Ethanol extract	Aqueous extract
Klebsiella ozaenae	4	4
Escherichia coli	5	0
Escherichia coli ATCC 25922	15	13
Pseudomonas aeruginosa	4	6
Proteus mirabilis	3	6
Yersinia enterocolitica	6	5
Salmonella typhi ATCC 14028	10	0
Salmonella typhi ATCC 700729	20	18
Serratia liquefaciens	5	6
Citrobacter diversus	6	5
Staphylococcus aureus	4	4
Citrobacter freundi	5	5

Antioxidant analysis

The DPPH scavenging activity was found to be highest at 0.0312 mg/mL for the aqueous extract and at 0.0625 mg/mL for the ethanol extract as shown in **Table 6**.

Table 6. Antioxidant analysis of ethanol and aqueous extracts of the bark of *P. kotschyi*

Concentration (mg/ml)	Mean ± SEM %DPPH scavenging activity		
	Ethanol extract	Aqueous extract	Ascorbic acid
0.031	91.40±1.39	52.40±1.39	21.53±0.07
0.063	88.40±0.81	64.40±8.43	43.32±0.90
0.125	84.90±1.55	53.73±2.27	76.41±1.02
0.250	79.00±3.46	26.70±5.71	84.67±1.10
0.500	57.30±6.87	9.30±0.40	95.41±0.08

SEM = Standard error of mean

The percentage $ABTS^{\bullet+}$ (cation) scavenging activity was found to be highest at 0.5 mg/mL for the aqueous extract and at 0.25 mg/mL for the ethanol extract as shown in **Table 7**.

Table 7. The ABTS•+ scavenging effect of ethanol and aqueous extracts of the bark of *P. kotschyi*

Extracts	Concentration (mg/mL)	Absorbance (734nm)			Mean ± SEM % ABTS•+ Scavenging effect
		1	2	3	
Ethanol extract	0.50 0.25	0.066 0.041	0.056 0.044	0.061 0.043	91.286 ±0.01 93.929 ±0.05
Aqueous extract	0.50 0.25	0.004 0.014	0.004 0.009	0.004 0.012	99.429 ±0.10 98.357 ±0.03

The percentage FRAP activity was found to be highest at 0.50 mg/mL for both the aqueous and ethanol extracts as shown in **Table 8**.

Table 8. FRAP Antioxidant analysis of ethanol and aqueous extracts of the bark of P. kotschyi

Extracts	Concentration (mg/mL)	Absorbance at wavelength (593nm)			Trolox Equivalent/g
		1	2	3	
Ethanol extract	0.50 0.25	2.503 2.443	2.459 2.396	2.481 2.420	5.384 10.198
Aqueous extract	0.50 0.25	2.339 2.163	2.277 2.217	2.308 2.190	5.801 11.305

The standard curve of different concentration of Trolox versus absorbance is shown in **Figure 1**.

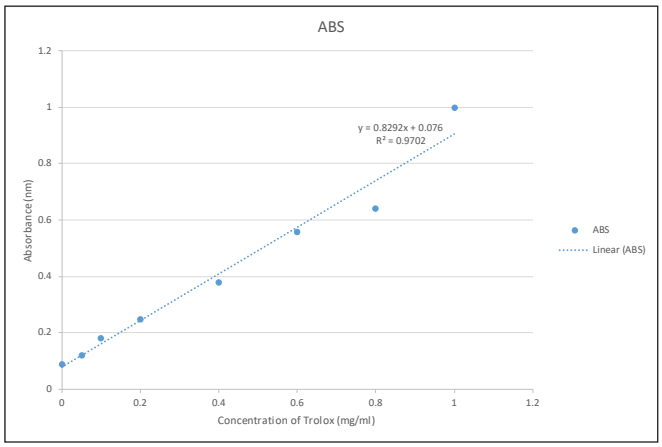

Figure 1. Standard curve of different concentrations of Trolox versus absorbance

Discussion

Literature has shown that extractive values vary with extracting solvents which in turn vary with different medicinal plants [22]. The OH group in both solvents (water and ethanol) can dissolve polar molecules and ionic compounds as well. The low extractive value or percentage yield of the aqueous compared to the ethanol stem extract may be due to low solubility of these compounds in water.

The qualitative phytochemical screening of the aqueous and ethanol stem extracts of *P. kotschyi* are in agreement with that of Otimenyin et al. [23]. The presence of alkaloids, cardiac glycosides, phenols and tannins, flavonoids, and terpenoids contributes to the antimalarial, anticonvulsant, antibacterial, antifungal and antidiabetic activity of both extracts of *P. kotschyi* [10, 24, 25]. Many tannin components have been reported to be anticarcinogenic and also antimutagenic for a number of mutagens. These anticarcinogenic and antimutagenic potentials have been suggested to be related to their antioxidative capacity, which is responsible for protecting against cellular oxidative damage and lipid peroxidation [26].

The result of the quantitative phytochemistry analysis shows the presence of phenols and flavonoids in adequate concentration. The lower flavonoid and phenolic contents of the aqueous compared to the ethanol stem extract may be due to low solubility of these compounds in water. The higher polyphenol contents in the ethanol stem extract may be due to the fact that unlike water, ethanol has a non-polar region that allows it to better dissolve phytochemicals which are polar, but also organic in nature.

Flavonoids have been reported to protect against free radical cellular damage (induced photolytically generating singlet oxygen and metabolic processes in living organisms) and act as antioxidants [27]. Many research outcomes corroborate the advantages of phenolic compounds, such as anti-inflammatory, anticancer, anti-aging, antioxidant and anti-proliferative agents [28].

Determination of antioxidant activity, percentage DPPH scavenging activity for both aqueous and ethanol extracts showed that the stem of *P. kotschyi* possessed antioxidant activity though that of the aqueous plant extract was significantly higher ($p<0.05$) than that of the ethanol plant extract.

Average percentage ABTS•+ scavenging effect for aqueous and ethanol extract showed the presence of antioxidants in high quantity, with the percentage antioxidant activity in aqueous extract higher than that of the ethanol extract though not statistically significant. FRAP antioxidant analysis of ethanol and aqueous extracts of *P. kotschyi* showed that the aqueous extract possesses more antioxidant activity expressed as Trolox equivalent than that of the ethanol extract.

Plants' antioxidant action is not entirely due to phenolic chemicals. Other water soluble elements such as reducing carbohydrates, tannins, saponins and ascorbates, as well as the synergistic impact between them, may contribute to total antioxidant activity [29, 30]. Thus this could explain the high antioxidant activity of the aqueous extract though having low flavonoid contents compared to the ethanol extract. It was also observed that the extract concentration was inversely proportional to the antioxidant activity.

Presence of antioxidants including phenolic compounds (e.g., flavonoids, phenolic acids and tannins), have diverse biological effects; they mitigate the effect of free radicals that facilitates the development of degenerative diseases [28], including cancer, cardiovascular diseases, Alzheimer's disease, neurodegenerative diseases and inflammatory diseases. Antioxidant compounds that are contained in natural plant sources could therefore serve as preventive medicine.

The antimicrobial activities of both ethanol and aqueous extracts against typed and clinical isolates as in **Table 5** showed the zones of inhibition for clinical isolates to be less than 7mm. This is an indication that the latter isolates are resistant to both extracts while the former isolates show higher and impressive values of zone inhibition.

Water is a universal solvent for extracting bioactive constituents from medicinal plants, yet, extracts obtained with organic solvent yielded more antimicrobial activity than water extracts [12]. Most of the antimicrobial bioactive moieties like flavonoid, phenols, saponins have been identified from organic solvent extracts [12] which is in agreement with this study. Water-soluble compounds, such as polysaccharides and polypeptides, are mostly inhibitors of pathogen adsorption without antimicrobial properties. Besides, water soluble flavonoids (mostly anthocyanins) possess no antimicrobial importance and water soluble phenolic compound have significant antioxidant activity [31].

This study also confirmed that efficacy of plant extracts evaluated as antimicrobial agents depend on the extraction solvent. The hydroxyl group as well as the non-polar part in ethanol is responsible for dissolving both polar molecules and ionizable constituents [32]. Thus the ethanol extract is more proficient in the degradation of cell walls which are made of non-polar substances and therefore results in polyphenols being released from cells. Furthermore, explanation on reduction in activity of aqueous extracts can be attributed to the enzyme polyphenol oxidase, which degrades polyphenols in aqueous extracts, but is normally inactivated in ethanol extract [33].

The results obtained from the well diffusion assay showed that there was a significant inhibition of standard cultures of test organisms by extracts of *P. kotschyi* when compared with clinical isolates. It was found that ethanol extract of *P. kotschyi* inhibited 80% of the pure isolates of bacterial strains under investigation while the aqueous extract inhibited less than 70% of the same isolates.

All clinical isolates of the test microorganisms used in this study showed resistance to both aqueous and ethanol extracts of *P. kotschyi* since the zones of inhibition were all less than 7mm which validated the suspicion that those organisms are resistant pathogens. Data obtained in this study showed that strains of microorganism isolated from nosocomial infection (clinical isolates) showed resistance to the *P. kotschyi* extracts than community acquired infection ones (standard cultures). This is usually due to exposure of patients in the hospital to varying infections which are usually caused by more than one pathogen. Studies have shown that resistance to antibiotics as well as mortality is about two times higher in case of nosocomial infections than in community acquired infections [34].

