KWAME NKRUMAH UNIVERISTY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

PREVALENCE OF COMMON BACTERIA ISOLATES AND THEIR SUSCEPTIBILITY TO ANTIBIOTICS IN MALNOURISHED CHILDREN UPTO 5 YEARS ADMITTED AT THE MATERNAL AND CHILD HEALTH HOSPITAL IN KEJETIA, KUMASI, GHANA

A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN MICROBIOLOGY

By

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SEPTEMBER, 2018

DECLARATION

I declare that this thesis is an original result from my personal effort. With the exception of other literally works of scholars duly being acknowledged, this thesis is the result of my own study done in KNUST under the supervision of Mr. John L. Terlabie.

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DEDICATION

This write up is dedicated to the Osei Young family and Enoch Osei Kuffour for their unfailing love and assistance, support and prayers throughout my two years of study and to the success of this work. I also dedicate this work to the staff of the laboratory unit of the Maternal and Child Health Hospital for their assistance and support. God richly bless you all.

ACKNOWLEDGEMENT

I personally wish to express my profound appreciation to the various persons who have contributed in diverse ways to make this study a success. I am personally indebted to God, for his faithfulness and grace given me to complete this study. I express my gratitude to my supervisor Mr. John L. Terlabie for the demonstration of patience in correcting my mistakes and offering invaluable advice. I remain grateful to Mr. Lawrence A. Adetunde of the University for Development Studies for his immense support. The staff of Department of Child Health, Nutrition Unit and Laboratory Unit of the Maternal and Child Health deserve commendation for their support during the sampling and data gathering phase of the study.

ABSTRACT

Malnutrition in children is the outcome of factors that are concerned poor food quality, insufficient food intake and recurring of infectious diseases. World Health Organization in 2011 estimated that 178 million children were stunted and 115 million children were underweight. Heikki in (2001) stated that the incidence and impact of life threatening bacterial infections in children across Africa have not been quantified and these bacterial infections can result in severe malnutrition.

The study was to determine the prevalence of common bacteria isolates in malnourished children who are upto 5 years admitted at the Maternal and Child Health Hospital. Samples of urine, blood and stool were taken from 200 malnourished children alongside with administration of questionnaires. Samples were subjected to laboratory analysis such as culture and sensitivity and biochemical test for identification of bacteria. Out of 200 malnourished children, severe acute malnutrition (SAM) was found in 71.1% and 27.7% were moderately malnourished. Children (65.0%) were marasmic and 35.0% suffered from kwashiorkor. Of the malnourished children, who 63.5% had diarrhoea, 49.5% presented with vomiting and 40.5% presented with fever. Bacteria isolated from urine were *Klebsiella* sp (43.3%), *Escherichia coli* (30.0%), *Pseudomonas* sp (13.3%), *Salmonella* sp (6.7%), *Enterobacter* sp (3.3%) and *Proteus* sp (3.3%). For blood samples, *Staphylococcus aureus* (53.6%) was the highest isolate, followed by *Streptococcus* sp (25.0%). Out of 38 stool samples, *Escherichia coli* (42.1%) was the highest isolated species followed by *Klebsiella* sp (21.1%) and *Proteus sp* (18.4%). *Escherichia coli*, *Proteus* sp, *Salmonella* sp and *Enterobacter* sp were resistant to 100% Ceftriaxone. *Pseudomonas* sp and *Salmonella* sp were resistant to 100% Ampicillin. *Staphylococcus aureus* (100%) was resistant to Ampicillin, Gentamicin and Cefuroxime. *Streptococcus* sp (100%) was susceptible to Gentamicin, 71.4% was susceptible to Azithromycin and 57.1% was susceptible to Chloramphenicol. Breastfeeding, complementary feeding, how the child is

fed, daily feeding periods, surrounding where food is bought or prepared and storage of feeding tools of malnourished children were found to be factors that predisposed children to malnourishment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

According to the WHO, malnutrition refers to deficiencies, excesses or imbalances in an individual's intake of energy and/or nutrients. The condition is categorized into two main groups: Undernutrition and Overweight. The former is defined by stunting growth – low height for age, wasting – low weight for height, underweight – low weight for age and micronutrient deficiencies or insufficiencies – a lack of important vitamins and minerals. The other is overweight, obesity and diet-related non-communicable diseases which include but not limited to heart disease, stroke, diabetes and cancer (WHO, 2017). According to the WHO, malnutrition is one of the most dangerous single menace to public health worldwide. With regards to global morbidity and mortality among children, malnutrition happens to be one of the most common factors, especially in the sub-Saharan Africa (SSA) and Southern parts of the Asian continent (WHO, 2017). About ten percent of children below five years worldwide suffer from acute childhood malnutrition. As a result, 50-60% of death among children globally is attributed to childhood malnutrition, and this is observed especially among people who live in regions of the world where there is abject poverty i.e. countries considered under-developed or developing by the World Bank (Black *et al*., 2003; 2010). Nearly half of all deaths in children under five years are attributable to undernutrition, translating into the loss of about three million young lives a year. Undernutrition puts children at greater risk of dying from common infections, increases the frequency and severity of such infections, and delays recovery (UNICEF, 2018c).

In Ghana, too many children suffer from health issues stemming from malnutrition which is an underlying cause of one third of all child deaths (UNICEF, 2018a). Generally, more than one in five children in Ghana is stunted, suffering from chronic malnutrition. Even though malnutrition has dropped, 23% of children are stunted and 57% are anaemic (Leonard et al., 2007). This condition is dire in some parts of Ghana. In the northern parts of Ghana in particular, nutrition is recorded to be poor and for every five children, almost two of them suffer stunted growth while 80% of the children may be suffering from low blood haemoglobin or anaemia (UNICEF, 2018b). A report released by the Regional Office of the World Health Organization (WHO) in Africa in 2017 revealed that undernutrition is still persistent in the region and the number of stunted children has increased. Malnutrition rates remain alarming: stunting is declining too slowly while wasting still impacts the lives of far too many young children (UNICEF, 2018c). According to the 2017 Global Nutrition report, the African continent faces serious nutrition-related challenges, stemming from both a deficiency in nutrients and obesity. Despite a decrease in the prevalence of stunting globally, about 60 million African children under five are not growing properly. At least 10 million others are also classified as overweight posing both a severe health burden on countries and hampering broader development efforts (UNICEF, 2018c).

The immune system, both innate or acquired defense processes of an individual, especially immune-compromised persons like children, pregnant women or people with immune disease condition is significantly affected in situations where there is severe malnutrition (Schaible and Kaufmann, 2007). Consequently, this may result in a number of weak health conditions including susceptibility to infections, more frequent and prolonged episodes, increased severity of disease, reactivation of viral infections, and development of opportunistic infections (CD-WGE, 2005; Cunninghan-Rundles *et al*., 2005).

Notwithstanding, sever acute malnutrition may often delay signs and symptoms of some disease conditions resulting in delayed or prompt diagnosis of the condition eventually making treatment difficult or impossible. Consequently, this may lead to high rate of morbidity and mortality resulting from communicable diseases (CD-WGE, 2005). Unfortunately, morbidities and mortalities resulting from bacterial infections among severe malnourished children in many parts of the world particularly developing and underdeveloped regions of the world is poorly researched.

The causalities of malnutrition can be explained from quite a number of diverse ways. Malnutrition can take its root cause from the social grounds, economic status of the sample in question and the health status. The cultural and environmental influences are as well vital as far as malnutrition is concerned. Besides these, there are controllable factors yet able to affect the entirety of a household. These include the educational level and the financial status of parents and guardians of malnourished children (D'souza and Bhuiya 1982). Malnutrition has been a global issue of topmost discussion. From the Western world draining to the African region, malnutrition is making the life of inhabitants unbearable especially in children. Nursing mothers and child malnutrition is very common among low-income and middle-income countries, consequently ending in substantial rise in mortality and overall disease burden (WHO, 2017).

Children for instance, do not decide what to eat or drink and this has been reviewed and documented evidence that parents in this situation are the main cause of malnutrition. Therefore, in most cases, children of such parents become vulnerable to malnutrition. A parent's level of education, financial status and the ability to make enough time to care or attend to their child has a great influence on exposure of children to malnutrition. (UNICEF, 2018c). As a child, the ability to eat, choice of food, when to eat and how to

eat the food, determines the child's physique and make-up. Detrimental effects of what children are fed with can manifest and be explained as malnutrition. The interaction between undernutrition and infection can create a potentially lethal cycle of worsening illness and deteriorating nutritional status. Poor nutrition in the first 1,000 days of a child's life can also lead to stunted growth, which is associated with impaired cognitive ability and reduced school and work performance (UNICEF 2018c).

Scanty information recorded or documented from the West African region in understanding the trend that links background of the home a child is raised and the nutritional status of the child has revealed low birth-weight as an important associated factor to growth failure though this has not been studied comprehensively. According to a study by Black *et al.,* (2003), one-tenth of children below the age of five in the world are affected with acute child malnutrition and it is the largest contributor to child deaths globally and a public health problem that needs to be given a serious attention (Leonard *et al*., 2007).

A study by Van de Poel *et al.,* (2007), points out that child malnutrition status in Ghana emanates from a wide range of sectors and these factors associated with the rates of malnutrition are not necessarily the same as those linked to the socio-economic difference. The malnourished state of the child exposes them to higher risk of pathogenic infections, lower academic achievement and on top of it all, worse health. A number of bacterial infections have been recovered from children in their malnourished state. Both localized and system bacteraemia have been reported to affect children who are malnourished.

In Chiang Mai, Thailand, malnourished children at the Anaemia and Malnutrition Research Centre were studied. Upon definition of the types and bacterial infections present, two-thirds of the patients were regarded to be in a potentially life-threatening state

(Prasitwattanaseree *et al*., 2016). According to Berman (1991), risk factors that elevate the occurrence and the intensity of lower respiratory infections among children in developing nations factored low birth weight and malnutrition. Research has indicated a huge potentiality of malnutrition posing the child to the dangers of bacterial infections. Certain strains would have been limited of growth in an ideal uncompromised immune system if not the unhealthy state of a malnourished child.

Children with juvenile idiopathic arthritis were studied to have a high vulnerability to the development of opportunistic infections including *Salmonella* just not disregarding that of malnutrition. The compromised immune state of the child at that tender age is of real issue as far as pathogenic disease is of concern (Beukelman *et al.,* 2013). Bacterial infections have been one of the reasons for significant morbidity among children in Africa. With reference to the ability of vaccines to reduce some bacterial infections like pneumococci, the vulnerability of malnourished children poses the threat of incapacitation of treatment methods hence high potential of resistance to treatment methods (Enwere *et al.,* 2006).

