KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF GRADUATE STUDIES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

PREVALENCE OF THE *PROTEUS SPECIES* CAUSING INFECTIONS AT THE KOMFO ANOKYE TEACHING HOSPITAL (KATH) AND THEIR ANTIMICROBIAL RESISTANCE PATTERNS

BY SOLOMON NII ARMAH QUAYE

MARCH, 2009

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A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE (MSC.) IN CLINICAL MICROBIOLOGY

BY

SOLOMON NII ARMAH QUAYE

DECLARATION

I hereby declare that this submission is my own work towards the MSc (Clinical Microbiology) and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other Degree of the University, except where due acknowledgement has been made in the text.

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(HEAD OF DEPARTMENT)

I am very much grateful to the Almighty God for His guidance, protection and provision. Indeed the Lord has been a shield around me, my glory and the lifter up of my head (Psalm3:3).

ACKNOWLEDGEMENT

I am very much grateful to the Almighty God for His guidance, protection and provision. Indeed the Lord has been a shield around me, my glory and the lifter up of my head (Psalm3:3).

I wish to express my sincere gratitude to Mr. P. K. Feglo, without whose encouragement, patience and perseverance this work would not have been possible.

My sincere thanks also go to my mother, Madam Gladys Amoo and all my siblings for their support and encouragement throughout the study. My profound gratitude also goes to my course mates especially Ike, Omane and Mr. Dompreh for their assistance and encouragement. I also appreciate the assistance and services of Mr. Ishmael Tetteh and all the staff of the Microbiology Department, KATH.

Finally, I wish to deeply thank my family and friends for their support, love, care and prayers. May God richly bless and keep you all. Amen.

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ABSTRACT

Various *Proteus species* are known to be responsible for causing several human infections. The various species may also present with different antimicrobial susceptibility patterns. However, in most of our hospitals, differentiation of the genus *Proteus* into species is not fully practised during bacteriological diagnosis because more materials, time and labour are required to carry out such tests. As a result, there is scanty information on the different types of *Proteus species* and how they vary with the types of infections they cause both in our communities and hospital environments. The level of resistance of the *Proteus species* to the commonly tested antimicrobial agents is also not known. This study therefore seeks to determine the prevalence of the various *Proteus species* causing infections and their antimicrobial susceptibility patterns at KATH. From 25th November, 2006 to 20th July, 2007, a total of two hundred (200) *Proteus species* were isolated from 2369 clinical specimens of patients suspected of bacterial infections. Three different *Proteus species* were identified and their antimicrobial resistant patterns determined. The *Proteus* isolates were also related to the patient's demographic data. *Proteus mirabilis* (61.5%) was identified as the commonest species causing the majority of *Proteus* infections followed by *Proteus vulgaris* (30.5%), with *Proteus penneri* (8%) being the least isolated species. The infection was distributed evenly among the sexes. The most susceptible age group to *Proteus* infections was the 1-9 age group. Blocks D (medical unit), B (childrens block) and C (accident/surgical unit) were identified as the most *Proteus* infection prone blocks at KATH. Antimicrbial susceptibility pattern of the *Proteus species* revealed that they were highly resistant to the antimicrobial agents tested with the following resistance values to ampicillin (77%), tetracycline (85%), chloramphenicol (82.5%) and co-trimoxazole (81%). Their

levels were low with resistance levels to amikacin being 4%, gentamicin (26.5%), ceftriaxone (36.5%) and cefotaxime (38%). Amikacin and gentamicin were therefore the most potent drugs with resistance levels being 4% and 26.5% respectively. Beta-lactamase was detected among the *Proteus species* with the highest beta-lactamase producing species being *Proteus mirabilis* (77%), followed by *Proteus penneri* (75%), with the least being *Proteus vulgaris* (64.5%). It is recommended that there should be continuous surveillance on antimicrobial resistance of bacterial isolates which should be identified to the species level in our hospitals. Information from these surveillances should be stored (on computers) to form a data base in the hospitals for health planners and quick reference guide for clinicians who prescribe antibiotics emperically and also for researchers.

CHAPTER ONE

1.0 INTRODUCTION

Proteus species are opportunistic pathogens responsible for a wide range of infections in humans (Gomez *et al.*, 1991). These diseases include urinary tract infections, wound and burn infections, pneumonia and septicaemia (Alaoui and Soussi, 2000). They also cause infections of the eye, ear, nose and throat (Gonzalez and Bronze, 2003). *Proteus species* associated with human infections are *Proteus mirabilis*, *Proteus vulgaris* and *Proteus penneri* (Yolken, 2003). The most common member is *Proteus mirabilis* which causes about 90% of all *Proteus* infections due to its high carriage rate of about 25% in the human intestines (Chow *et al.*, 2005). *Proteus species* are also responsible for causing both nosocomial and community acquired infections (Emori and Gaynes, 1993). Vehicles for the transmission of nosocomial *Proteus* infections include food, equipment, intravenous solutions and the hands of hospital personnel (Emori and Gaynes, 1993). Community acquired *Proteus* infections also occur when the organism living in the environment gains access into humans through contaminated water, soil and occasionally food (Douglas *et al.*, 2000). Direct contact of *Proteus species* through the hands of carriers can also serve as a means of transmitting *Proteus* infections within the environment (Heinzelmann *et al.*, 2002).