Conclusion

This research further confirms that *P. kotschyi* stem extracts possess antioxidants in high concentration and can serve as a beneficial effect on human health. The phytochemical analysis shows the presence of phytochemicals which are also responsible for antioxidant activity of this plant. Presence of phytochemicals such as flavonoids, phenols, alkaloids, tannins, terpenoids, glycosides and saponins contributes to the therapeutic and medicinal value of *P. kotschyi*. It was found that extracts of *P. kotschyi* inhibited the standard cultures of bacterial strains under investigation more than the clinical isolates. The antimicrobial activity of aqueous and ethanol extracts of *P. kotschyi* has been investigated and established against orodental, respiratory and urinary pathogens. The ethanol extract of *P. kotschyi* inhibited the pure isolates of bacterial strains under investigation more than the aqueous extract. Therefore, it can be concluded that aqueous and ethanol extracts of *P. kotschyi* can be used in treatment of community acquired infections caused by orodental, respiratory and urinary pathogens.

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Bacterial profile and Antibiogram for Hemodialysis Patients at Muhimbili National Hospital, Tanzania: A cross section study

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Abstract

Objective: The purpose of this study was to determine the prevalence of bacterial infection and antimicrobial susceptibility patterns among patients attending hemodialysis at Muhimbili National Hospital (MNH) with a 1,500-bed facility located in Dar es Salaam, Tanzania.

Participants: A cross-sectional study involving 18 inpatients and 146 outpatients, making 164 eligible participants, was performed. Of these participants, 53 were female and 111 were male. This study recruited patients who underwent hemodialysis (HD) at the high dependence unit regardless of chronic kidney disease (CKD) stage, patients who had a catheter inserted over 72 hours, and patients who were 18+ years. The Primary and secondary outcome measures included two primary outcome variables prevalence of bacterial infection and bacterial isolates and the secondary outcome was antimicrobial susceptibility patterns of isolated bacteria.

Results: In total, 29.9% of participants had positive blood culture of which 44.4% were inpatient and 28.1% were outpatients. Prevalence was highest in the female gender (32.1) and higher in younger (75%) compared to older patients. Gram-positive isolates comprised 56.2% of the total isolates, and infection was found to be high in patients with diabetes (33.8%). Among gram-negative isolates, *Escherichia coli* were more (24.6%) than other isolates. More than 60.9% of patients with blood culture positive reported having fever and 47.8% reported skin itching. *Klebsiella* species were the most resistant among isolates tested for amikacin, ceftriaxone, and amoxicillin/clavulanic acid. In this study, the prevalence of infection was high among admitted patients compared to outpatients.

Conclusion: The current study observed a high prevalence of infection in admitted patients compared to outpatient. Gram positive bacteria remain frequent isolates that cause infection in dialysis patients, and *Staphylococcus aureus* alone contributed a high percentage when compared to other gram-positive bacteria with high resistance to gentamycin, amoxicillin-clavulanic acid, ceftriaxone, erythromycin, and penicillin. Therefore, more measures are needed to protect patients undergoing HD from infections from resistant bacteria.

Key Words: *chronic kidney disease; Hemodialysis; Bacterial Infection, Antibiotics susceptibility; Antibiotic Resistance.*

Introduction

Patients with chronic renal failure (CRF) and having a glomerular filtration rate (GFR) of 15 ml/min/1.73m^2 usually undergo hemodialysis (HD) as a means of assisted kidney functions[1,2] For patients with kidney failure, dialysis is continuous and a permanent process until they undergo a kidney transplant. Due to the process of dialysis, patients are at high risk of bacterial infections [3]. Infections from HD catheters cause rise of >50% mortality in HD patients equated to patients on native fistulas [4]. It is estimated that 55% of HD catheters are colonized by one or more microorganisms; mainly from the skin (58%), intraluminal (17%), and others (6.8%)[5]. The common bacterial isolates reported are *Staphylococcus epidermidis* and *Staphylococcus aureus*(5) but this varies between individuals.

Many factors such as comorbidities, hygiene practices, types, and sites of insertion of catheters contribute to the occurrence of these infections[6]. Patients may get other infections in hospital settings other than their primary infection through direct or indirect contact with devices like catheters and professionals' hands [7]. Various studies have established that health care workers' (HCW) contaminated hands are some of the key modes of transmission of health-care-associated infections[8] to patients.

In HD therapy, vascular access procedure is among the causes of infection, hospitalization, and death of patients with renal disease[7]. In addition, the risks of infection associated with vascular access, are contributed by problems with the intravascular connection, white blood cell, and complement dysfunction from contact with HD membranes[9]. Regarding the dialysis process itself, there are other factors contributing to the infection in HD patients including, contaminated equipment, water, environmental surface in the treatment areas, and the infectious patients who pose a threat to other nearby patients being treated in the unit[10].

Host factors are also contributing to the pathogenesis of bacterial infection in HD patients. Many studies disclose a significant relationship between iron overload in HD patients and bacteremia. Two main mechanisms are involved: the first one is the augmentation of bacterial growth in an iron-enriched medium and stimulates bacterial virulence; the second is the decreased host defense, particularly the function of polymorphonuclear granulocytes and impaired bacterial killing capacity when hemosiderosis is present[11,12]. Uremia is also associated with alteration in primary host defense mechanisms, which raise the risk of bacterial infections[12].

The problem of bacteremia is compounded by the emergence of bacterial resistance among patients who undergo HD [12]. Reports indicate that 40% of hemodialysis patients were colonized by methicillin resistant S. aureus (MRSA) in the year 2019 compared to 27% in the general population [13]. A study conducted at Muhimbili National hospital indicated that the threat of colonization by multidrug-resistant organisms in HD patients is very high, ranging from 3% to over 20% [14]. However, there were no studies done to determine the antimicrobial susceptibility and bacteriological pattern in patients on dialysis in Tanzania. Therefore, it was necessary to conduct this study in Tanzania to know the prevalence of infections, types of bacteria, and antimicrobial susceptibility patterns. The knowledge of this study would be used for improving the treatment of HD patients at MNH, United Republic of Tanzania, and the whole continent at large

Methodology

Materials

Mueller Hinton Agar, 0.5 MacFarland tube, Sterile cotton swabs, Wire loop, Gloves medium size, Universal bottles screen cap aluminum 20ml, Disposable plastic culture plates, Antibiotic disks.

Study Setting

This study was conducted at Muhimbili National Hospital (MNH) dialysis unit, Dar es Salaam, Tanzania. MNH is a National, Teaching and Referral hospital with a 1,500-bed facility.

Study design

A cross-sectional study involving 18 inpatients and 146 outpatients making a total of 164, out of whom, 53 were female and 111 males. Prior to dialysis, the patient had to fulfil pre-dialysis vital condition check-ups such as Haemoglobin level (HB), blood pressure that should be normal BP, temperature, and blood glucose. During three months from June to August 2019, all hemodialysis patients with a central venous catheter inserted over 72 hours and arteriovenous fistula were recruited for the study.

Eligibility criteria

This study recruited 164 patients who underwent Hemodialysis at MNH regardless of chronic kidney disease (CKD) stage. The study included patients who had a catheter inserted over 72 hours and aged 18 years and above.

Patient and public involvement

Patients were not involved in the design of the study.

Patient recruitment and consenting

Patients included in the study were those with clinician appointments on their respective dates. After patient consultation and review from the clinician, consent was taken from the patient before study participation.

Patients' information

Demographic data: age, gender were collected via interviews and review of the patients' case files. Clinical information: co-morbidities like diabetes mellitus, blood pressure, and presence of fever, chills, and discharges, insertion date, insertion site, and antibiotic use were collected, and HD modalities were collected from patient charts.

Sample size and blood sample collection

All the patients that attended HD at MNH that met inclusion criteria were sampled. We had two primary outcome variables; the prevalence of bacterial infection and bacterial isolates and the secondary outcome was antimicrobial susceptibility patterns of isolated bacteria. A volume of 20mL of blood was drawn from each patient before the dialysis session or after the dialysis session. Two sets of blood cultures were collected from each patient, where one set of blood samples (anaerobic and aerobic) from a peripheral vein and the other from the venous catheter. For patients with arteriovenous vascular (AV) access, the blood sample was obtained from the arterial needle before connecting the arterial blood tubing or flushing the needle. We made sure no saline or heparin was in the arterial needle. A volume of 5ml of blood for aerobic culture and 5ml for anaerobic culture was then collected from both sides. A sterile zone was then determined and cleaned with 70% alcohol followed by 10% povidone-iodine in a round motion starting from

the center and moving outwards, and then allowed the site to dry. Blood was also collected from the catheter in a similar manner whereby a 10mls syringe was used to withdraw heparin and normal saline from the arterial port of the catheter, along with the blood to a total of 3mls. The syringe contents were discarded in a bio-hazardous container. Then 10mls of blood were collected by using a new syringe. For post dialysis, the samples were collected immediately after completion of HD. The dialysate flow was turned off; blood flow was decreased to 100mL/min for 15 seconds. Then the blood specimen was obtained from the arterial sample port on the arterial bloodline. The specimen bottle was labeled with a patient number, date, and time. The blood samples were then assigned for culture and antibacterial sensitivity tests. Clinical and Laboratory Standards Institute (CLSI) protocol[15] was used to test the isolated cultures.