1.2 PROBLEM STATEMENT

According to Collins *et al.,* (2006), several millions of children across the world die each year where majority is contributed by child malnutrition. Nutrition has been a vital factor in curbing morbidity and mortality caused by certain infections. This being that malnutrition has been proposed to affect the susceptibility to and expression of bacterial infections (Shankar, 2000). An initiation of the WHO in a community-based program like the Integrated Management of Childhood Illness has tried to develop the relation or the link between malnutrition and bacterial infection in children (WHO, 1999). Heikki, (2001) stated that the incidence and impact of life-threatening bacterial diseases in children across

Africa have not been quantified. In most cases in the sub-Saharan African province, where malnutrition related morbidity is at its highest, certain life-threatening diseases can result in secondary severe malnourishment. This raises the attention to children malnourished as a result of food shortage and others as a result of other life-threatening diseases. A collaborative study by the WHO in 2013, to design a guideline for these sub-Saharan countries to yield a reduce percentage of mortality related malnourishment in children was not as easy as can be explained (WHO, 2013). Opportunistic infections associated with malnutrition increases the risk of morbidity and mortality of vulnerable children in many regions of Africa particularly in Ghana. Looking at the dangers and the cost of therapy in the cases of bacteria prevalence can actually be frustrating. Muller *et al.*, (2010), stated categorically that the resistance of antimicrobials to infectious diseases and childhood anemia has been closely connected to the nutritional status of children.

1.3 JUSTIFICATION

The increasing burden of diseases and infection in the African province has become severe as the number of malnutrition escalates. This can be explained as low weight for height or mid-upper arm circumference according to international criteria. Malnutrition has a negative influence on child morbidity and on their education. The worsening part is the infections created by opportunistic bacteria in these children. These children have an increasing potential of dying. Studies conducted over the years have indicated a high possibility of therapy application to children with uncomplicated or non-severe malnutrition (Joshi *et al*., 2000). These children are mostly taken care of on out-patient basis. At the extreme end are the children with complicated malnutrition which has been colonized by certain opportunistic bacteria like *Salmonella, Shigella, E. coli* etc. They are prone to infections because of their weak immune system. There have been numerous

reports that microorganisms keep developing multidrug resistance to commonly used antibiotics due to over the counter sale of antibiotics, inadequate legislation on the control and management of the usage of these antibiotics (Joshi *et al*., 2000). This has been of much concern, therefore, proper data needs to be managed for adequate measures to be taken. This work sought to investigate the bacterial infection commonly associated with malnourished children admitted at the Maternal and Child Health Hospital in Kejetia, Kumasi and their antimicrobial susceptibility patterns.

1.4 OBJECTIVE

1.4.1 Main objective

The study aims to determine bacterial infection commonly associated with malnourished children zero to five years of who are admitted at the Maternal and Child Health Hospital (MCHH) in Kejetia, Kumasi.

1.4.2 Specific objectives

1. To determine the common bacteria found in urine, stool and blood of children zero to five years who are malnourished.
2. To determine the antibiotic sensitivity patterns of urinary tract infections and bacteraemia isolated from these children.
3. To assess the feeding habits or behaviour of malnourished children as well as personal hygienic practices of guardians at MCHH in Kejetia, Kumasi.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MALNUTRITION

Malnutrition can appear in many forms regarding which vital food element is missing either in diet or been supplied, and the four most common types include malnourishment due to protein-energy, iron deficiency and anaemia, deficiency in vitamin A as well as deficiency disorders by the absence of iodine (Stephenson *et al.*, 2000).

According to the WHO (2005), a huge percentage of child mortality occurs within the first month of child birth. This mortality rate has in part been attributed to child malnutrition which in turn makes them vulnerable to infections. The new born child being deprived of vital dietary elements and for that matter, the new body tissues of these children waste out to deprive them of proper growth. The total being of these children is altered and a possible end up in disease progression and subsequently child mortality. In developing countries like Ghana, improper nutrition can be associated with great percentage of child mortality (United Nation Systems, 2004). The improvement of probiotics to curb the huge menace of child malnutrition has been applicable in medical science for some time now (WHO, 2001). Its effects have been magnanimous in reducing the risk infectious diseases (Saran, 2004). The risk factors to malnutrition has been reported by some organizations such as United Nations International Children's Emergency Fund (UNICEF), but the expected link correlating that to care given to newborn such as breastfeeding and weaning food introduction still remains under review. It has been projected that more than 820,000 deaths of children below age 5 yearly across all the continents in the world can be subdued during breastfeeding (Victoria *et al*., 2015). Breastfeeding decreases the risk of respiratory tract infections and diarrhoea and also improves cognitive development (WHO, 2014).

2.2 GLOBAL MALNUTRITION AMONG CHILDREN

The WHO in 2011 made a projection to describe the state of global malnutrition. One hundred and fifteen million forming 18 percent of a study population were described as

underweight whilst 178 million constituting 28 percent had stunted growth (WHO, 2011). Solis *et al.,* (2002) also stated that, almost one fourth of all children born in the developing countries are malnourished. This throws the light on this cruel childhood menace. The situation of malnutrition is similar in most countries across the continent. In the African and Asian continents, the situation is so propounding, that the life of these children are endangered. The case in the sub-Saharan African nations explains these phenomena with an increasing fatality of malnutrition, AIDS and tuberculosis (TB) which have led to an epidemic of secondary severe malnutrition associated with these co-morbidities (Kessler *et al.,* 2000). In Ghana the situation has a similar reflection where deaths among children between age 0 months to age 59 months was 32,052 and age 0 days to age 27 days was 22,672 after an estimation was made with neonatal sepsis accounting for 4,923 deaths in 2008 (Black *et al.,* 2010).

Socioeconomic, environmental and cultural influences can result in Stunting. (Stewart et al., 2013). Stunting as a result of malnutrition has affected children with huge impacts in different parts of the globe like South Central Asia (48%), Eastern Africa (48%), South Eastern Asia (38%), and Latin America (13–24%). With the predicament of child death in 1995, the WHO reported that, malnutrition contributed to almost half (WHO, 1995). A sample population of eight hundred and fifty children between the ages of 3 to 36 months were studied in Southern Ethiopia in the year 2000. The report showed the highest percentage of the effect of malnourishment being stunted, followed by underweight and finally wasted (Grimes *et al*., 2017). In the Sub-Saharan Africa, children infected with HIV were studied for malnutrition. The study reported that, children faced with severe acute malnutrition were significantly more likely to die than those without HIV infection

(Fergusson and Tomkins, 2008). A similar report was put forward by Berkley *et al*., (2005). In a survey conducted in some villages located in western Kenya, the incidence of malnutrition among children was similar. The cross-sectional study revealed that 47% of the study population were stunted, 30% were underweight and 7% were wasted. After logistic regression analysis, it was reported that, children who are 2 years old are more likely to be underweight and stunted and if left with non-biological parents, they are likely to be under weight as well (Bloss *et al.,* 2004). Although the situation of stunting is on the decline in African malnutrition among children is of serious public health concern which needs to be tackled (UNICEF, 2009)

During a quantitative survey in a community setting in West Ethiopia to determine the situation of under-nutrition and related factors among children aged 6-59 months, stunting among these children recorded higher numbers than wasting and underweight. In the males, stunting was significantly higher especially those born to illiterate and financially challenged parents (Demissie and Worku 2013, Zewdie and Degnet, 2013).

2.3 EFFECTS OF MALNUTRITION IN CHILDREN

Literature has much focused on the consequences and the link between malnourishment and behaviour (Rutter *et al*., 1998). Public health measures and medical care facility have given an increasing attention to the long-term consequences of early malnutrition. According to this study, those likely to be disadvantaged are mostly less severely malnourished as compared to the long-term effects in the more severely malnourished population which is difficult to identify by present procedures (Galler, 1984). Malabsorption is sometimes seen in children with protein-calorie malnutrition. The progress of malabsorption can result in proliferation on the intestinal wall by bacteria as well as the overgrowth of these bacteria in the lower bowel of these children (Mata *et al.,*

1972). In a study by Wynn *et al*., (1995), they recorded significant effects of malnutrition on patients with various health issues. In their survey, they concluded that malnutrition remains common in hospitalized children with congenital heart disease.

2.4 COMMON MICROORGANISMS ASSOCIATED WITH MALNUTRITION

High percentage of death of children according to research has been associated to diarrhoea related illness, malaria, respiratory diseases and illness that require immunization. Though the protocol employed in the study (O' Reilly *et al*., 2012) did not identify malnutrition as the major cause of such type of death among developing nations, the strong force employed together by the co-morbidity of infection and malnutrition recorded a high and recognized morbidity in the study.

2.4.1 *Shigella* sp

Infection with *Shigella* sp. among children has been recorded in Asia and Africa and manifest in a wide array of different clinical signs of which diarrhoea is a major component (Guerrant *et al.*, 1990). *Shigella* over the past decades has acquired a great resilient to the application of antimicrobials that were effective to combat its menace. The prevalence of clinical symptoms caused by *Shigella* has been recorded among children in diverse ways. *Shigella* sp are usually transmissible through stool by direct contact with an infected person. Instances were reported from a study where child care practices of mothers who do not properly handle baby diapers tend to contaminate food and water for consumption (Ferri, 2016).

2.4.2 Salmonella sp

Salmonella infection has been linked to food and water as sole vehicles for its transmission. This is only by oral-faecal route. Infected persons suffer a huge blow of pathological symptoms ranging from diarrhoea to vomiting. Salmonella infection may occasionally to bleeding in the intestine and perforation of intestinal walls. Upon recovery, a person may still pass the bacteria in faeces and this could serve as a potential point of infection to other people if it is not properly handled sanitary-wise (www.cliffsnotes/ guide/bacteria).

2.4.3 Vibro cholera

Cholera is a disease transmissible basically by contaminated water and food. Substantial diarrhoea accompanies *Vibro cholera* infection and dehydration frequently results in death. The most efficient cure is frequent water intake and this is most effective when received intravenously and orally achieved by intravenous and oral rehydrating results (www.cliffsnotes/guide/bacteria).

2.4.4 Escherichia coli

Certain strains of *E. coli* generate toxins and some also have the potential to invade tissues, causing infections in humans. Urinary tract infections and diarrhoea in infants are few pathological manifestations. *E. coli* 0157:H7 has been involved in several food-borne outbreaks in recent years. Haemorrhage, particularly in the kidneys, and other infections that patients suffer can be serious (www.cliffsnotes/guide/bacteria).

2.4.5 Staphylococcus aureus

Over millions of deaths among children by acute respiratory infections occur every year. More of these have been recorded in developing countries (Mtango, 1986) *Staphylococcus* sp. forms part of the majority of these infections. A survey was conducted on MRSA

carriage in people who have previous exposure and re-hospitalised. Almost forty percent of the seventy-eight participants were noted as carriers (Scanvic *et al.,* 2001).

In most cases of *Staphylococcus aureus*, antibiotic treatment is not recommended, instead, fluid substitute is required if severe diarrhoea is presented or observed. Cautious ways of handling food are vital in preventing food poisoning.

2.5 ASSOCIATION OF URINARY TRACT INFECTION (UTI) WITH MALNUTRITION IN CHILDREN

2.5.1 Epidemiology of Urinary Tract Infection

Urinary tract infection (UTI) has for years been familiarised with difficulties in malnourished children. Bagga *et al.,* (2003), prospectively cross checked the occurrence of bacterial infection among malnourished children. The menace of bacterial infection increased significantly as malnutrition becomes severe in patients experiencing diarrhoea. Their observations indicated that malnourished children, principally those with high temperatures are at risk for UTI. Among children under five years old, urinary tract infection forms one of the commonest diseases where infants and younger children are presented with feverishness and vomiting; nonetheless, such symptoms are often misdiagnosed with malaria particularly in regions endemic with malaria cases. According to Festo *et al*., (2011), fevered babies and young children in malaria endemic areas have recorded high prevalence of urinary tract infections.