IDENTIFICATION OF BACTERIA

Protocol and Materials

Aerobic and anaerobic bottles were incubated in an automated continuous monitoring system. The BD BACTEC FX40 (BD and Company USA) was used during the study, and the alert system and colorimetric signal notified the growth of bacteria, the systems monitor CO_2. All the researchers and study assistants had put on plastic aprons, facemasks, and sterile gloves to avoid contamination of the blood culture.

The MNH laboratory protocol adopting techniques used by previous studies [15,16] was used during the isolation and identification of organisms. Isolated organisms were identified through colonial morphology, relevant biochemical tests, and Gram staining. Identification was undertaken using gram staining, single iron agar test, sulfide-indole motility (SIM) test and catalase and oxidase tests.

Antibiotic susceptibility testing

Antibacterial susceptibility testing was conducted on both aerobic and anaerobic isolates on Muller-Hinton agar using the Kirby-Bauer disc diffusion method at a central pathology laboratory, MNH. Antibacterial susceptibility on the selected antibiotic discs was performed according to the criteria of the CLSI. From a pure culture, colonies of bacteria (three to five) were taken and moved to a tube having 5ml of sterile normal saline and mixed moderately to a homogenous suspension and adjusted to Mcfarland 0.5. A sterile cotton swab was used to allocate the bacteria steadily over the entire surface of Mueller Hinton agar. The inoculated plates were left at room temperature to dry for 3-5 minutes, and a set of antibiotic discs were then placed on the surface of a Muller-Hinton plate. Drugs for disc diffusion testing for gram positive bacteria were Ciprofloxacin (5µg), Cefoxitin (30µg), Gentamycin (10µg), Doxycycline (30µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg), Clindamycin (2µg), Vancomycin (30µg), Penicillin (10units), and Erythromycin (15µg). Drugs for gram negative were Amikacin (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), Gentamycin (10µg), Ceftriaxone (30µg), Chloramphenicol (30µg), and Amoxicillin-clavulanic acid (20/10µg). The diameter of a zone of inhibition around the disk was measured with the ruler and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI.

CONTROLS

The quality of blood culture was ensured by proper antiseptic techniques during the blood samples collection, by taking appropriate sample volume. Sterility testing of the culture media was done by incubating overnight at 37°C and for performance by inoculating known standard strains. Standard strains of Escherichia coli American Type Culture Collection (ATCC) 25922 and S. aureus ATCC 25923 were used during culture and antimicrobial susceptibility testing

DATA ANALYSIS

Prevalence was obtained based on the positivity of blood culture and presented in terms of percentage. Bacterial isolates are presented as proportions in terms of percentages. Antimicrobial susceptibility pattern results are presented in terms of percentages of given bacteria isolate to a given type of antibiotic being sensitive, resistant, or multidrug resistant. Data were analyzed by Chi-square test using social statistical package for social sciences (SPSS software version 20.0). The p-value< 0.05 was set as a significant level in statistical associations (**Table 1**).

Table 1. Analysis plan

Objective	Analysis plan
To determine the prevalence of bacterial infection in HD patients at MNH	Proportion/ frequency and percent-age
To determine the type of bacterial isolates among patients attending HD at MNH.	Frequency and percentage
To determine the antimicrobial susceptibility patterns of isolated bacteria in HD patients at MNH.	Frequency distribution/ percentage

ETHICAL CONSIDERATION AND ETHICAL CLEARANCE

Ethical clearance was obtained from the Institutional Research Board of Muhimbili University of Health and Allied Sciences (MUHAS) and MNH (MNH/TRC/Permission/2019/085), before the initiation of the study. The study imposed minimal risk to the participants like a puncture of the skin and sensing transient pain. All patients gave consent prior to blood sample collection. Patients benefited from the study, as their obtained results were communicated to their respective medical doctors and patients with culture positive results were arranged for treatment.

Confidentiality

All the information obtained from this study was used for the research purpose only and was not shared with anyone without participants' consent.

Results

Participants' social demographics and clinical information

Data analysis was done to a total of 164 samples from patients on dialysis out of 165 who were recruited as one participant withdrew the consent. The baseline characteristics of the patients are shown in **Table 2**. Participants were mostly adults, predominantly males (67.7%). Among 164 participants, 49(29.9%) were culture positive. Additionally, the mean dialysis catheter permanence was 107 days, and the mean age of the study population was above 50 years as shown in **Table 2**. However, in terms of proportion, the proportion of females who had infection was large compared to males (p-value=0.671), Infections were seen more among outpatients aged below 20 years as well as among inpatients as shown in **Table 3**. There was similar comparability between infections incidence in the pre-and post-dialysis group. The most-reported symptom among these patients who had infections was fever with 60.9%.

Table 2. Study participant's baseline information

Variable		n (%)
Sex	F M	53(32.3) 111(67.7)
Age	<=20 21-30 31-40 41-50 51-60 61+	4(2.4) 19(11.6) 21(12.8) 24(14.6) 49(29.9) 47(28.7)
Hospitalization	Yes No	18(11.0) 146(89.0)
Vascular access	Jugular Fistula Subclavian Femoral	71(43.3) 56(34.1) 35(21.3) 2(1.2)
Dialysis	Pre dialysis Post dialysis	82(50.0) 82(50.0)
Isolates found	Yes No	49(29.9) 115(70.1)
Median of hospitalization days (Range) Mean age of study population in years (±SD)		11 50.7 (±15.0)
Median of catheterization days (Range)		71
Mean of dialysis hours (±SD) 4 (0)		

Table 3. Determinants of infection status.

		Infection status			
		Infected n (%)	Not infected n (%)	Total N(%)	p-value
Sex	F M	17 (32.1) 32 (28.8)	36(67.9) 79(71.2)	53 (100.0) 111 (100.0)	0.671
age	<=20 21-30 31-40 41-50 51-60 61+	3 (75.0) 2 (10.5) 8 (38.1) 8 (33.3) 12(24.5) 16 (34.0)	1 (25.0) 17 (89.5) 13 (61.9) 16 (66.7) 37 (75.5) 31 (66.0)	4 (100.0) 19 (100.0) 21 (100.0) 24 (100.0) 49 (100) 47 (100.0)	0.103
Hypertension	Yes No	47 (31.5) 2 (13.3)	102 (68.5) 13 (86.7)	149 (100.0) 15 (100.0)	0.142
Diabetes	Yes No	26 (33.8) 23 (26.4)	51 (66.2) 64 (73.6)	77 (100.0) 87 (100.0)	0.306
Vascular access	Jugular Fistula Subclavian Femoral	22 (31.0) 14 (25.0) 13 (37.1) 0(0.0)	49 69.0) 42 (75.0) 22 (62.9) 2 (100)	71 (100.0) 56 (100.0) 35 (100.0) 2 (100.0)	0.492
Hospitaliza-tion	Yes No	8(44.4) 41 (28.1)	10 (55.6) 105 (71.9)	18 (100.0) 146 (100.0)	0.152
Dialysis	Pre dialysis Post dialy-sis	29(35.4) 20 (24.4)	53 (64.6) 62 (75.6)	82 (100.0) 82 (100.0)	0.125
Cough	Yes No	9(37.5) 40 (28.6)	15 (62.5) 100 (71.4)	24 (100.0) 140 (100.0)	0.377
Fever	Yes No	14 (60.9) 35 (24.8)	9 (39.1) 106 (75.2)	23 (100.0) 141 (100.0)	<0.0001
Joint Pain	Yes No	7 (30.4) 42 (29.8)	16 (69.6) 99 (70.2)	23 (100.0) 141 (100.0)	0.950
Edema	Yes No	2(20.0) 47 (30.5)	8 (80.0) 107 (69.5)	10 (100.0) 154 (100.0)	0.481
Skin Itching	Yes No	11(47.8) 38 (27.0)	12 (52.2) 103 (73.0)	23 (100.0) 141 (100.0)	0.043
Vomiting	Yes No	7(38.9) 42 (28.8)	11 (61.1) 104 (71.2)	18 (100.0) 146 (100.0)	0.376

Generally, hypertension was the most commonly found co-morbidity among patients, while for those who had co-morbidities, 33.8% of diabetic patients were infected as compared to the hypertensive patient, 31.5%. Patients with fever and skin itching were found statistically significant (p <0.05) among symptoms that their association with infections were ascertained.

Distribution of bacteria isolated in patients attending HD at MNH

After positive cultures were recorded, further studies were done to determine the type of organisms isolated. Among isolated organisms with their proportion in brackets were *S. aureus* (35.1%) *E. coli* (24.6%) *Pseudomonas* (10.5) and *Klebsiella* accounted for 24.6% (**Figure 1**). The bar chart below shows proportions and types of isolated bacteria in patients attending HD at MNH, both gram negative and gram positive were obtained.

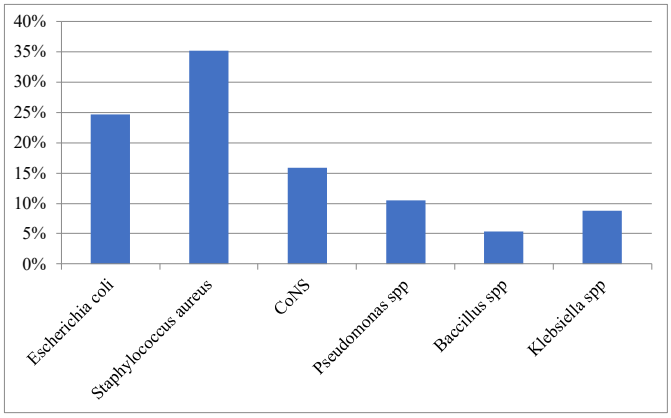

Figure 1. Proportion of the isolated pathogens from N = 49 (57 Isolates) participants with positive blood cultures at MNH HD unit from June through August 2019 [CoNS - Coagulase Negative Staphylococcus]

Antibacterial Susceptibility for Gram Positive Isolates from Patients Attending HD at MNH

After isolation, susceptibility of the strains on the available antibiotics was done. From the isolated gram-positive strains, Vancomycin resistance in *Staphylococcus* aureus isolate was 10%, coagulase negative *Staphylococcus* strain showed 11.1% resistance to vancomycin, whereas all of them were sensitive to ciprofloxacin. Both *Staphylococcus* aureus and Coagulase negative *Staphylococcus* showed resistance to erythromycin and penicillin, while there was the least resistance to doxycycline as shown in **Table 4**.

Table 4. Antibacterial Susceptibility for Gram Positive Isolates from Patients Who attended Hemodialysis at MNH from June through August 2019

		Coagulase negative Staphylococcus n (%)	Staphylococcus aureus n (%)
Ciprofloxacin	Sensitive Sensitive	9 (100.0) 3(33.3)	20 (100) 18(90.0)
Cefoxitin	Resistant Sensitive	6(66.7) 8(88.9)	2(10.0) 18(90.0)
Gentamycin	Resistant Sensitive	1(11.1) 8(88.9)	2(10.0) 17(85.0)
Doxycycline	Intermediate Resistant	0 (0) 1(11.1)	2(10.0) 1(5.0)
Trimethoprim-Sulfamethoxazole	Sensitive Resistant	3(33.3) 6(66.7)	10(50.0) 10(50.0)
Clindamycin	Intermediate Resistant Sensitive	1(11.1) 3(33.3) 8(88.9)	1(5.0) 7(35.0) 17(85.0)
Vancomycin	Intermediate Resistant Resistant	0 (0) 1 (11.1) 9 (100.0)	1(5.0) 2(10.0) 20 (100.0)
Erythromycin	Sensitive Resistant	0(0) 9 (100.0)	1(5.0) 19(95.0)

Antibacterial Susceptibility for Gram Negative Isolates from Patient attending HD at MNH

Of the gram-negative isolates, *Klebsiella spp*. showed high resistance among isolates tested for amikacin, ceftriaxone, and amoxicillin/clavulanic, while sensitive to ciprofloxacin, chloramphenicol, and meropenem. *E. coli* was resistant to gentamycin, ceftriaxone, amoxicillin/clavulanic while sensitive to ciprofloxacin, meropenem, trimethoprim-sulfamethoxazole, and chloramphenicol. *Pseudomonas* was resistant to gentamycin, amoxicillin/clavulanic, and ceftriaxone but sensitive to amikacin, ciprofloxacin, and meropenem as shown in **Table 5**.

Table 5. Antibacterial Susceptibility for Gram Negative Isolates from Patients Who attended Hemodialysis at MNH from June through August 2019

		Pseudomonas species	E. coli	Klebsiella
		Yes n (%)	Yes n (%)	Yes n (%)
Amikacin	Sensitive Intermediate Resistance	5(83.3) 0 (0) 1(16.7)	11(78.6) 1(7.1) 2(14.3)	1(20.0) 0 (0) 4(80.0)
Ciprofloxacin	Sensitive Resistance	6 (100.0) 0 (0)	14(100.0) 0 (0)	3(60.0) 2(40.0)
Imipenem	Sensitive Intermediate Resistance	4(66.7) 0 (0) 2(33.3)	12(85.7) 1(7.1) 1(7.1)	3(60.0) 0 (0) 2(40.0)
Meropenem	Sensitive Intermediate Resistance	5(83.3) 0 (0) 1(16.7)	12(85.7) 1(7.1) 1(7.1)	3(60.0) 0 (0) 2(40.0)
Gentamycin	Sensitive Intermediate Resistant	1(16.7) 0 (0) 5(83.3)	3(21.4) 2(14.3) 9(64.3)	0 (0) 0 (0) 5 (100.0)
Ceftriaxone	Sensitive Intermediate Resistant	1(16.7) 1(16.7) 4(66.7)	4(28.6) 0 (0) 10(71.4)	0 (0) 0 (0) 5 (100.0)
Chloram-phenicol	Sensitive Resistants	4(66.7) 2(33.3)	8(57.1) 6(42.9)	5 (100.0) 0 (0)
Amoxiclav	Resistant	6 (100.0)	14 (100.0)	5 (100.0)

Discussion

Bacterial infections are an essential cause of morbidity and mortality among patients with end-stage renal disease (ESRD). The need for routine hemodialysis is a risk factor for bacterial infection for patients with ESRD. Septicemia due to hemodialysis accounts for more than 75% of these infectious mortalities. Moreover, the presence of comorbidities like diabetes mellitus has been associated with an additional risk for sepsis-related mortality. HD is an infectious prone procedure that over time might introduce infection to the patient who initially had no prior infections.

Results from this study showed that the prevalence of infections during dialysis was found to be 29.9%. In terms of the prevalence of infections, the results of this study are slightly higher than the study reported in Ethiopia 19.7% [17]. Of those that were found to have bacterial infections due to HD, the most common bacteria to cause infections were found to be gram positive (56.2%). This result was observed by Suzuki et al. who reported a half to two-thirds of the causative agents of bacteremia in hemodialysis patients are from Gram-positive bacteria[18] This may be due to inappropriate use of antibiotic and the use of catheters, to improve this, strong infection control policies in haemodialysis canters should be instituted.

Out of the 56.2% of the gram positive isolates, uniquely 35.1%, were contributed by *Staphylococcus aureus* tailed by *coagulase negative staphylococcus*, 15.8% (**Figure 1**). This is relatively similar to the study reported by Bouza et al. [19] where the proportion of isolated gram-positive bacteria noted was 55.2% [19]. Although on the subject of specific isolate, the results of this study were somewhat low compared to *Staphylococcus aureus* (53%)[20] and somewhat high compared to *Staphylococcus aureus* (23.5%)[2]. This might be because it's a normal flora bacterium on human skin; moreover, different strains of the bacterial are equipped with adhesin that facilitates surface attachment. They also produce toxins as well as immune evasion molecules; that might expedite transmission and colonization in hemodialysis settings [13].

The potential reason for this slight difference in the proportion of specific isolates could be explained by

hospitalization criteria where, a study was done among only hospitalized patients [20]. The hospitalized patients had a high chance of adding infection as is evident from this study, though not statistically significant (p-value=0.15); among all hospitalized patients, 8/18 (44.4%) had infections compared to outpatients, 41/146 (28.1%) who were infected [20]. The risks related to nosocomial infections in admitted HD patients are associated with severity of the disease, nurse procedures on dressing manipulations, dressing type, co-morbid condition, recurrent use of antibacterial agents, and prolonged contact with health care setting[21,22].

On the other hand, this study results were supported by the study done in Malaysia where it was reported 44.4% of the patients on HD were found with gram negative infections[23]. Nevertheless, it considerably differed with another study in Algeria which found *Klebsiella pneumonia* (26.5%) as gram negative bacteria with a relatively high proportion linked with HD-related infections and 2.9% *E. coli* linked least[2].

Catheter-related infections are the major predisposing factors among patients on HD[24,25]. Older age and co-morbidities, diabetes in particular are independent risk factors for infections in patients who are on HD[12,26,27]. Given co-morbidity as an escalating factor for acquiring infection in HD, diabetes has been linked quite often tailed by hypertension. This scenario has become compatible with the results of this study, where diabetes has been more substantial than hypertension (**Figure 1**). Diabetic patients on HD are more vulnerable to infections because of the high level of blood sugar that enhances the growth of bacteria. Compared with other studies, Grothe and colleagues[28] reported hypertension and diabetes 22% and 37% respectively which is low compared to (31.5%) for hypertension and higher for diabetes (33.8%) of this study results.

Furthermore, age is another independent factor for HD's vulnerability to infection. Old age (persons who are over 60 years) is associated with deterioration of body immunity an increased chance of developing complications such as infections and thrombosis. The elderly tend to be immunologically incapacitated and often have co-existing chronic diseases such as diabetes and systemic hypertension[21]. Although in this study majority of the patients who got infection were below twenty years (75%). This could be explained by the sampling error since this study employed a convenience sampling technique, and this group comprise the least proportion of the study participants (**Table2**).

In addition to catheter related infections, vascular access during HD also triggers the infection. Vascular access can be a central venous catheter or arteriovenous fistula where central venous catheters (CVC) is associated with the potential incidence of infections[29,30]. With these in mind, these infections are critical for the hospitalization among patients on HD[31].

Several mechanisms suggest CVC be associated with a high rate of bacteremia and septicemia, including the provision of lower blood flow which may lead to low dialysis dose and hence infections[32]. According to reports [33], dialysis patients experience illnesses caused by retained waste solutes (uremia) that cannot be removed by dialysis as native kidney function can eliminate. Uremia is associated with impairing body defense mechanism (immunity) which increases the risk of bacterial infection[12]. Immunocompromised patients get a bacterial infection due to the immune system not being able to manage bacterial infection from a wound, gastrointestinal tract, or urinary tract[17]. These catheters related infections are suspected to develop from the bacterial biofilms on their inner surface as early as 24h after their placement. Catheter-related bacteremia often arises from these biofilms[34].