In Africa, particularly in the Sub-Sahara, fevered illness in children continues to be the primary cause of hospital reported attendance and death (Bryce *et al*., 2005). Additionally, bacteraemia contributes to high mortality rate in children. In Tanzania, a study conducted by Bloomberg *et al*. (2007) in the Muhimbili National Hospital confirmed bacterial infection among in-patients and out-patients with a death rate of 40%. UNICEF (2018b)

has reportedly indicated about 4.6 million (76%) of children under the age of five worldwide die probably due to invasion of a bacterial infection that was not identified. This is due to the fact that, many health centres situated under the umbrella of underdeveloped and developing countries fall short of some substantial primary and secondary conveniences for carrying out routine bacterial culture test to identify bacteraemia (WHO, 2006). According to Hausdorff *et al*., (2001), physicians are compelled to consult to symptoms of children directly for antimicrobial prescription without the involvement of the clinical laboratory. Reed and Wegerhoff, (2016), reported from an eight month period study that 134 children whose age were less than 5 admitted with all marks of malnutrition to a rural hospital were examined for urinary tract infection. Their report showed no significant variation in the incidence rates amongst malnutrition categories.

2.5.2 Diagnosis

The clinical diagnoses of UTIs are essential likewise its proper treatment. When left untreated or not properly treated, its end result can be rapid onset of severe illnesses ranging from abnormal functioning of the kidney to hypertension. Management of children with UTI comes with high cost. They have to go through the stress of repeated visits to health facility, antimicrobial use, and radiation treatment. According to Dolan and Meyers, (1973), with increasing chances of developing kidney failure, babies and young children with urinary tract infections are of greater worry. Report on clinical signs of UTI has indicated variations which is dependent on child's age.

Clinical symptoms which range from sepsis, jaundice, failure to thrive, and strong smelling urine to new onset of urinary incontinence are most often present in neonates (Bhutani *et al*., 2013). Signs and symptoms may be profound in cases of severe bacterial infection.

School-going children may encounter similar exhibit signs and symptoms of UTI common to adults, and are more specific to the urinary system comprising dysuria with foul smelling urine, urgency and frequency. Smellie *et al*., (1964) also reported of other symptoms such as fever, abdominal pain and vomiting, suggestive of pyelonephritis.

2.5.3 Prevention and Treatment

The common proverb in health, 'prevention is better than cure' is paramount as far as children health is concern. The child needs to receive proper health care to avoid infections and its complications. Basic health hygiene needs to be practiced and be done properly. Some children after using the toilet may not empty their bladders frequently enough therefore flushing out germs in urine will not be achieved which can result in an infection. Children should be offered drinks or watery meals and clean water frequently throughout the day since taking adequate amount of fluid would provide enough water for urine formation for frequent bladder emptying in a day, and this can facilitate frequent urination, thus reducing infection risks involved. More water for the child also ensures efficient electrolyte balance.

Improper digestion of ingested food as a result of dietary lacking roughage is likely to cause constipation in such children. Regular habit of visiting the washroom and consuming roughage-rich diets or meals that facilitate frequent emptying of bowels is a good way of putting such infections under control. UTIs and their prevention become a challenge to prevent in children who are usually infected. Antibiotics recorded after sensitivity test of the underlining bacteria is the appropriate medication for infected children. Clinicians usually would order or advise antibiotics treatment to stop the infection and prevent it from occurring again while they wait for laboratory test results usually for a child who encounters this kind of infection for the first time. Intravenous treatment is continual until

systemic signs resolve, thereafter antibiotics will be given orally up to 7 to 10 days (Navarro *et al*., 1984). Children less than 2 years who get ill should begin with intravenous antibiotics after taking the urine sample to the laboratory for culture and sensitivity where treatment can be revised after the results and oral antibiotics can be administered (Clinical Practice Guideline, 2011).

However, children who are not systemically ill should have the urine sent for culture and sensitivity test under close observation until report is ready for additional management. Oral antibiotics are effectual treatment for acute pyelonephritis (Clinical Practice Guideline, 2011).

B-lactam antibiotics and aminoglycosides have proven to resist the thriving of the most probable pathogens responsible for UTIs in infants. These include the *Escherichia coli* and *Enterococcus faecalis*. Second and third-generation cephalosporins are effective against pyelonephritis. *Escherichia coli* and some few causative bacteria have a high ability of developing resistance to therapeutic drugs hence augmented drugs effective against them should be carefully administered (Igarashi *et al.*, 1999) Trimethoprim and sulfamethoxazole (TMP-SMX), amoxicillin and clavulanate, as well as first-generation cephalosporins can be employed in cases of single drug resistance (Fabre *et al*., 2010). The survey by Saadeh and Mattoo (2012), recorded gentamicin as an effective antimicrobial in complementary therapy for treatment of pathogens that are resistant after identifying the renal functions. Cephalosporins and aminoglycosides are preferred in experimental and observatory treatments. Treatment options including ampicillin are more effective in situations where enterococcal UTI is found in association with urinary catheter, abnormalities of the genitourinary organ or urinary bladder instrumentation.

2.6 CHILD NUTRITION

Children at the weaning-age require a tentative food supplement. Exactly the time when the mother decides to switch from breastfeeding to solid meal needs proper attention. The babies need high good food nutrients both micro and macro food nutrients. A study by Besten *et al.,* 1998 to assess the contribution of α-amylase to weaning foods to uplift the dietary intake recorded a significant increase in the nutritional value. In Nigeria, an educational program in nutrition was undertaken to improve upon the standard of infant feeding practices and the nutrition of children in the weaning age. This survey was conducted to structure a culturally appropriate and nutritionally standard weaning food (Nnanyelugo, 1985). Horton, 2006 combines a number of literatures on the cost effectiveness of well-structured food for children especially at the weaning age. The survey shifted much attention to the commercial enrichment as well as on the home and bio enrichment. The idea of weaning then boils to the fact that the child receives optimum food supplement to help in growth and development. This coupled with other reasons of parent's preference of foods for their babies many at times results in potentially contaminated foods. Parents in Sub-Sahara Africa prepare locally produced foods much in liquid state to curb and supplement breastfeeding. These foods differ from place to place.

2.7 CONTAMINATION FROM WEAN FOODS

Poor hygienic practices seen in the African territory exposes, the new born and their wean foods to microbial contamination. The child at this stage also begins to explore its environment. This further exposes them to infections through certain fomites. Literature has given the evidence that, making weaning foods known in less developed states with little or poor insufficient resources can lead to preparing nutritionally insufficient meals that may be contaminated by pathogens or chemically unsafe, resulting in several

deficiencies in the infant's nutritional value (Gibson, 2001). Diarrhoea in children is a major set-back as far as unhygienic foods are introduced to the child. According to Motarjeni *et al*., 1993, pathogens such as *Vibrio cholerae*, *Escherichia coli*, *Shigella* sp, *Entamoeba histolytica* and *Campylobacter* are carried conveniently by houseflies. These microorganisms can thrive on the wings of flies for days. By the direct contact of these flies on the food of the child and through their feacal matter, microorganisms can be transmitted to the new child. Literature has recorded some link between the personal hygiene of mothers and the increased contamination by microbes of the child's food (Imong *et al*., 1989). Using soap and water to wash the hands is ideal in this situation and some mothers were reported to neglect this practice especially after defecating. This contributes to diarrhoeal infections (Khing-Maung *et al*., 1994).

2.8 DIARRHOEA ASSOCIATED FOODS

Enteropathogens get introduced into the system of babies once mothers start feeding with unhygienically prepared weaning food. According to Kaul *et al*., 1996, studies have proven that, some mothers in the Sub-Sahara Africa struggle to purchase continental wean foods for their babies. Cost barrier compels these mothers to resort to locally designed solid and semi-solid foods that are vulnerable to microbial contamination. Diarrhoea still remains one of the leading causes of child mortality (Black *et al*., 2003). Mortarjemi *et al*., (1993), stated in the report of their study that diarrhoeal diseases, aside respiratory infections are the commonest illnesses with the extreme undesirable impact upon the growth of infants and young children.

A longitudinal study in Bangladesh to determine the degree of contamination of weaning foods that are given to children reported that about 41 percent of the weaning feeds were contaminated with *Escherichia coli*. Its presence is an indication of faecal contamination.

Milk foods were more contaminated than rice. This was explained by the storage conditions of the weaning foods which recorded higher numbers for *E. coli* in foods stored under unhygienic environmental conditions. *E. coli* contamination of child's food samples of children and the annual incidence of diarrhoea was significantly related (Black *et al*., 1982).

According to a report by Mortarjemi *et al.,* (1993), there is often heavy contamination of weaning foods designed by mothers under unhygienic conditions with pathogens which is major factor causing diarrhoeal diseases and associated malnutrition. Molbak *et al.,* 1994, examined the influence of breast feeding on diarrhoeal disease and persistence in children in Guinea-Bissau. They recorded a high occurrence of diarrhoea in weaned children than in moderately breast fed babies. Partially breast-milk fed babies suffered less number of days of diarrhoea as compared to weaned babies. Infant mortality was reported to be high when children were fed with one-type of milk due to respiratory and diarrhoea infections. This study compared with infants breast-fed with no milk supplements and those fed with milk supplements. Those completely weaned had 14.2 and 3.6 times higher the risk of death from diarrhoea and respiratory infections respectively after regulation of confounding variables. Part-weaning likewise corresponded with associated relative risk (Victoria *et al.,* 1987).

A study was conducted to understand the occurrence of different kinds of diarrhoeal diseases between the period 1991 and 1992 to clinically evaluate 211 infants including young children in Bolivia Santa General Hospital. The highest group was observed in children that were a year old, of which a higher percentage had acute diarrhoea and with the less recording chronic diarrhoea. Some clinically significant bacteria identified in this

study included enteropathogenic *E. coli O517*, *Klebsiella*, *Shigella* and *Vibrio cholerae* (Ise *et al*., 1994).

It has been reported that diarrhoea has severe effects on nutritional status (Motarjemi *et al*., 1993). The relationship between malnutrition and diarrhoea diseases has been the focus of conversation and regardless of a complex inter-relationship, it is by and accepted that diarrhoea has some stern impact on a child's growth and development. The structure of enteric infectious disease on malnutrition and its long-term consequence on child development has been reported as much as diarrhoeal related illness make children susceptible to malnutrition as well as growth defects, malnutrition in-turn makes these affected children susceptible to prolong occurrence of diarrhoea and the length or recovery is as well extended. Considerable percentage of global malnourishment is as a result of weakened intestinal absorptive function emerging from numerous and recurring enteric infections (Guerrant *et al*., 2008). This weakens both the innate and adaptive immune ability to fight common infections, hence disrupts intestinal function as a result of malnutrition and diarrhoeal diseases rendering weaning children very vulnerable to recurrent enteric infections.

It has been reported that there is a higher reduction in taking diets during diarrhoeal diseases than periods of respiratory infections (Martorell *et al.,* 1994). Diarrhoeal diseases in infants and young children affect their proper growth and development to a greater extent (Martorell *et al.,* 1994) hence children with suppressed immunity are at greater risk to opportunistic infections including respiratory infections which could readily lead to death. Diarrhoea and malnutrition dilemma can be wrecked by mediations that will curtail the prevalence of infection, thus reducing malnutrition (Keusch and Scrimshaw, 1986). Prevention and/or treatment of malnourishment and enhanced hygienic practices would

save up to 800000 children who are exposed to malnutrition and diarrhoeal diseases annually (Jones *et al*., 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY SITE

The study was conducted at the Maternal and Child Health Hospital situated at Kumasi (Pampaso) in the Ashanti Region of Ghana. The Maternal and Child Health Hospital (MCHH) is a public hospital which is popularly known as the CWC or the Kwashiorkor clinic. It is located in the Subin Sub Metro within the Kumasi Metropolis.