Biofilms formation involves extracellular polysaccharides produced by bacteria. In the presence of CVC, it is more likely to develop and can potentiate the pathogenicity of the skin bacteria flora like coagulase- negative staphylococci. The biofilms render the bacteria less susceptible to antimicrobial agents and enhance bacteria survival and antimicrobial resistance [12]. In this study, a total of 108 patients were on CVC, out of this 68.1% were infected while Native fistula total was 56 and 25% were culture positive. This proportion of the isolates in the fistula (arteriovenous) differs from that at CVC by 43.1%. These results are supported by what has been articulated in literature[10,18].

Vancomycin is an antibiotic that has an action on the majority of gram-positive bacteria[35]. *Coagulase negative staphylococci* and *Staphylococcus aureus* are the most reported resistant isolates to vancomycin[27]. Although this may be true, vancomycin has been over utilized in hemodialysis patients for more than 35 years [27] and is so likely to develop resistance. Although the pattern of susceptibility in this study varied from these two isolates still resistance of these isolates was evident. *Staphylococcus aureus resistance* to vancomycin was found to be 10% while *Coagulase negative staphylococcus* strain showed resistance to vancomycin of 11.1%. However, this trend of resistance among *Staphylococcus aureus* to vancomycin lies similar with studies done in Ethiopia [17] and Algeria[2].

Similarly, *Klebsiella spp.* showed high resistance to amikacin, Ceftriaxone, and amoxicillin/clavulanic acid, while sensitive to ciprofloxacin, ceftazidime, chloramphenicol, and meropenem. In Algeria, Sahli reported the *Klebsiella* isolates were resistant to gentamycin[2]. In this study, *Escherichia coli* were resistant to gentamycin, ceftriaxone, and amoxicillin/clavulanic acid, while sensitive to ciprofloxacin, meropenem, trimethoprim-sulfamethoxazole, and chloramphenicol. These bacterial isolates resistance pattern, although shows predictable trend sometimes disagrees across the literature. This variation is due to disparity and prolonged use of drugs in different HD settings following local guidelines; however, WHO encourages the use of universal guidelines to local context and experience.

Strengths and limitations of this study

- This study provided a real understanding into prevalence

of bacterial infection and antimicrobial susceptibility pattern among patients attending hemodialysis (HD) in Muhimbili National Hospital.

- The sample size in the cross-sectional study was large enough to provide correlation of the results to the larger number of patients undergoing HD and generalization of the findings.
- The study was done in a hospital setting, which may affect the generalization of the data to the entire patients undergoing HD in the country.
- A wider study in a different hospitals setting in Tanzania/ East Africa region would be better to accurately describe and validate the results in this study.

Conclusion

The current study observed a high prevalence of infection in admitted patients compared to outpatient. Gram positive bacteria remain frequent isolates which cause infection in dialysis patients, and *Staphylococcus aureus* alone contributed high percentage than other gram positives. The most sensitive drugs for gram positive isolates were vancomycin, ciprofloxacin, and cefoxitin, while for gram negative bacteria were ciprofloxacin, chloramphenicol, and meropenem. The study also revealed high resistance of isolates when tested to gentamycin, amoxicillin/clavulanic acid, ceftriaxone, erythromycin, and penicillin. We noted that these antibiotics are still widely used and put the greater risk of persistence of infection and antimicrobial resistance.

Ethical statement

Directorate of Research and Publications (MUHAS), and Institutional Review Board and Department of Teaching, Research and Consultancy of Muhimbili National Hospital with approval number MNH/TRC/Permission/2019/085 on 14th June 2019.

Competing Interests

The authors declare no conflict of interest.

Authors' Contributions

George Musiba; conception and designed the study, data acquisition, analysis and interpretation, drafting the first draft of the manuscript and revision. Raphael Z. Sangeda; conception and design of the study, analysis, data interpretation, and revised the manuscript. Calvin A. Omolo; ideate and designed the study, analyzed, interpreted data, and revision of the manuscript. Omary Minzi; design of the study, interpretation of data, and supervision. George Bwire; design of the study and data interpretation. Paschal Ruggajo; study design, interpretation of data, and manuscript revision. Rajabu M. Kingo; a collection of data, data interpretation, and revised the manuscript. Doris Chuwa; data collection and revision of the manuscript. Lawrence Museru; provided the resources, data interpretation, and revision of the manuscript.

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Management of Peptic Ulcers and Treatment Outcomes Following Withdrawal of Ranitidine in Hospitalized Patients at Thika Level V Hospital

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Abstract

Introduction: Peptic ulcer disease is the discontinuity in gastric mucosa with inflammation. Management involves the use of acid-suppressive medications alone or in combination therapy with antibiotics. Ranitidine, a H_2 receptor antagonist used for acid suppressive therapy, was withdrawn from the market as of April 2020 following its association with increasing levels of N-methyl-D-aspartate, a potent carcinogen.

Objectives: To study the changes in management of peptic ulcers, length of hospitalization and adverse events in hospitalized patients with peptic ulcer disease at Thika Level V Hospital following the withdrawal of ranitidine. The socio-demographic factors of patients that required hospitalization were also evaluated.

Methodology: A retrospective hospital-based comparative pre-post study using files of patients hospitalized with peptic ulcer diagnosis at Thika Level V Hospital from June 2019 to February 2021. A data collection sheet was used for data collection. Data was entered into Microsoft excel 2013. Statistical Package for the Social Sciences software version 20 was used for data analysis.

Results: One hundred and three (103) files of hospitalized peptic ulcer patients were sampled. Males had a higher incidence of peptic ulcers (63 patients, 61.2%). Blue-collar workers had a higher incidence of peptic ulcers (44 patients, 42.7%). After ranitidine withdrawal there was predominant use of high dose PPI therapy (23 patients, 57.5%). Similarly, there was an increase in diarrhea (28 cases, 82.4%) with most of the cases being associated with high dose PPI therapy (22 cases, 78.6%). PPI + Metronidazole dual therapy was a popular regimen both before and after ranitidine withdrawal with the patients having an average hospital stay of 16 days with a standard deviation of ±7.1. Triple therapy of choice after ranitidine withdrawal was PPI + Levofloxacin + Amoxicillin with the patients having an average hospital stay of 6 days with a standard deviation of ±3.2.

Conclusion: After Ranitidine withdrawal, there was increased use of high dose PPI therapy which was associated with increased diarrhea cases. The use of PPI + Levofloxacin + Amoxicillin was a comparative alternative to Ranitidine + Amoxicillin + Metronidazole. Most patients on PPI + metronidazole dual therapy had a prolonged hospital stay.

Keywords: *Peptic ulcers, Proton Pump Inhibitors, Ranitidine withdrawal.*

Introduction

The effective management of Peptic Ulcer Disease (PUD) has been realized over the past years owing to the efficacy of the various drug therapies advanced for its eradication. The general improvement of socioeconomic status amongst individuals has also contributed to the decline in the prevalence of PUD [1]. Ranitidine, a H_2 receptor antagonist, was commonly used as an acid suppressive therapy for the management of PUD due to its efficacy in inhibiting production of gastric acid by about 70% for 24 hours as well as its efficacy in inhibiting nocturnal and basal acid production [2]. However, it was withdrawn from the market as of April 2020 for the safety of patients following its association with increasing levels of N-methyl-D-aspartate (NMDA), a potent carcinogen [3]. This withdrawal of Ranitidine forced both patients and prescribers to shift their preference to alternative acid-suppressive medication especially the Proton Pump Inhibitors (PPIs) [4].

However, prior to the withdrawal of ranitidine, the PPIs had been listed in the FDA Adverse Event Reporting System (October - December 2019 issue) [5]. This is after their long term therapy was associated with rising concerns of adverse effects such as pseudomembranous colitis [6], gastric and esophageal cancer [7], and increased risk of chronic kidney disease [8]. A correlation has also been shown between PPI use and a fatal course of COVID-19 [9].

These developments coupled with the worldwide increase in antimicrobial resistance to *Helicobacter pylori,* which threatens human health since the efficacy of such antibiotics is lowered [10], led to the rise in advocacy for appropriate use and stewardship of PPIs and antibiotics. This has created a need for more information on the use and safety of agents used in PUD treatment in order to advance strategies to improve on the general outcome of PUD treatment.

Objectives

The main objective of this study was to determine the changes in management of peptic ulcers, length of

hospitalization and adverse events in hospitalized patients with peptic ulcer disease at Thika Level V Hospital (TL5H) following the withdrawal of ranitidine. The socio-demographic factors of patients that required hospitalization were also evaluated.

Methodology

Study design, site, and study population

This study was done at the Thika Level V Hospital (TL5H). It is a County referral hospital located in Kiambu County, Kenya that serves approximately five million residents, including those from Nairobi, Kirinyaga, Murang'a, and Machakos counties. The study employed a retrospective hospital-based comparative pre-post study design where files of hospitalized patients diagnosed with PUD at TL5H for the period June 2019 to February 2021 were thoroughly scrutinized.

Sample size determination

All files (103) of the hospitalized patients diagnosed with PUD at TL5H for the period June 2019 to February 2021 were included in the study. This was arrived at after a reconnaissance at TL5H indicated 103 files of hospitalized patients diagnosed with PUD were available for the period June 2019 to February 2021.