Maternal and Child Health Hospital is the only hospital in the northern sector of the country that is dedicated to the treatment and management of malnourished children 0-5 years of age who suffer from severe acute malnutrition including kwashiorkor, marasmus and marasmic kwashiorkor, anaemia, HIV/AIDS and other health related problems. Maternal and Child Health Hospital (MCHH) offers clinical care to inhabitants of the Subin metro and its surrounding districts and also serves as a referral hospital to all other hospitals. The hospital has a nutrition unit that admits, treats and provides nutritional rehabilitation to children with acute malnutrition. The Maternal and Child Health Hospital has a Bacteriology unit that runs culture and antimicrobial sensitivity on samples from the hospital.

3.2 STUDY POPULATION

Malnourished children who were under age five years admitted at Maternal and Child Health Hospital were eligible for the study.

3.2.1 Inclusion criteria

All malnourished children under 5 years showing symptoms of UTIs and sepsis who are able to produce urine, stool and blood. Informed consent was obtained from parents and caregivers who consented to the study. Questionnaires were administered to parents/guardians of the malnourished children who fulfilled the inclusion criteria for the study.

3.2.2 Exclusion criteria

Children who could not produce urine, stool and blood and had parents and caregivers not consenting were exempted from the study.

3.3 SAMPLE SIZE

A total of 200 malnourished children admitted were enrolled in the study with a confirmed consent given by parents/caregivers as well as medical professionals at MCHH. The study was conducted from September, 2016 to April, 2017.

3.4 ETHICS APPROVAL

Ethical clearance for the study was obtained from the Committee on Human Research Publication and Ethics of the Kwame Nkrumah University of Science and Technology. Permission to embark on the study at the Maternal and Child Health Hospital was sought and approved by the management and the head of the laboratory. Consent forms were given to relatives of the target group to consent to or reject before they were included in the study.

3.5 BLOOD CULTURE AND SENSITIVITY

Skin of malnourished children was prepared and cleaned with 70% alcohol for blood sample to be taken. Blood (5 -10ml) was collected into the commercial blood culture bottles (BD Bactec bottles). Samples were transported to the laboratory and culture bottles were barcoded into the Culture Analyzer and incubated at 35°C for five days after which positive growth culture bottles were inoculated onto Blood, Chocolate and MacConkey agar plates and incubated at 35°C for 18-24 hours and examined for growth. A microscope slide smear of the blood was gram stained, followed by biochemical testing for classification and identification of bacteria. Kirby-Bauer disk diffusion antimicrobial susceptibility testing was performed using Mueller Hinton agar and zone of inhibition was measured to determine the resistivity and susceptibility of the cultured bacteria using the breakpoints given by the CLSI (2013) (Cheesbrough *et al*., 2006).

3.6 URINE CULTURE AND SENSITIVITY

Second portion (mid-stream) of early morning urine was collected into sterile containers and transported to the bacteriology laboratory of the Maternal and Child Health Hospital (MCHH). Urine samples were swirled gently and loopful was inoculated onto Cystine Lactose Electrolyte Deficiency (CLED) Agar plate and incubated at 35°C for 18-24 hours and examined for growth. Gram staining was performed for classification and identification. Sign of no growth was reported as no bacterial growth. Lactose fermenters appeared as yellowish while non-lactose fermenters took the colour of the agar. Biochemical testing was performed on agar plates with significant growth for identification of bacteria. Other confirmatory tests such as catalase, oxidase and coagulase were also performed (Cheesbrough *et al*., 2006).

3.7 STOOL CULTURE AND SENSITIVITY

Sterile containers were given to caregivers for stool samples. They were transported to the laboratory for analysis. A small amount (2 - 4g) was picked with a loop and inoculated onto SS Agar, MacConkey agar and Selenite F broth at 35°C - 37°C for 18-24hours. Agar plates were examined for growth. Sign of no growth was recorded as no bacterial growth. Pinkish colonies (lactose fermenters) on agar plates and non-lactose fermenters were reported as such. Various biochemical tests and other confirmatory test such as oxidase, catalase and coagulase test were performed for identification of bacteria. Kirby-Bauer disk diffusion antimicrobial sensitivity was performed using Mueller Hinton agar (Cheesbrough *et al*., 2006).

3.8 DATA ANALYSIS

Data was keyed into WHONET 5.6 software and analysed with CLSI (2013) breakpoints and Microsoft excel was used for graph processing.

CHAPTER FOUR

4.0 RESULTS

This chapter presents the study findings in relation to the objectives of the study. The results are presented according to the key specific objectives of the study.

4.1 AGE AND SEX DISTRIBUTION OF MALNOURISHED CHILDREN

Table 1 presents the number, age and sex distribution of malnourished children used for the study. Age group 12 – 23 months recorded the highest with (34.0%) of the total population studied.

Table 1: Age and sex distribution of patients

Age (months)	Male (n)	%	Female (n)	%	Total(n)	%
≤ 11	31	51.7	29	48.3	60	30.0
12-23	34	50.0	34	50.0	68	34.0
24-35	14	43.8	18	56.3	32	16.0
36-45	13	54.1	11	45.8	24	12.0
46-60	9	56.3	7	43.8	16	8.0
Total	**101**	**50.5**	**99**	**49.5**	**200**	**100**

4.2 SYMPTOMS SHOWED BY MALNOURISHED CHILDREN

The data presented by in Figure 1shows that (63.5%) of the total population had the highest complain of diarrhoea whilst the least (40.5%) had fever.

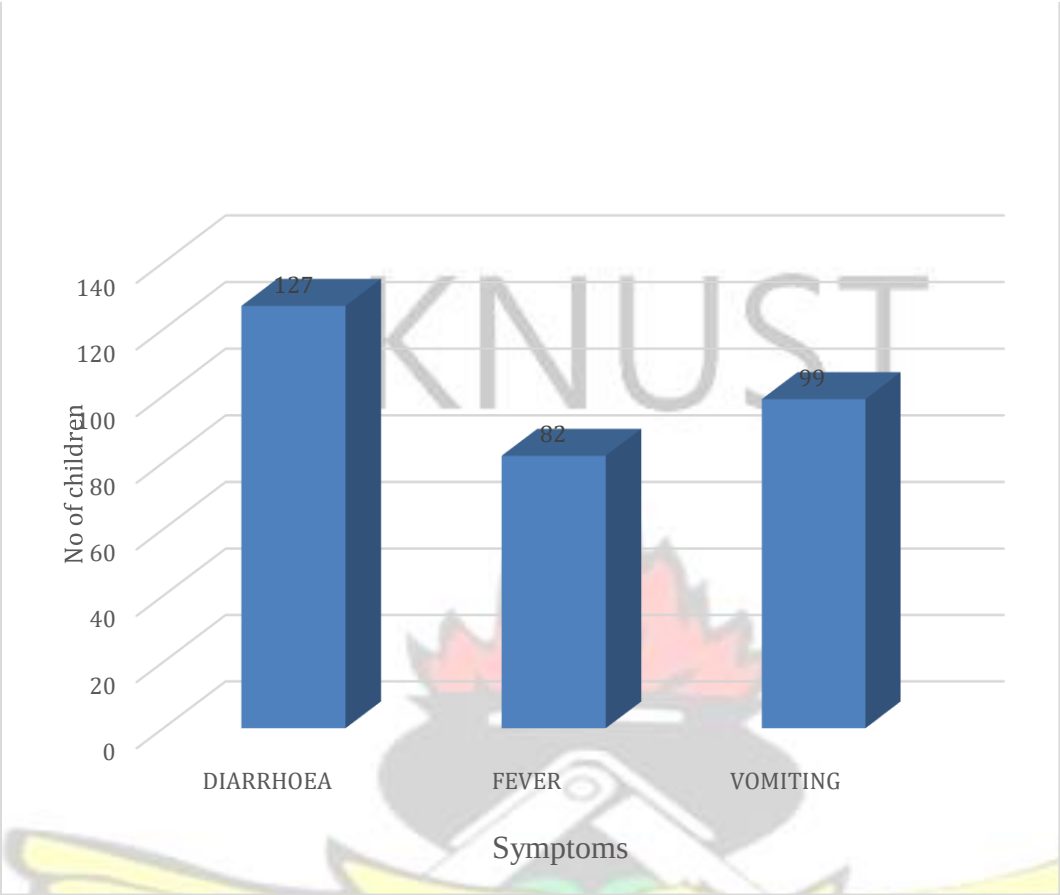

Figure 1: Symptoms recorded in malnourished children at the MCHH

4.3 MID UPPER ARM CIRCUMFERENCE (MUAC) MEASUREMENT OF MALNOURISHED CHILDREN

Figure 2 shows that majority of the children had Severe Acute Malnutrition (SAM), 123(71.1%) and 2(1.2%) of the population had Adequate Nutrition being the least.

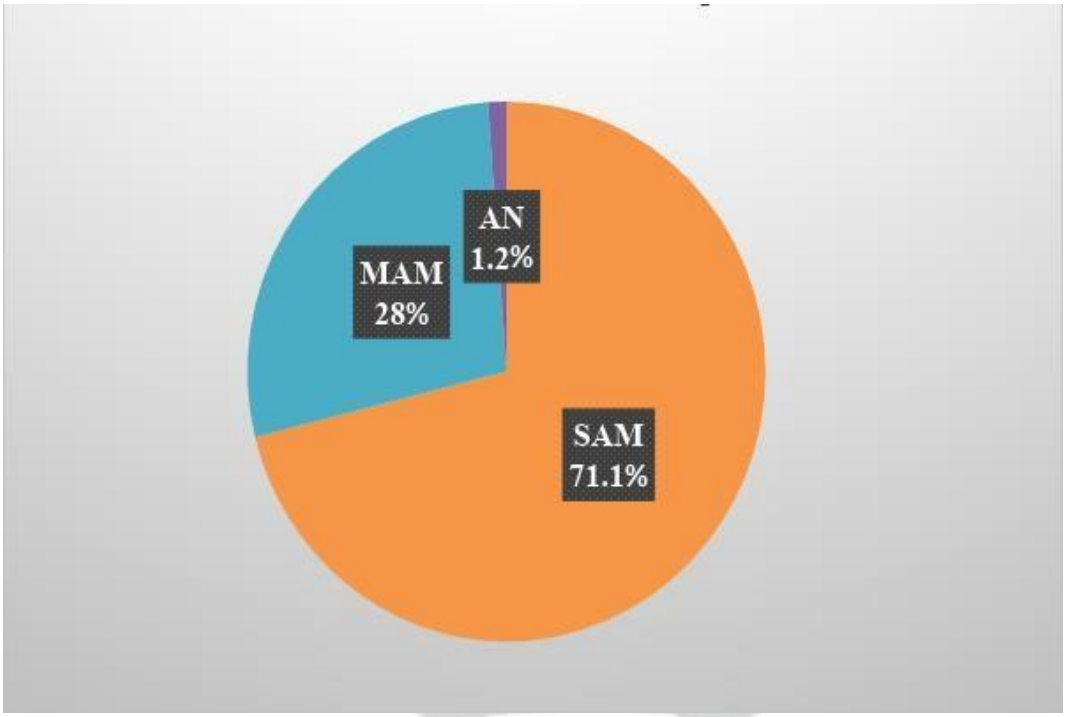

Figure 2: MUAC measurement of patients

MUAC length for Severe Acute Malnutrition (SAM) is < 11.5cm, Moderate Acute Malnutrition ≥ 11.5cm < 12.5cm. Mild Acute Malnutrition (MAM) is ≥ 12.5cm ≤ 13.5cm, Adequate Nutrition (AN) is ≥ 13.5cm.

4.4 SEVERE ACUTE MALNUTRITION IN CHILDREN UNDER FIVE YEARS

Table 2 shows that 65.0% of the target population were Marasmic and 35.0% suffered from Kwashiorkor. Males were highly Marasmic with 35.5% while 20.0% of females formed the highest with Kwashiorkor.

Table 2: Sex distribution and severe acute malnutrition in children under five years

Age(months)	**Marasmus**				**Kwashiorkor**			
	Male (n)	**%**	**Female (n)**	**%**	**Male (n)**	**%**	**Female (n)**	**%**
≤ 11	25	41.7	21	35.0	6	10.0	8	13.3

12-23	28	41.2	19	27.9	6	8.8	15	22.1
24-35	4	12.5	10	31.3	10	31.3	8	25.0
36-47	9	37.5	7	29.2	4	16.7	4	16.7
48-60	5	31.3	2	12.5	4	25.0	5	31.3
Total	**71**	**35.5**	**59**	**29.5**	**30**	**15.0**	**40**	**20.0**

P value = 0.0017

Plate 1: Some Severe Acute Malnutrition cases seen at the Maternal and Child

Health Hospital

Plate 2: Mid Upper Arm Circumference (MUAC) taken by a Nutritionist at MCHH

4.5 NUMBER OF BACTERIA ISOLATED FROM URINE SAMPLES WITH AGE

Isolation was highest in age group 12-23 months representing 37%, followed by age group 24-25 months with 23% in Table 3. No particular trend was observed.

Table 3: Number of bacteria isolated from urine samples with age

	Age in months (n %)					
Bacteria	**≤ 11**	**12 -23**	**24 _ 35**	**36 -47**	**48 -60**	**Total**
Klebsiella sp	4(67)	3(27)	3(27)	3(60)	1(100)	13(43)
Escherichia coli	2(33)	3(27)	4(57)	0	0	9(30)
Salmonella sp	0	1(9)	1(14)	0	0	2(7)
Enterobacter sp	0	0	0	1(20)	0	1(3)
Pseudomonas sp	0	3(37)	0	1(20)	0	4(13)
Proteus sp	0	1(9)	0	0	0	1(3)
Total	**6(20)**	**11(37)**	**7(23)**	**5(17)**	**1(3)**	**30(100)**

4.6 BACTERIA CULTURED IN URINE WITH SEVERE ACUTE

MALNUTRITION

Table 4 shows that 43.3% of female children, out of the total population suffering from Marasmus were associated with bacteria isolates while 23.3% represented male children. Female children 16.7% with Kwashiorkor were associated with bacteria and 16.7% represent male children.

Table 4: Bacteria cultured in urine with acute malnutrition

	Marasmus				Kwashiorkor				
Bacteria	**Male (n)**	**%**	**Female (n)**	**%**	**Male (n)**	**%**	**Female (n)**	**%**	**Total**
Escherichia. coli	1	11.1	5	55.6	2	22.2	1	11.1	9
Klebsiella sp	3	23.1	6	46.2	2	15.4	2	15.4	13
Salmonella sp	0	0.0	0	0.0	0	0.0	2	100	2
Enterobacter sp	1	100	0	0.0	0	0.0	0	0.0	1
Pseudomonas sp	1	25.0	2	50.0	1	25.0	0	0.0	4
Proteus sp	1	100	0	0.0	0	0.0	0	0.0	1
Total	**7**	**23.3**	**13**	**43.3**	**5**	**16.7**	**5**	**16.7**	**30(100)**

4.7 ANTIMICROBIAL SUSCEPTIBILITIES OF BACTERIA ISOLATES IN URINE

Table 5 indicates that 100% *Proteus sp* was susceptible to Cefotaxime, Gentamicin, Ciprofloxacin and Chloramphenicol. *Klebsiella* sp *(*84.6%), *Escherichia coli* (55.6%), *Salmonella* sp (50%) and *Pseudomonas* sp (50%) were susceptible to Gentamicin. *Salmonella* sp (100%) and *Pseudomonas* sp (100%) were resistant to ampicillin. 100% *Proteus* sp, *Escherichia coli*, *Enterobacter* sp and *Salmonella* sp were resistant to ceftriaxone. *Enterobacter* sp was resistant to ciprofloxacin and tetracycline. *Klebsiella* sp (100%) was resistant to Chloramphenicol.

Table 5: Antimicrobial susceptibilities of bacteria isolates in urine

BACTERIA ISOLATES	ANTIBIOTICS AMP (n)		CRO (n)		CTX (n)		GEN (n)		CIP (n)		CHL (n)		TCY (n)	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Escherichia coli	1(11)	8(89)	0	9(100)	1(11)	5(56)	5(56)	4(44)	4(44)	3(33)	1(11)	8(89)	1(11)	8(89)
Salmonella sp	0	2(100)	0	2(100)	0	1(50)	1(50)	1(50)	0	1(50)	1(50)	0	1(50)	1(50)
Pseudomonas sp	0	4(100)	0	1(25)	0	4(100)	2(50)	1(25)	2(50)	0	1(25)	3(75)	1(25)	3(75)
Klebsiella sp	1(8)	5(39)	0	10(77)	0	9(69)	1(85)	1(8)	1(8)	1(8)	0	13(100)	0	9(69)
Proteus sp	0	0	0	1(100)	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0	0
Enterobacter sp	0	0	0	1(100)	0	1(100)	0	0	0	1(100)	0	0	0	1(100)

S – Sensitive R – Resistant AMP – Ampicillin CRO – Ceftriaxone CTX – Cefotaxime GEN – Gentamicin

CIP – Ciprofloxacin CHL – Chloramphenicol TCY - Tetracycline

4.8 BACTERIA ISOLATED FROM BLOOD WITH VARIOUS AGE GROUPS

Table 6 indicates that isolation was high in children of age group 12-23 months (28.6%) and children within age group 36-47 months had the least number of isolation (7.1%). *Staphylococcus aureus* representing 53.6% was the commonest isolate, followed by *Streptococcus* sp (25.0%), *Escherichia coli* (10.7%), *Salmonella* sp (7.1%) and *Enterobacter* (3.8%).

Table 6: Bacteria isolated from blood with various age groups

	Age in months (n %)					
Bacteria	**≤ 11**	**12-23**	**24-35**	**36-47**	**48-60**	**Total**
Escherichia coli	1(17)	1(13)	1(!7)	0	0	3(11)
Salmonella sp	0	2(25)	0	0	0	2(7)
Staphylococcus aureus	5(83)	3(38)	2(33)	1(50)	4(67)	15(54)
Enterobacter sp	0	0	0	1(50)	0	1(4)
Streptococcus sp	0	2(25)	3(50)	0	2(33)	7(25)
Total	**6(22)**	**8(29)**	**6(21)**	**2(7)**	**6(21)**	**28(100)**

4.9 ANTIMICROBIAL SUSCEPTIBILITIES OF BACTERIA IN BLOOD

Table 7 shows all the isolates except *Streptococcus* sp (100%) were resistant to Ampicillin and Cefuroxime. *Escherichia coli* showed maximum susceptibility to all the antibiotics. *Staphylococcus aureus* was susceptible to Azithromycin and Gentamicin and *Enterobacter* sp (100%) were susceptible to Gentamicin. *Salmonella* sp (100%) was susceptible to Trimethoprim/Sulfamethoxazole with *Enterobacter* 100% having susceptibility to Chloramphenicol.

Table 7: Antibiotic susceptibilities of bacteria in blood

	ANTIBIOTICS											
BACTERIA	**AZM (n %)**		**AMP (n %)**		**CHL (n %)**		**CXM (n %)**		**GEN (n %)**		**SXT (n %)**	
	S	R	S	R	S	R	S	R	S	R	S	R
E. coli	3(100)	0	0(0)	3(100)	2(66.7)	1(33.3)	0(0.0	3(100)	3(100)	0(0.0)	3(100)	0(0.0)
Salmonella sp	1(50.0)	1(50)	0(0)	2(100)	1(50.0)	1(50.0)	0(0.0)	2(100)	1(50.0)	1(50.0)	2(100)	0(0.0)
Staphylococcus aureus	14(93.3)	1(6.7)	0(0)	15(100)	8(53.3)	7(46.7)	0(0.0)	15(100)	15(100)	0(0.0)	8(53.3)	1(6.7)
Streptococcus sp	5(71.4)	2(28.6)	0(0)	0(0.0)	4(57.1)	3(42.9)	2(28.6)	0(0.0)	7(100)	0(0.0)	0(0.0)	0(0.0)
Enterobacter sp	0(0.0)	1(100)	0(0)	1(100)	1(100)	0(0.0)	0(0.0)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)

(S – Sensitive R – Resistance) AZM – Azithromycin AMP – Ampicillin CHL – Chloramphenicol CXM – Cefuroxime GEN – Gentamicin SXT – Trimethoprim/Sulfamethoxazole

4.10 TYPES OF MALNUTRITION AND NUMBER OF BACTERIA IN BLOOD

The data in Table 8 shows that, 42.9% of the total population who suffered from kwashiorkor were infected with bacteria while 57.1% who were marasmic were associated with bacterial infection.

Table 8: Types of malnutrition and number of bacteria in blood

Malnutrition Category	No. of isolates	Percentage (%)
Kwashiorkor	12	42.9
Marasmus	16	57.1
Total	**28**	**100**

4.11 BACTERIA ISOLATES FROM STOOL SAMPLES

Escherichia coli (43%) was the highest bacteria isolated from stool samples and the least was *Pseudomonas* species (5%), and *Enterobacter* species (5%) in (Figure 5).