Inclusion and exclusion criteria

All files of hospitalized patients diagnosed with PUD at TL5H for the period June 2019 to February 2021 were included in the study. Files of hospitalized patients without a diagnosis of PUD were excluded from the study.

Data Collection, Management, and Analysis

Data from the patient files was recorded using a data collection sheet aligned with the objectives of this study. The data was then entered into Microsoft Excel 2013. This was password protected for data protection. Data analysis was done using SPSS version 20 software. Categorical variables were summarized as descriptive statistics of frequencies and percentages. Numeric variables were summarized as means and standard deviation of the mean. The data files were synchronized to google drive for automatic backing up of the data.

Ethical consideration

Ethical approval was granted by Jomo Kenyatta University of Agriculture and Technology (JKUAT) Ethical Review Committee (Approval No. JKU/IERC/02316/0089). Permission for data collection was granted by TL5H (Ref; MOH/TKA/GEN/VOL. V/271). All the data from the files was coded to ensure patient confidentiality.

Results

Socio-demographic Factors of patients presenting at TL5H with PUD

Incidence, gender, and age

Of the 103 files of PUD patients reviewed, 63 (61.2%) were male and the remaining cases, (40, 38.8%) were female. This gives a ratio of 1.58:1 (male to female). The highest incidence of PUD was in the age group of between 36 to 50 years. This age group presented with 39 patients (37.9%) (**Table 1**).

Table 1. Age distribution of PUD cases

Age distribution	Frequency	Percent
Below 18 yrs.	12	11.7
18 - 35 yrs.	28	27.2
36 - 50 yrs.	39	37.9
Above 50 yrs.	24	22.3
Total	**103**	**100.0**

Incidence of PUD relative to occupation

Diverse occupations were seen amongst the PUD patients presenting at TL5H. Of these, 44 (42.7%) patients had blue-collar jobs. The least frequency was seen among pink-collar workers (2, 1.9%). The occupation status of 33 patients (32.0%) was unknown (**Figure 1**). A sub-group analysis of blue-collar jobs showed farmers had a higher incidence of PUD (24 patients, 54.5%) while security workers had the least incidence with 2 patients (4.5%) (**Table 2**).

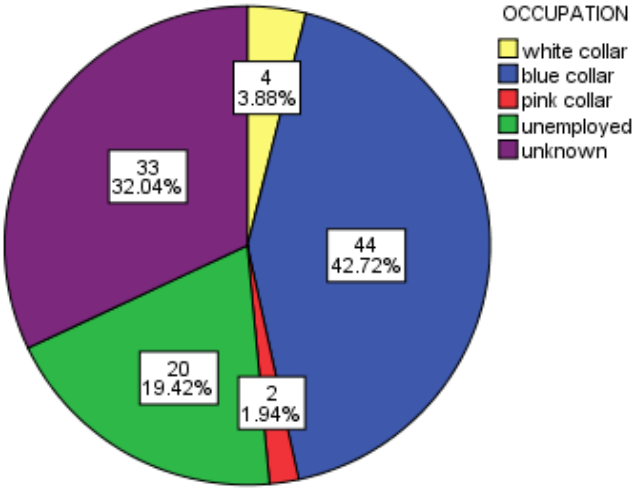

Figure 1. Occupation of patients presenting with PUD at TL5H.

Table 2. Frequency of PUD amongst blue-collar workers

Blue-collar job	Frequency	Percent
Factory worker	4	9.1
Farmer	24	54.5
Mason	6	13.7
Machine operator	8	18.2
Security	2	4.5
Total	**44**	**100.0**

Drug regimens

Various drug regimens were used in PUD management at TL5H. Before ranitidine withdrawal, the monotherapy of choice was ranitidine with a frequency of 18 cases (30.5%). After ranitidine withdrawal (April 2020), PPIs were the preferred monotherapy presenting with 12 cases (27.3%). Before ranitidine withdrawal, the popular triple therapy was Ranitidine + Amoxicillin + Metronidazole with a frequency

of 9 cases (15.3%). After ranitidine withdrawal, PPI + Levofloxacin + Amoxicillin was popular presenting with 12 cases (27.3%). Quadruple therapy of choice before ranitidine withdrawal was Ranitidine + Amoxicillin + Metronidazole + Clarithromycin with a frequency of 4 cases (6.8%) whereas after ranitidine withdrawal, PPI + Amoxicillin + Metronidazole + Clarithromycin was preferred (4 cases, 9.1%). PPI + Metronidazole dual therapy had a relatively high frequency of use both before and after ranitidine withdrawal with 6 cases (10.2%) and 8 cases (18.2%) respectively (**Table 3**).

Table 3. Drug Regimens used in PUD management.

Regimen	Number of Patients Per Period		Entire Study Period
	Before ranitidine withdrawal	After ranitidine withdrawal	
PPI alone	8 (13.5%)	12 (27.3%)	20 (19.4%)
PPI + Metronidazole	6 (10.2%)	8 (18.2%)	14 (13.6%)
PPI + Levofloxacin + Amox	0	12 (27.3%)	12 (11.7%)
PPI + Amox + Clari	0	2 (4.5%)	2 (1.9%)
PPI + Amox + Metro	0	2 (4.5%)	2 (1.9%)
PPI + Amox + Metro + Clari	2 (3.4%)	4 (9.1%)	6 (5.8%)
Ranitidine	18 (30.5%)	0	18 (17.5%)
Ranitidine + Amox + Metro	9 (15.3%)	0	9 (8.8%)
Ranitidine + Amox + Metro + Clari	4 (6.8%)	0	4 (3.9%)
Metronidazole	10 (16.9%)	3 (6.8%)	13 (12.6%)
Other	2 (3.4%)	1 (2.3%)	3 (2.9%)
Total	**59** (100%)	**44** (100%)	**103** (100%)

Before ranitidine withdrawal (June 2019 to April 2020), After ranitidine withdrawal (May 2020 to February 2021)

key: Amox = Amoxicillin, Metro = Metronidazole, Clari = Clarithromycin, Other = treatment with IV fluids and analgesics alone with discharge within 1 week.

For the entire period of the study 56 patients (54.3%) patients had been initiated on PPIs, 31 patients (30.1%) were on ranitidine while 16 patients (15.6%) were not given any acid-suppressive medication. On dividing the study into 2 periods that is before ranitidine withdrawal (April 2020) which had 59 patients and after ranitidine withdrawal where 44 cases were present, Ranitidine was predominantly used before its withdrawal (31 patients, 52.5%) and after its withdrawal, there was an increase in the use of PPIs for acid suppression (40 patients, 90.9%) (**Figure 2**).

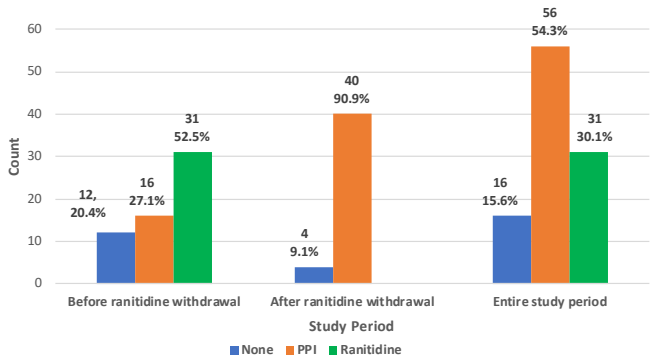

Figure 2. Acid suppressive therapy used in PUD management

A comparison of the dose of PPI used before and after ranitidine withdrawal showed standard dose PPI therapy (40 mg daily) was predominantly used before ranitidine withdrawal (11 patients, 68.8%). However, after ranitidine withdrawal there was a shift of preference to high dose PPI therapy (23 patients, 57.5%) (**Table 4**).

Table 4. Comparison of PPI dose use before and after ranitidine withdrawal.

PPI dose	Number of Patients Per Period	
	Before ranitidine withdrawal	After ranitidine withdrawal
High dose therapy	5 (31.2%)	23 (57.5%)
Standard dose therapy	11 (68.8%)	17 (42.5%)
Total	**16** (100.0%)	**40** (100.0%)

Standard dose = 40mg per day
High dose = 80mg per day

Comparison of the Length of hospitalization of patients on various treatment regimens

For the entire study period, PUD patients on PPI + Metronidazole dual therapy had an average hospital stay of 16 days with a standard deviation of ±7.1. Most of the patients had a hospital stay of 3 weeks (7 patients, 50.0%). The average hospital stay for this regimen before ranitidine withdrawal was 18 days with a standard deviation of ±7.2 and after ranitidine withdrawal it was 15 days with a standard deviation of ±5.3. The average hospital stay for patients on Ranitidine + Amoxicillin + Metronidazole was 12 days with a standard deviation of ± 6.5. Most patients had a hospital stay of 2 weeks (5 patients, 55.6%). The average hospital stay for patients on PPI + Levofloxacin + Amoxicillin triple therapy was 6 days with a standard deviation of ±3.2. Most patients had a hospital stay of 1 week (9 patients, 75.0%). The average hospital stay for patients on Ranitidine + Amoxicillin + Metronidazole + Clarithromycin was 8 days with a standard deviation of ±4.1. After ranitidine withdrawal, the average hospital stay for patients on PPI + Amoxicillin + Metronidazole + Clarithromycin was 11 days (**Table 5**).