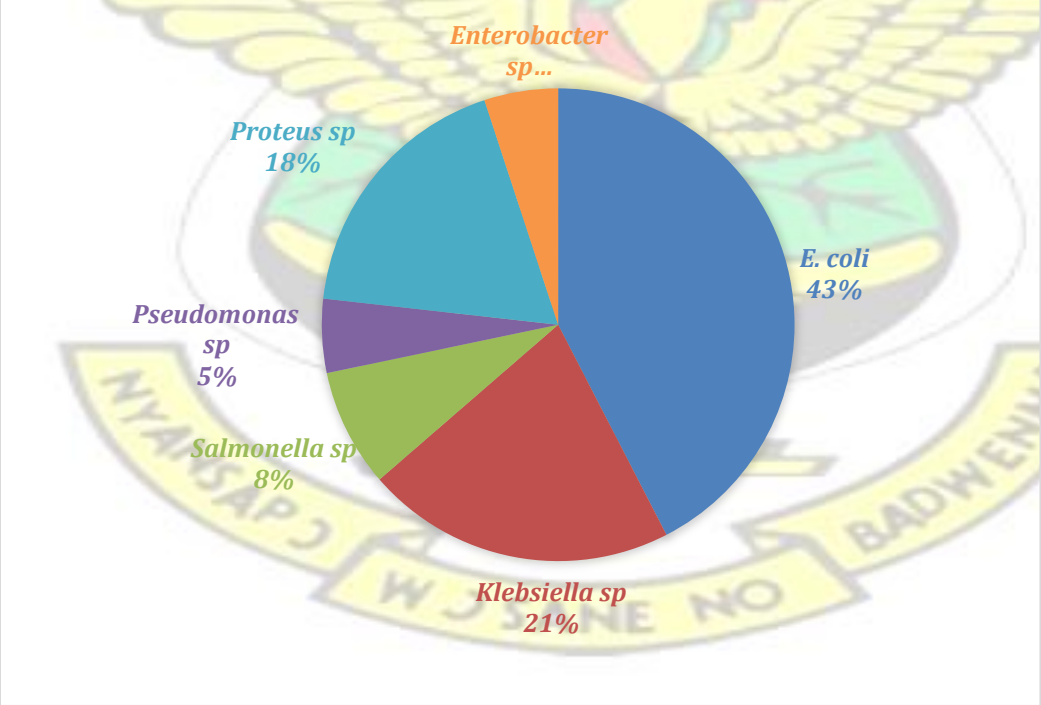

Figure 3: Bacteria isolates from stool samples of malnourished children at MCHH

4.12 FREQUENCY OF BACTERIA CULTURED FROM STOOL SAMPLES WITH AGE

Figure 4 shows that majority of the malnourished children in the age group 12-23 months had the highest number of isolation 14 (37%) while age group 48-60 months had the least 4 (11%) number of isolates. *Escherichia coli* 16 (42%) and *Klebsiella* sp 8 (21%) were the most common bacteria cultured.

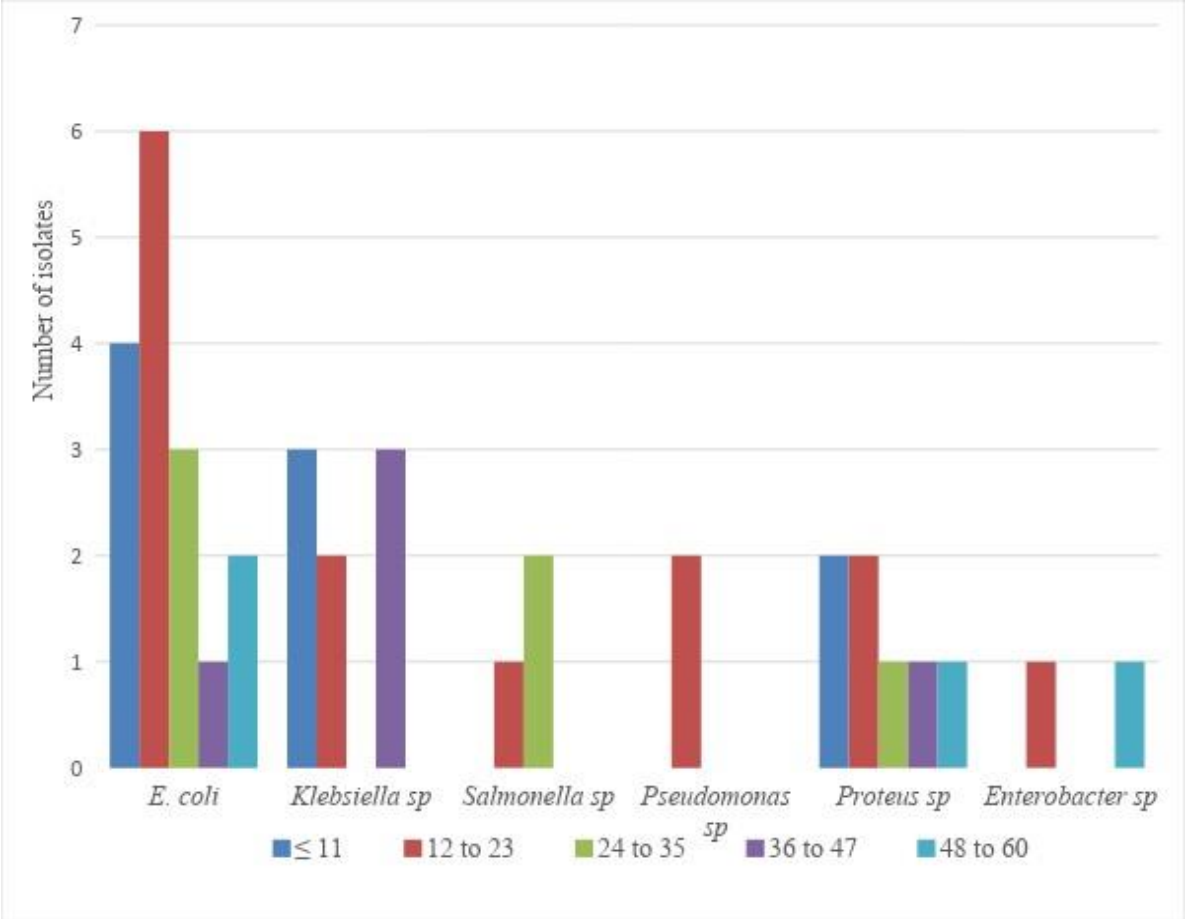

Figure 4: Frequency of bacteria cultured from stool samples among the age groups

4.13 BACTERIA ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITIES IN STOOL SAMPLES OF MALNOURISHED CHILDREN

All the bacteria isolates (100%) were resistant to Ampicillin. *Salmonella* sp (100%) *Pseudomonas* sp (100%) and *Enterobacter* sp (100%) were resistant to Cefotaxime. *Pseudomonas* sp (100%) Enterobacter sp (100%) were susceptible to Chloramphenicol and *Escherichia coli* (81%) was susceptible to Trimethoprim/Sulfamethoxazole. *Enterobacter* sp (100%) was susceptible to Ciprofloxacin. About 33.3% of *Salmonella* sp was susceptible to Amikacin, Gentamicin and Chloramphenicol.

Table 9: Antimicrobial susceptibilities of bacteria in stool samples

	AMP (n%)		CTX (n%)		AMK (n%)		GEN (n%)		SXT (n%)		CHL (n%)		TCY (n%)		CIP (n%)	
BACTERIA	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
E. coli	0	16(100)	5(31)	6(37)	10(63)	0	7(44)	5(31)	13(81)	0	9(56)	7(44)	3(19)	11(69)	7(44)	9(56)
Klebsiella sp	0	8(100)	2(25)	4(50)	4(50)	0	2(25)	3(38)	2(25)	0	2(25)	6(75)	2(25)	3(38)	1(13)	7(87)
Salmonella sp	0	3(100)	0(0)	3(100)	1(33)	0	1(33)	2(67)	0(0)	2(68)	1(33)	2(67)	0	2(67)	0	3(100)
Pseudomonas	0	2(100)	0(0)	2(100)	1(50)	0	1(50)	1(50)	0(0)	1(50)	2(100)	0	0	2(100)	1(50)	1(50)
Proteus sp	0	7(100)	3(43)	0(0)	2(29)	2(29)	2(29)	4(57)	0(0)	4(57)	4(57)	3(43)	0	6(86)	5(71)	2(29)
Enterobacter	0	2(100)	0(0)	2(100)	1(50)	0	0	1(50)	1(50)	1(50)	2(100)	0	0	1(50)	2(100)	0

AMP – Ampicillin CTX – Cefotaxime AMK – Amikacin GEN – Gentamicin SXT – Trimethoprim/Sulfamethoxazole CHL – Chloramphenicol TCY – Tetracycline CIP – Ciprofloxacin S –Sensitive R - Resistant

4.14 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF CAREGIVERS OF MALNOURISHED CHILDREN

Table 10 shows 62.0% of the total number of the parents/guardians were married with 2% being widowed and 36.0% being single parents. Caregivers who completed secondary school formed 53.0% and 3.0% completed tertiary education. About 84.5% were employed and 21.5% were unemployed. Biological mothers of the malnourished children who were interviewed were 71.0% with 29.0% being other relatives.

Table 10: Socio-demographic characteristics of parents/guardians of malnourished children

Variable	**Frequency (n = 200)**	**Percentage (%)**
Marital Status		
Married	124	62.0
Single	72	36.0
Widowed	4	2.0
Educational level		
Primary	70	35.0
Secondary	74	37.0
Tertiary	6	3.0
None	50	25.0
Occupation		
Employed	169	84.5
Unemployed	31	15.5
Person interviewed		
Mother	142	71.0
Other relatives	58	29.0
Total	**200**	**100**

4.15 FEEDING HABITS AND TYPE OF MALNUTRITION IN MALNOURISHED CHILDREN

In Table 11, 70.7% of children being breastfed were severely malnourished and 63.7% of the children who were not breastfeeding had severe acute malnutrition. Almost all the malnourished children were introduced to complementary feeding before the age of 6 months representing 97.7%. Only 1.2% had complementary food introduced after 6 months. 64.2% of children whose food were prepared by parents/guardians were severely malnourished and 1.6% had adequate nutrition. 60.0% of foods were prepared and bought from outside and 64.2% of children having foods prepared by parents had severe malnutrition. Children (66.7%) who feed themselves were severely malnourished and 65.8% of children who were fed by parents had severe acute malnutrition. Severely malnourished children (73.4%) are fed one to three times daily with 27.7% of them having parents/guardians being mindful of the surrounding that food is bought and prepared while 1.2% of the parents were not mindful of the surrounding. Out of the total population, 62.43% of the children had their feeding equipments stored separately and 37.6% placed feeding equipments among others. Values were represented as n% and p- value was determined by one way ANOVA.

Table 11: Feeding habits and type of malnutrition in malnourished children under 5 years

Variable	**Type of malnutrition**			**Total**	**P-value**
Breastfeeding	**SAM (N %)**	**MOAM (N %)**	**AN (N %)**		
Yes	53(70.7)	21(28.0)	1(1.3)	75(43.4)	
No	62(63.7)	35(35.7)	1(1.0)	98(56.6)	0.0015
Introduction of Complementary food					
≤ 6 months	113(65.3)	54(31.2)	2(1.2)	169(97.7)	
>6 months	2(1.2)	2(1.2)	0(0.0)	4(2.3)	0.0226
How child is fed					

Prepared by parent	79(64.2)	42(34.1)	2(1.6)	123(71.1)	
Prepared from outside	21(60.0)	14(40.0)	0(0.0)	35(20.2)	0.0059
Prepared by both	15(100)	0(0.0)	0(0.0)	15(8.7)	
Mode of feeding					
By parent	77(65.8)	39(33.3)	1(0.9)	117(67.6)	
By child	36(66.7)	17(31.5)	1(1.9)	54(31.2)	0.3885
By both child & parent	2(100)	0(0.0)	0(0.0)	2(1.2)	
Feeding periods in a day					
1 – 3 times	79(62.2)	46(36.2)	2(1.6)	127(73.4)	
4 – 6 times	34(77.2)	10(22.7)	0(0.0)	4425.4)	
7 – 9 times	2(100)	0(0.0)	0(0.0)	2(1.2)	0.0017
Mindful about surrounding where food is prepared or bought					
Yes	31(64.6)	17(35.4)	0(0.0)	48(27.7)	
No	2(100)	0(0.0)	0(0.0)	2(1.2)	
N/A	82(66.7)	39(31.7)	2(1.6)	123(71.1)	0.1383
Storage of feeding tools of children					
Placed among other tools	43(66.1)	21(32.3)	1(1.5)	65(37.6)	
Separately kept	72(66.7)	35(32.4)	1(0.9)	108(62.4)	0.0001
Total	**115(66.5)**	**56(32.4)**	**2(1.2)**	**173(100)**	

4.16 CAREGIVERS KNOWLEGDE ON BALANCED DIET

In Table 12, (49.5%) of mothers/caregivers had an idea of what balanced diet was and (50.5%) had no idea. About 35.0% of the mothers fed their children with balanced diets while 65.0% of children were fed with non-balanced diets

Table 12: Caregivers knowledge on balanced diet

Variable	Frequency	Percentage (%)

Heard of balanced diet		
Yes	99	49.5
No	101	50.5
Quality of food given to child		
Balanced diet	70	35.0
Non-balanced diet	130	65.0
Total	200	100

CHAPTER FIVE

5.0 DISCUSSION

The study on 200 malnourished children showed the types and prevalence of bacteria isolates associated with the study population and their antimicrobial susceptibility properties. Prevalence of bacteria isolates in urine was 15.0%, stool was 19.0% and blood was 14.0%.

In this study, 50.5% of the total population were male children and 49.5% females with age group 12-23months forming majority of (34.0%) of the study population. Malnourished children who were males were higher than females. Male children turn to be more vigorous in moving and playing about with all kinds of objects exposing them to various infections from the environment. This finding corresponds to a study conducted among children in Sudan (Mamoun *et al*., 2005).

The data presented in Figure 1 shows the symptoms exhibited by the malnourished children. About 63.5% of the total population had the highest complain of diarrhoea, 49.5% presenting with vomiting and the least being fever with 40.5%. This may be attributable to the introduction of contaminated complementary foods during the weaning period (Dewey and Adu, 2008). Polluted water sources and contaminated liquids could be common sources of bacterial infection in children under 5 years of age. Furthermore, poor

and unhealthy surroundings expose children to poor health and nutritional status. Children begin to crawl at this age and the possibility of ingesting contaminated objects and materials may cause a lot of havoc. Black *et al*., (2003) reported that about 88% of child deaths as a result of diarrhoea cases worldwide are due to poor hygiene and sanitation, unhealthy water intake and contaminated foods.

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The data in Table 3 shows that *Klebsiella* sp was the most predominant bacteria (43%) in malnourished children followed by *Escherichia coli* (30%). *Pseudomonas* sp also present in 13.3% of the children and the least were *Enterobacter* and *Proteus* sp (3%). Isolation was high in the age group 12-23(37%) with the age group 48-60 having the least number (3%) of bacteria isolates. This decrease may be due to the fact that the immunity of children gets stronger as they age hence, the immune system is able to fight against infections and diseases (Thiem *et al*., 2004) This finding concurs with a similar study conducted in Sudan (Mamoun *et al*., 2005). Female children (43%), out of the total population suffering from Marasmus were associated with bacteria isolates while 23% represented male children. About 17% of female children with Kwashiorkor were associated with bacteria while in male children it was 17%. This may be due to the fact that female children are at higher risk of bladder infections because females have a shorter urethra. The use of diapers often keeps genitals always moist and warm which facilitates the breeding of bacteria and other microorganisms due to the movement of bacteria contained in faecal matter from the bowel to the genitals (http://www.Mayoclinic.org/diseases).

It has been observed that UTI can be a common complication among malnourished children (Bagga *et al*., 2003) and can further complicate malnutrition because of associated poor feeding, diarrhoea and vomiting (Isaack *et al*., 1992). Numerous studies have reported that uncircumcised males turn to be susceptible to urinary infections than circumcised

males because microorganisms such as bacteria can hide under the foreskin and this makes cleaning very difficult (Ginsburg and McCracken, 1982, Winberg *et al*., 1986).

Males were highly marasmic with (35.5%) while females formed the highest with kwashiorkor (20%). Marasmus may result from poor child feeding practices and nutritionally inadequate diets (Neumann *et al*., 2004). This study corresponds to a similar study which was done in Nigeria where there were higher cases of marasmus among malnourished children (Hamidu *et al*., 2003). This may be due to a number of factors such as inability to process or absorb nutrients such as calories, proteins, carbohydrates and other essential nutrients properly due to infections. Studies have shown that 20 million children below 5 years suffer from severe forms of malnutrition such as marasmus at certain points in their lives, with about 500,000 to 2 million child death. (UNICEF, 2007).

Table 5 indicates that *Proteus* sp (100%) was susceptible to Cefotaxime, Gentamicin, Ciprofloxacin and Chloramphenicol. *Klebsiella* sp (84.6%), *Escherichia coli* (55.6%), *Salmonella* sp (50%) and *Pseudomonas* sp (50%) were susceptible to Gentamicin. *Salmonella* sp (100%) and *Pseudomonas* sp (100%) were resistant to ampicillin. *Proteus* sp (100%), *Escherichia coli* (100%), *Enterobacter* sp (100%), and *Salmonella* sp *(*100%) were resistant to ceftriaxone. *Enterobacter* sp was resistant to ciprofloxacin and tetracycline. *Klebsiella* sp (100%) was resistant to Chloramphenicol. Increased use of antibiotics has surfaced as one of the greatest threats to the health of human beings and a serious public health problem (WHO 2007). Antimicrobial resistance has been reported to include practices of abuse and misuse of antibiotics in humans and animals (Aarestrup, 2005).

In Table 6, *Staphylococcus aureus* (54%) was predominant in all the age groups with age group 12-23 months having the highest number (29%) of bacteria isolates in blood of

malnourished children. *Streptococcus* sp was the second highest (25%) bacteria isolated, followed by *Escherichia coli* (11%) and *Salmonella* sp (7%). The least bacteria isolated was *Enterobacter* sp (4%). *Staphylococcus aureus* was the common cause of bacteraemia in malnourished children admitted at MCHH and this concurs to a similar studies conducted by Phillips and Wharton (1968) and Bachou *et al*., (2006). This may be due to skin infection and vitamin A deficiency in children admitted (Wiedermann *et al*., 1996). Bacteraemia in children can be caused by both gram negative and positive bacteria (Bearman and Wenzel, 2005).

In Table 7, 75% out of the total number of cultured bacteria isolated from blood samples of malnourished children admitted at MCHH had maximum resistance to Ampicillin and Cefuroxime. Increased use of antibiotics has been one of the greatest threats to the health of human beings and a serious public health problem (WHO 2007). Antimicrobial resistance has been reported to include practices of abuse and misuse of antibiotics in humans (Aarestrup, 2005). However, 82% of these bacteria isolates were susceptible to Azithromycin, 100% to Gentamicin, 46% to Trimethoprim/Sulfamethoxazole and 57% to Chloramphenicol All the bacteria isolates were susceptible to Gentamicin and Chloramphenicol. This corresponds to a similar study conducted in Nairobi by (Noorani *et al*., 2005) but different from that of Babirekere-Iriso *et al*., (2006)

Figure 5 shows that out of the total number of bacteria isolated from stool samples of malnourished children, *Escherichia coli* (43%) was the commonest cultured isolate, followed by *Klebsiella* sp (21%), *Proteus* sp (18%), *Salmonella* sp (8%), *Pseudomonas* sp and *Enterobacter* sp (5%). More often than not, raw foods are major sources of microbial substances because food substances may contain microbial pathogens due to improper

processing. This subjects infants to great risk. Contaminated water for food preparation can also be a source for pathogens (Kung'u *et al*., 2009).

Table 8 indicates that, all the cultured bacteria from stool were resistant to Ampicillin. About 66% and 44% of the bacteria were resistant to Tetracycline and Cefotaxime respectively. Over the last two decades, organisms have developed a multi-drug resistance (Maryam *et al.,* 2001) because of over the counter sale and arbitrary use of antibiotics and inability of legislation to control their use (Rehman *et al*., 2001). The isolates were mainly susceptible to Chloramphenicol (52%), Amikacin (50%), Ciprofloxacin (42%) and Gentamicin (34%). This concurs to a similar study conducted in Nigeria by (Musa-Asien *et al*., 2003) but differs from that of (Caksen *et al*., 2000).

In Table 11, 70.7% of children being breastfed were severely malnourished and 63.7% of the children who were not breastfeeding had severe acute malnutrition. Almost all the malnourished children were introduced to complementary feeding before the age of 6 months representing 97.7%. Only 1.2% had complementary food introduced after 6 months. This may be due to the fact that caregivers do not practice exclusive breastfeeding and improper feeding methods during weaning periods in poor hygienic surroundings could be a factor (Sheth *et al*., 2000).

Children whose food were prepared by parents/guardians (64.2%) were severely malnourished and 1.6% had adequate nutrition. About 60.0% of foods were prepared and bought from outside and 66.7% of children who feed themselves were severely malnourished. About 65.8% of children who were fed by parents had severe malnutrition. Severely malnourished children (73.4%) were fed one to three times daily. Parents of severe malnourished children (27.7%) were mindful of the surrounding that food is bought and prepared with 1.2% of parents not mindful of the surrounding. 62.43% of the total

population had their feeding tools stored separately and 37.6% placed tools among others. During the era of introducing weaning foods, children are at great exposure to under nutrition (Shrimpton *et al*., 2001) since those foods are mostly inadequate in terms of nutritional quality, either they are introduced too early or too late or not sufficient enough. Microbial agents in food can cause diarrhoea and all forms of diseases in infants (WHO, 1989, Motarjemi, 2000). Again children are very vulnerable to foodborne diseases and when these unhygienic foods are consumed, they are likely to contract all forms of infections and illnesses. Weaning foods prepared under unhygienic conditions are profoundly contaminated with microbes serving as a risk factor in disease transference especially diarrhoea. Indications are that weaning food contamination commonly eventuates as a result of poor hygiene of food handlers, household equipments and the surrounding where food preparation is done (Sheth *et al*., 2000).

Table 12 shows 50.5% of the parents/guardians had no idea about what balanced diet was and 49.5% were abreast with what balanced diet meant. Parents who had an idea of what balanced diet meant (35.0%) do consider feeding their children with balanced meals whereas 65.0% of the caregivers do not consider feeding their children with diets that contain the required nutrients. Arya *et al.,* (1991) reported in a study carried out in Parbhani, India that, the children of mothers with basic knowledge on what food to give a child have better anthropometric measurements than mothers with no knowledge on what balanced diet and its essence on child growth and development is. Again, D'souza *et al*., (1982) also believes that the effect of mothers' education on the nutritional status of children is made known through their roles as providers of household health and nutrition.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Prevalence of bacteria isolates in urine, stool and blood of malnourished children admitted at the Maternal and Child Health Hospital was 15%, 19% and 14% respectively. Majority of the children (34.0%) who were within the age group of 12 to 24 months recorded the highest number of the population being malnourished with bacteria isolates. *Escherichia coli* and *Klebsiella* sp being the commonest bacteria in urine whilst *Staphylococcus aureus* and *Streptococcus* sp predominated in blood. There was a high level of antimicrobial resistance to commonly used antibiotics such as Ampicillin, Cefuroxime, Cefotaxime and Tetracycline. However, bacteria isolates were susceptible to antibiotics such as Gentamicin, Chloramphenicol, Ciprofloxacin, Azithromycin, Amikacin and Trimethoprim/sulfamethoxazole. Nutritional problems observed were due to poor knowledge on feeding practices. Majority of the mothers could not practice exclusive breastfeeding for 6 months, therefore majority of the children were introduced to complementary feeding during the weaning period, which is a likely to expose most children to be malnourished and being infected with various forms of diseases if the child's meal is not balanced, mode of child feeding is not considered appropriately, not being mindful about the surrounding where food is prepared or bought, storage of feeding tools as well as mothers not practicing good hygiene.