The PUD patients hospitalized before ranitidine withdrawal had an average hospital stay of 9 days with a standard deviation of ±6.4. After ranitidine withdrawal the average hospital stay for the PUD patients was 9 days with a standard deviation of ±5.4. The average hospital stay of PUD patients for the entire period of study was 10 days with a standard deviation of ±6.0 (**Table 5** - Next Page).

Table 5. Different drug regimens in relation to hospital stay of PUD patients

Period	Regimen	Hospital stay						Total
		Mean (days)	S.d	Number of Patients Per Week				
				week 1	week 2	week 3	week 4	
Before Ranitidine withdrawal	PPI alone	15	5.2	0	4(50%)	3(37.5%)	1(12.5%)	8(100%)
	PPI + Metronidazole	18	7.2	0	2(33.3%)	2(33.3%)	2(33.3%)	6(100%)
	PPI + Amox + Metro + Clari	11	0	0	2(100%)	0	0	2(100%)
	Ranitidine	8	4.9	10(55.6%)	6(33.3%)	2(11.1%)	0	18(100%)
	Ranitidine + Amox + Metro	12	6.5	2(22.2%)	5(55.6%)	1(11.1%)	1(11.1%)	9(100%)
	Ranitidine + Amox + Metro + Clari	8	4.1	2(50%)	2(50%)	0	0	4(100%)
	Metro	4	0	10(100%)	0	0	0	10(100%)
	Other	4	0	2(100%)	0	0	0	2(100%)
	Total	**9**	**6.4**	**26(44.1%)**	**21(35.6%)**	**8(13.5%)**	**4(6.8%)**	**59(100%**
After Ranitidine withdrawal	PPI alone	9	6.0	6(50%)	3(25%)	3(25%)	0	12(100%)
	PPI + Metronidazole	15	5.3	1(12.5%)	2(25%))	5(62.5%)	0	8(100%)
	PPI + Levofloxacin + Amoxicillin	6	3.2	9(75%)	3(25%)	0	0	12(100%)
	PPI + Amox + Clari	4	0	2(100%)	0	0	0	2(100%)
	PPI + Amox + Metro	11	0	0	2(100%)	0	0	2(100%)
	PPI + Amox + Metro + Clari	11	0	0	4(100%)	0	0	4(100%)
	Metro	4	0	3(100%)	0	0	0	3(100%)
	Other	4	0	1(100%)	0	0	0	1(100%)
	Total	**9**	**5.4**	**22(50.0%)**	**14(31.8%)**	**8(18.2%)**	**0**	**44(100%**
Entire Study Period	PPI alone	12	6.4	6(30%)	7(35%)	6(30%)	1(5%)	20(100%)
	PPI + Metronidazole	16	7.1	1(7.1%)	4(28.6%)	7(50%)	2(14.3%)	14(100%)
	PPI + Levofloxacin + Amoxicillin	6	3.2	9 (75%)	3 (25%)	0	0	12(100%)
	PPI + Amox + Clari	4	0	2(100%)	0	0	0	2(100%)
	PPI + Amox + Metro	11	0	0	2(100%)	0	0	2(100%)
	PPI + Amox + Metro + Clari	11	0	0	6(100%)	0	0	6(100%)
	Ranitidine	8	4.9	10(55.6%)	6(33.3%)	2(11.1%)	0	18(100%)
	Ranitidine + Amox + Metro	12	6.5	2(22.2%)	5(55.6%)	1(11.1%)	1(11.1%)	9(100%)
	Ranitidine + Amox + Metro + Clari	8	4.1	2(50%)	2(50%)	0	0	4(100%)
	Metro	4	0	13(100%)	0	0	0	13(100%)
	Other	4	0	3(100%)	0	0	0	3(100%)
	Total	**10**	**6.0**	**48(46.6%)**	**35(34.0%)**	**16(15.5%**	**4(3.9%)**	**103**

Before ranitidine withdrawal (June 2019 to April 2020), After ranitidine withdrawal (May 2020 to February 2021)

S.d = Standard deviation
Key: Amox = Amoxicillin, Metro = Metronidazole, Clari = Clarithromycin, Other = treatment with IV fluids and analgesics alone with discharge within 1 week.

After Ranitidine withdrawal, the average hospital stay for PUD patients on high dose PPI therapy was 13 days with a standard deviation of ± 5.8. During this period, the average hospital stay for patients on standard dose PPI therapy was 7 days with a standard deviation of ± 4.3 (**Table 6**).

Table 6. PPI dose in relation to hospital stay of PUD patients

Period	PPI dose	Hospital stay						Total
		Mean (days)	S.d	Number of Patients Per Week				
				week 1	week 2	week 3	week 4	
Before Ranitidine withdrawal	High dose therapy	13	3.2	0	4(80.0%)	1(20.0%)	0	5(100%)
	standard dose ther-apy	14	7.3	2(18.2%)	4 (36.4%)	3(27.2%)	2(18.2%)	11(100%)
	Total	**14**	**6.2**	**2(12.5%)**	**8(50.0%)**	**4(25.0%)**	**2(12.5%)**	**16(100%)**
After Ranitidine withdrawal	High dose therapy	13	5.8	5(21.7%)	9(39.1%)	8(34.8%)	**1(4.4%)**	23(100%)
	standard dose ther-apy	7	4.3	11(64.7%)	5(29.4%)	1(5.9%)	**0**	17(100%)
	Total	**10**	**5.9**	**16(40.0%)**	**14(35.0%)**	**9(22.5%)**	**1(2.5%)**	**40(100%)**
Entire Study Period	High dose therapy	13	5.5	5 (17.9%)	13(46.4%)	9(32.1%)	1(3.6%)	28(100%)
	standard dose ther-apy	10	6.6	13(46.4%)	9 (32.1%)	4(14.3%)	2(7.1%)	28(100%)
	Total	**11**	**6.2**	**18(32.1%)**	**22(39.3%)**	**13(23.2%)**	**3(5.4%)**	**56(100%)**

Standard dose = 40mg per day
High dose = 80mg per day
S.d = Standard deviation

Gastrointestinal side effects

A total of 34 diarrhea cases were recorded amongst the PUD patients presenting at TL5H. Before ranitidine withdrawal, 6 patients (17.6%) presented with diarrhea. However, after ranitidine withdrawal, PUD patients presenting with diarrhea increased to 28 cases (82.4%). On comparing diarrhea cases relative to PPI dose, high dose PPI therapy presented higher diarrhea cases (23 patients, 67.6%) than standard-dose therapy (9 patients, 26.5%) for the entire study period. After ranitidine withdrawal high dose PPI therapy was associated with most of the diarrhea cases (22 patients, 78.6%) (**Table 7**)

Table 7. Frequency of diarrhea cases relative to PPI dose.

Period	PPI dose	Number of Patients with Diarrhea
Before ranitidine withdrawal	high dose therapy standard dose therapy none **Total**	1 (16.7%) 3 (50.0%) 2 (33.3%) **6 (100%)**
After ranitidine withdrawal	high dose therapy standard dose therapy none **Total**	22 (78.6%) 6 (21.4%) 0 **28 (100%)**
Entire Study Period	high dose therapy standard dose therapy none **Total**	23 (67.6%) 9 (26.5%) 2 (5.9%) **34 (100%)**

Discussion

Socio-demographic factors and risk of PUD

Males had a higher risk of PUD than females (ratio 1.58:1). This finding is similar to a study done by Anu Realo from the Estonian Biobank cohort where the PUD male to female ratio was 1.62:1 [11]. However, these results are in contrary to a recent study in the USA by BS Anand, a member of the American Gastroenterological Association, which suggested a shift of PUD prevalence from male predominance to similar occurrences in males and females [12]. These suggest regional differences in gender susceptibility to PUD between developed and developing countries where in developing countries (Kenya and Estonia) males have a higher incidence of PUD while in developed countries (USA) both genders have similar PUD occurrence. Hence, the need to conduct local investigations to improve health care strategies on the susceptible gender.

This study showed PUD was widely distributed across the various age groups, nevertheless, patients between ages 36 to 50 years presented the higher PUD risk while those below 18 years had a lower risk. A study done in Harare, Zimbabwe showed an increase in PUD prevalence with age with children < 5 years having a low PUD risk and adults aged between 35 and 39 years having a higher risk [13]. A similar study done at Mbagathi Hospital, Kenya showed patients of age group between 31 to 40 years had an increased risk of *H. pylori* infection [14]. The low risk for patients below 18 years could be as a result of limited exposure to sources of *H. pylori* as suggested by a study in South western Uganda that showed similar trends amongst children 1 – 15 years [15].

The incidence of PUD was more common amongst individuals of blue-collar jobs. These findings are similar to those of a study done in Korea that also showed blue-collar workers had a higher PUD incidence. The study in Korea attributed this to working-environment factors such as inadequate cleanliness, danger involved, and loading of heavy items [16]. A subgroup analysis of blue-collar jobs showed farmers presented with higher PUD cases. These findings are consistent with a previous literature that reported an association between farming occupation and *H. pylori* infection among dyspeptic patients in Ghana [17]. An identical study conducted in Egypt. found that farmers acquired *H. pylori* infection more significantly than non-farmers. It further suggested potential zoonotic transmission of *H. pylori* especially for dairy farmers as well as inadequate living resources and poor sanitation as the possible predisposing factors [18].