6.2 RECOMMENDATIONS

- Routine nutritional counselling sessions should be organized for parents/ guardians of children in order to prevent Severe Acute Malnutrition in children.
- Exclusive breastfeeding should be practiced extensively and improve complementary feeding practices of children from 6 months to reduce the rate of malnutrition in children.

- Organisms keeps developing resistance to indiscriminate use of antibiotics hence there is the need for clinical trials to determine the most feasible combination of antibiotics for the management of bacteraemia in malnourished children since it still remains a leading cause to morbidity and mortality in children.

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APPENDICES

APPENDIX I

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

QUESTIONNAIRE

TOPIC: PREVALENCE OF COMMON BACTERIA ISOLATES IN MALNOURISHED CHILDREN UNDER 5 YEARS ADMITTED AT THE MATERNAL AND CHILD HEALTH HOSPITAL AT KEJETIA (PAMPASO).

Background

1. Age (nearest age) of child …… Sex M/F

2. Age of parent/guardian ……..

3. Year of study …….

Socio economic characteristics (Please thick where appropriate)

4. Marital Status	• Married • Single • Divorced • Separated • Widowed

5. Educational Level	• None • Primary • Secondary • Tretiary

6. Mother/Guardian's Occupation	• House wife • Self employed • Paid employment

7. Clinical presentations

Diarrhea	*Yes*	*No*		Vomiting	*Yes*	*No*
Others						

Weight/*Kg*		Height/*cm*		MUAC/*cm*	

Malnutrition Types	Marasmus	Kwashiorkor	Marasmus kwashiorkor

Laboratory samples

Feeding practices and health seeking characteristics

8. Breastfeeding Yes / No

9. Child's age when weaning (complementary) food was introduced ...……

10. First solid food introduced	• Porridge • Mashed kenkey • Cereal **specify** …….

Blood sample ID	
Stool sample ID	
Urine sample ID	

11. Frequency of feeding (in a day)	• 1 – 3 times • 4 – 6 times • 7 – 9 times • 10 – 12 times

12. How is the child fed?	• Often buying already prepared meal from outside

	• Often prepared meal by parent/guardian

13. Are you mindful about the surrounding where food is bought? Yes / No

14. Manner in which child is fed?	• Child is mostly left alone to feed him/herself • Child is fed by parent/guardian

15. Storage of child feeding tools e.g. feeding bottles, plates, spoons	• Separately kept at a place • Placed amongst other kitchen tools

16. Have you heard of Balanced Diet? Yes / No

17. If yes, do you consider that when feeding your child? Yes / No

18. Quality of food	• Balanced diet • Non balanceddiet
19. Growth monitoring by parent/guardian e.g. attending weighing…	• Being undertaken • Not being undertaken

20. How often is growth monitored by parent/guardian?	• Once - twice every month • Once in every two months

APPENDIX II

2.1 MATERIALS USED

2.2 REAGENTS

- Blood Agar Base Powder
- MacConkey Agar
- Mueller Hinton Agar
- Nutrient Agar
- Cystine Lactose Electrolyte Deficient Agar
- Salmonella Shigella Agar
- Sheep blood
- Distilled water
- Peptone water agar
- Simmons Citrate agar

- Tripple Sugar Iron agar
- Kovac's reagent
- Crystal violet
- Ethanol
- Iodine
- Acetone
- Safranin
- Hydrogen peroxide
- Oxidase reagent

2.3 EQUIPMENTS ●

Gloves

- Frosted slides
- Cover slips
- Sterile calibrated wire loop
- Swab sticks
- Autoclave
- Long and short tube
- Petri dish
- Media bottles
- Sterile containers
- BD bactec bottles
- Staining rack
- Microscope
- Burner

APPENDIX III

3.1 CULTURE MEDIA PREPARATION

3.2 Salmonella Shigella Agar

15g of SS agar powder was weighed and dissolved in 250mls of distilled water and was heated until particles dissolved completely. Medium was sterilized by autoclaving at 121 degree Celsius for 15 minutes and 15mls were poured into petri dishes and allowed to solidify Flaming was done to remove bubbles from the plates. Media was packed and stored. Preparation was done in the Media room. This agar is a differential and selective isolation of *Salmonella* and *Shigella* species from pathological specimens.

3.3 Cystine Lactose Electrolyte Deficiency (CLED)

36.2g of CLED agar was weighed and dissolve in 1000mls of distilled water. The medium was heated until the solution dissolved completely. Media was autoclaved at 121 degree Celsius for 15 minutes and poured into sterilized petri dishes. Flaming was done to remove bubbles from the media. Plates were allowed to solidify and stored. Media was placed in the incubator for the ice to be taken off anytime it is going to be used. This medium is for isolation and differentiation of urinary pathogens on the basis of lactose fermentation.

3.4 Blood Agar

40g of blood agar base was weighed and dissolved in 950mls of distilled water. The medium was heated until complete dissolution was attained. Medium was autoclaved at 121 degree Celsius 15 minutes. The base was allowed to cool to about 45 degree Celsius and mixed thoroughly with sheep blood. 15mls was poured into the media plates and allowed to solidify and stored in the fridge. This medium is an effective base for preparation of blood agar for maximum recovery of fastidious pathogens.

3.5 Chocolate Agar

2.8g of blood agar base was dissolved in distilled water and heated until it dissolved completely. Medium was poured into media bottle and autoclaved for 15 minutes a 121 degree celsius. Sheep blood was added and mixed gently to attain chocolate and pouring was done onto petri dishes and allowed to solidify. Flaming was done to take off bubbles. Media was stored in the fridge.

3.6 Nutrient Agar

28g of agar powder was suspended into 1000mls of distilled water. It was boiled to dissolve completely and sterilized by autoclaving at 121 degree celsius for 15 minutes. Pouring of 15mls each was poured into petri dishes allowed to solidify and stored. Nutrient agar is for cultivation of less fastidious microorganisms and can be enriched with blood or other biological fluids. **3.7 MacConkey Agar**

55g of the powder was suspended into 1000mls of distilled water. Medium was boiled to dissolve completely and autoclaved for 15 minutes a 121 degree celsius. Pouring of 15mls each was poured into petri dishes allowed to solidify and stored. Medium is for cultivation and differentiation of enteric bacteria restricting swarming of *Proteus sp*from specimens as urine which may contain large number of *Proteus sp* as well as pathogenic gram positive organisms.

APPENDIX 1V

4.1 SENSITIVITY TEST AND MEDIA PREPARATION

Kirby Bauer disk diffusion method was used for the Antimicrobial sensitivity testing.

4.2 Mueller Hinton Agar

38g of Mueller Hinton powder was weighed with a scale and dissolved in distilled water. It was heated until the medium dissolved completely. Sterilization was done by autoclaving at 121 degree celsius for 15 minutes. Pouring was done into petri dishes and medium was allowed to solidify and stored.

4.3 Peptone water

15g of peptone water powder was dissolved in 1000mls of distilled water. It was gently mixed and heated until complete dissolution was attained. Medium was sterilized by autoclaving at 121 degree celsius for 15 minutes. 15mls each of the medium was measured and poured into the petri dishes and allowed to solidify. This medium is used as a growth medium and as the basis of carbohydrate fermentation.

APPENDIX V

5.1 BIOCHEMICAL TEST MEDIA PREPARATION

5.2 Simmons Citrate Agar

24.2g of citrates agar powder was suspended in 1000mls of distilled water. Medium was thoroughly mixed and heated until it dissolves completely. Medium was autoclaved at 121 degree celsius for 15 minutes. 5mls was poured into tubes to cool in slanted position, allowed to solidify and stored.

5.2.1 Citrate Test

Straight sterile wire loop was used to pick an organism, stabbed and streaked along the slop and incubated at 35 -37 degree celsius for 48 hours. Change of colour from green to blue indicated positive citrate test which explains that bacteria was able to make use of carbon. Change of colour from green to green indicates negative results.

5.3 Triple Sugar Iron (TSI)

64.5g of TSI agar powder was dissolved n 1000mls of distilled water and heated until medium completely dissolved. 7-8mls of the solution was poured into long tubes and caped. Medium was autoclaved with an indication of the autoclave tape to ensure sterilization has been achieved. It was allowed to set in sloped form with a butt about I inch long and stored. This medium is for identification of gram negative enteric bacilli on the basis of dextrose, lactose, sucrose fermentation and hydrogen sulphide production.

5.3.1 Triple Sugar Iron (TSI) Test

Straight sterile wire loop was used to pick isolates and stabbed into long tubes containing TSI agar and streaked along the slop. Incubation was done at 35-37 degreecelsius for 48 hours.

Results: acidic reaction is indicated as A while alkaline reaction is indicated as K

- ✓ Slant red/butt yellow indicates that organism fermented glucose but not lactose hence its written as K/A
- ✓ Slant yellow/butt yellow indicates that organism fermented both lactose and glucose hence written as A/A.
- ✓ Slant red/butt red indicates that organism was not able to ferment both glucose and lactose hence written as K/K
- ✓ Presence of black precipitate indicates hydrogen sulphide (H2S) production.
- ✓ Presence of bubbles and splits in the medium also indicates gas production.

5.4 Indole Test

Straight sterile wire loop was used to pick a colony and inoculated by emulsifying into the peptone water and incubated for 48 hours at 35-37^{o}C. Kovac's reagent was added and shaked thoroughly. Red or pink coloration indicated a positive reaction. Indole test demonstrates the ability of a particular bacterium to split amino acid tryptophan to form the compound indole and is detected by Kovac's reagent which contains 4 pdimethylaminobenzaldehyde which reacts with indole to produce a red coloured compound.

5.5 Coagulase Test

This test is used to differentiate *Staphylococcus aureus* which produces Coagulase which is enzyme-like protein causing plasma to clot by converting fibrinogen to fibrin.

Staphylococcus aureus produces two forms of Coagulase:-Bound Coagulase, cell wall directly reacts with fibrinogen resulting in precipitation causing the cells to clump when mixed with a bacterial suspension. Free Coagulase also involves activation of plasma Coagulase factor and reacts with fibrinogen to produce fibrin clot.

5.6 Catalase Test

This test is used to differentiate bacteria producing enzyme Catalase from non-producing Catalase enzyme bacteria. A small inoculum is introduced into hydrogen peroxide and rapid oxygen bubbles occurred showing a positive reaction. Lack of bubbles was an evidence of negative reaction.

5.7 Oxidase Test

This test is for identification of cytochrome c oxidase which oxidizes the reagent tetramethyl-p-phenylenediamine to (indophenols) purple colour as end product conferring positive. Absence of enzyme causes the reagent to be colourless.