Drug regimens and their effects on PUD management outcomes

Proton pump inhibitor + Metronidazole dual therapy was a popular regimen both before and after ranitidine withdrawal. The average hospital stay for patients on this regimen was 16 days with most of the patients having a hospital stay of 3 weeks. This prolonged hospital stay could be an indicator of poor prognosis of PUD due to the low efficacy of the dual therapy as reported by previous literature that attributed the low efficacy to increasing resistance by *H. pylori* [19,20]. In addition, dual therapy use in PUD management has been highly discouraged, with only high dose PPI + amoxicillin dual therapy having a conditional recommendation in ACG guidelines as salvage therapy [21]. These recommendations arose after studies suggested that high dose PPI + amoxicillin is the only dual therapy that is as efficacious as standard triple therapy for *H. pylori* eradication [22-24]. Hence, the high frequency of use of PPI + Metronidazole dual therapy at TL5H remains controversial.

Before ranitidine withdrawal, Ranitidine + Amoxicillin + Metronidazole was the triple therapy of choice, with patients having an average hospital stay of 12 days. Previous literature has shown high *H. pylori* eradication rates achieved by this regimen [25]. Others have shown similar efficacy as PPI + Amoxicillin + Metronidazole regimen with variations occurring only in Metronidazole resistant patients where the later regimen is more efficacious [26,27]. After ranitidine withdrawal, PPI + Levofloxacin + Amoxicillin triple therapy was the most prescribed. Patients receiving it had an average hospital stay of 6 days, which indicates a better prognosis of PUD than the case of Ranitidine + Amoxicillin + Metronidazole. This levofloxacin-based triple is an ACG-recommended first-line therapy for *H. pylori* eradication [21]. Various reports exist on its efficacy as compared to standard clarithromycin-based triple therapy. The majority of the reports show equivalence in efficacy and tolerability of these two regimens [21,28-30], whereas some show its higher efficacy than the standard triple therapy [31].

However, another study showed the supremacy of standard triple therapy over levofloxacin triple therapy in Asian countries [32]. In this current study, a few patients received the clarithromycin triple therapy, which was not significant for comparison with levofloxacin triple therapy. Nevertheless, the shorter average hospital stay of patients on levofloxacin triple therapy, in addition to evidence from previous studies on its efficacy, supports the use of this therapy as a comparable alternative to Ranitidine + Amoxicillin + Metronidazole.

A significant number of PUD patients were on metronidazole monotherapy with all having a hospital stay of 4 days both before and after ranitidine withdrawal. Although metronidazole monotherapy has been shown to be effective in the treatment of mild Clostridium difficile diarrhea [33], none of the patients in this study was recorded to have had diarrhea. Since various studies have discouraged the use of metronidazole monotherapy for *H. pylori* eradication due to a rise in microbial resistance [21], and the files of PUD patients at TL5H had insufficient information to justify this prescription, the choice of metronidazole monotherapy for PUD management at TL5H remains debatable.

After ranitidine withdrawal, the use of high-dose PPI therapy at TL5H increased to a level that surpasses the use of standard-dose PPI therapy. However, the average hospital stay for the PUD patients on high dose PPI therapy was 13 days whereas the average hospital stay for patients on standard dose PPI therapy was 7 days. This indicates a better prognosis for patients on standard dose PPI therapy. Previous studies have shown standard dose PPI therapy to be as effective as a high-dose regimen in PUD management, especially in reducing the risk of rebleeding [34,35]. However, this is still debated as other studies have shown standard-dose to be inferior to high-dose therapy in preventing rebleeding events after endoscopic surgery in PUD patients [36]. With one of the PPI deprescribing strategies aimed at reducing the risk of adverse effects being the use of standard-dose therapy [37], the predominant use of high dose PPI therapy at TL5H after ranitidine withdrawal is debatable, especially since only 8 patients had endoscopic surgery after ranitidine withdrawal at TL5H. A significant number of the hospitalized patients had not been given acid-suppressive medications these were either on metronidazole alone or they were initiated on analgesics and IV fluids. The IV fluids were given as perioperative fluid therapy for endoscopic procedures.

There was an increase in diarrhea cases after ranitidine withdrawal with most of the cases being associated with high dose PPI therapy. Previous studies have shown high dose PPI therapy, rather than standard-dose PPI therapy, to be associated with increased risk of various PPI adverse effects [38-40]. Amongst these adverse effects, *Clostridium difficile* diarrhea has been reported to be common in most cases of PPI use [41-43]. Hence the spike in diarrheas cases associated with high dose PPI therapy is a potential alarm of *Clostridium difficile* diarrhea adverse effect.

A significant limitation of this study was that the etiology of PUD at TL5H was not defined as to whether it was NSAID-induced, stress induced or H. pylori-associated. In addition, no culture was done to confirm the etiology of the diarrhea cases.

Conclusion

The male gender, age group 36 to 50 years, blue-collar jobs, and farmers were associated with a high risk of developing peptic ulcers. After Ranitidine withdrawal, there was increased use of high dose PPI therapy which was associated with increased diarrhea cases. The use of PPI + Levofloxacin + Amoxicillin was a comparative alternative to Ranitidine + Amoxicillin + Metronidazole. Most patients on PPI + metronidazole therapy had a prolonged hospital stay.

Recommendations

Health care strategies aimed at lowering the incidence of PUD in males as well as farmers should be initiated. Use of PPI + metronidazole dual-based therapy for *H. pylori* eradication should be discouraged. Alternatively, the use of triple and quadruple-based therapy for *H. pylori* eradication should be investigated. High-dose PPI therapy deprescribing should be advocated.

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Need for action on tramadol by pharmacists

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Tramadol is an opioid analgesic prescribed for the management of moderate to severe pain in various conditions including cancer and osteoarthritis due to its reduced risk for respiratory depression as well as addiction compared to other opioids [1]. Tramadol possesses a dual mechanism of pain relief conferred by its 2 enantiomers. First is (+)-tramadol and its O-desmethyltramadol (M1) metabolite which alter nociceptive neurotransmitter release through their selective agonist action on mu-receptors. (+)-tramadol is also a serotonin reuptake inhibitor. The second enantiomer is (-)-tramadol which inhibits norepinephrine reuptake and intensifies its release by activating the auto receptor [2,3]. Tramadol is administered orally or rectally as a sustained release formulation, and in solution for IV/IM administration. Tramadol has a half-life of 5 - 6 hours while the M1 metabolite has a half-life of 8 hours [1]. Tramadol has also shown significant benefits in the management of Covid-19 [4].

In 2017, United Nations Office on Drugs and Crime sounded an alarm on the increase in non-medical use of tramadol in Central, North and West Africa as well as the Middle East region [5]. In 2019, the WHO Expert Committee on Drug Dependence in its 41st report. issued an alert highlighting the worldwide increase in cases of tramadol abuse especially in low to middle income countries [6]. According to a study that utilized the French Addicto-vigilance network to analyze data from 2013 to 2018, there was an increase in problematic use of Tramadol in terms of high rate of dependence, increased non-medical use, and high death rates compared to other opioid analgesics [7]. A 2018 study in Nigeria revealed that 4.6 million people practiced non-medical use of opioids especially tramadol [8]. In Kenya, a study done in 2020 covering 18 counties highlighted a significant non-medical use of tramadol amongst other prescription drugs [9].

A number of negative health implications have also been associated with tramadol use despite its efficacy in pain management. Literature has shown Tramadol to be associated with increased risk of hip fractures, venous thromboembolism in osteoarthritis, and all-cause mortality when compared to commonly used NSAIDs [10–12]. A study comparing tramadol and codeine have shown that tramadol has an increased risk of fractures, cardiovascular events, and all-cause mortality [13]. Tramadol has also been shown to be associated with increased risk of severe hyponatremia [14], bleeding peptic ulcer [15], hypoglycemia [16,17], in vitro hepatotoxicity [18], gonadotoxic effect [19], acute kidney disease and rhabdomyolysis [20].

A research done in Kenya highlighted unethical health care providers and unethical persons managing pharmacies as key contributors towards non-medical use of prescription drugs [9]. Hence, such drugs are easily and readily accessible to individuals for non-medical use. This reveals a gap in the pharmacy practice suggesting nonadherence to the code of ethics for pharmacists and the Pharmacy and Poisons Act (CAP 244) of the constitution on Kenya. A Pharmacist in a clinical setting should advocate for and collaborate with prescribers to ensure tramadol is only prescribed where the pain is severe enough to require it, alternate regimens are not tolerated or are inadequate for the pain. The benefits of using tramadol should outweigh the associated risk. In addition, injectable tramadol formulations should be administered at the point of dispensing in the outpatient setting.

Pharmacists in manufacturing and research should develop more abuse deterrent formulations of tramadol. These would include incorporation of physical or chemical barriers that prevent crushing, grinding or mechanical manipulation of formulations. Development of delivery systems that confer resistance to abuse such as the tramadol ER. Pharmacists should also create awareness about abuse deterrent formulations in outpatient settings.

To prevent adverse drug effects, enhance safety and efficacy of tramadol, Pharmacists in clinical and community settings should provide medication reconciliation for tramadol by collecting its accurate medication history, verifying the prescribed doses in terms of its frequency, dose strength and compatibility with other prescribed drugs. Also, documentation of any changes to initial prescription is key. Pharmacist should also provide medication therapy management as well as pharmacovigilance activities around tramadol use.

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