According to Douglas *et al.*, (2000), the important epidemiological risk factors for infections caused by *Proteus species* include age, sex, race and socio-economic factors. Other factors are genetic, environmental, nutritional and poor hygiene. Based on some of

these factors, various studies have been carried out from different parts of the world to find out the prevalence of the different *Proteus species* and their distribution in different age groups, sex, in-patients and out-patients. For instance, in India, out of the 1500 uropathogens isolated, 30(2%) were found to be *Proteus mirabilis* whilst 22(1.5%) were found to be *Proteus vulgaris* (Abdumani *et al.*, 2005). They also found 5% of the *Proteus species* isolated to be from inpatients whilst 7% were from outpatients. Distribution of the pathogens in sex and different age groups revealed significant bacteriuria in females more than in males, except in the 0-10 year age group where 62(4%) pathogens were isolated from males and 44(3%) from females. In Ghana, a study by Newman *et al.*, (2006) in nine of the ten regions, showed that the prevalence of *Proteus species* was about 9.2%. A similar work by Ohene, (1997) at the Komfo Anokye Teaching Hospital, Ghana, also quoted the prevalence of *Proteus* infections at 14.6%

Proteus infections are treated with antibiotics such as the penicillins, cephalosporins, aminoglycosides and co-trimoxazole (Yao and Moellering, 1999). However, *Proteus species* causing many infections have become multi-drug resistant strains due to their ability to produce various enzymes which have the ability to degrade antibiotics used for treatment (Tenssaie, 2001). According to Patterson *et al.*, (1999) certain strains of *Proteus species* have become resistant to second and third generation cephalosporins and broad spectrum penicillins. Such strains are more common in developing countries where indiscriminate antibiotic use is rampant (Tenssaie, 2001). The production of beta-lactamases which are enzymes that hydrolyse the beta-lactam ring in antimicrobial agents

such as the penicillins and the cephalosporins is one of the ways by which *Proteus species* develop resistance to such antibiotics (Doern and Tubert, 1987).

Several studies have shown that *Proteus mirabilis* are generally more susceptible to antimicrobial agents than are *Proteus vulgaris* and *Proteus penneri* (Vourli *et al.*, 2006). *Proteus mirabilis* has intrinsic resistance to nitrofurantoin and tetracycline but in many cases has shown susceptibility to the broad spectrum penicillins, cephalosporins, aminoglycosides, ciprofloxacin, and trimethoprim-sulfamethoxazole (Fuchs *et al.*, 1996; Yao and Moellering, 1999). High levels of ciprofloxacin resistance have been reported for *Proteus mirabilis* in hospitals where the use of this agent is unrestricted (Thompson *et al.*, 1994). In 2005, Chow *et al.*, reported an outbreak of infection caused by *Proteus mirabilis* that was resistant to ampicillin, cephalothin, tetracycline, chloramphenicol, carbenicillin, colistin, trimethoprim-sulfamethoxazole, streptomycin, and the aminoglycosides. *Proteus penneri* is generally more resistant to broad spectrum penicillins than is *Proteus vulgaris*, but both may be susceptible to cefoxitin, broad spectrum cephalosporins and aminoglycosides (Yao and Moellering, 1999).

1.1 JUSTIFICATION

Various *Proteus species* are known to be responsible for causing several human infections. The various species may also present with different antimicrobial susceptibility patterns. However, in most of our hospitals, differentiation of the genus *Proteus* into species is not fully practised during bacteriological diagnosis because more materials, time and labour are required to carry out such tests. As a result, there is scanty

information on the different types of *Proteus species* and how they vary with the types of infections they cause both in our communities and hospital environments. The level of resistance of the *Proteus species* to the commonly tested antimicrobial agents is also not known. This study therefore seeks to determine the *Proteus species* responsible for the various infections and their antimicrobial susceptibility patterns. Results from this study will serve as baseline data to help health planners and other health authorities in their strategies towards the control and management of *Proteus* infections in our communites.

1.2 AIM OF STUDY

To investigate the prevalence of the *Proteus species* causing infections at the Komfo Anokye Teaching Hospital (KATH) and their antimicrobial resistance patterns.

1.3 OBJECTIVE

To determine the various *Proteus species* causing infections at KATH and their antimicrobial susceptibility patterns to the commonly used antimicrobial drugs at KATH.

1.4 SPECIFIC OBJECTIVES

i. To determine the *Proteus species* responsible for causing infections at KATH.

ii. To determine the in vitro antimicrobial susceptibility patterns of the different *Proteus species* isolated to the commonly used antimicrobial drugs at KATH.

iii. To determine the extent of beta-lactamase production within the various *Proteus species*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GENERAL CHARACTERISTICS OF THE GENUS *PROTEUS*

The genus *Proteus* consists of Gram negative, facultative anaerobic, non-spore-forming, non-capsulated bacilli classified among the enterobacteriaceae (O'Hara *et al.*, 2000). They possess peritrichous flagella and therefore are actively motile at 37°C (Forbes *et al.*, 2002). *Proteus* bacilli measure from 0.4 to 0.8 μm in diameter and 1.0 μm to 3.0 μm in length (Abott, 2003). They grow at temperatures between 10 and 43°C, and within pH range of 7.0-7.4 (Coker *et al.*, 2002). *Proteus species* exhibit proteolytic activity (Mahon and Manuselis, 2000). They often produce a highly characteristic thin translucent sheet of growth on non-inhibitory agar such as blood agar. This phenomenon is known as "swarming" (Allison *et al.*, 1992). The swarming phenomenon coupled with the ability to produce hydrogen sulphide gas when inoculated into kligler iron agar are the characteristic features that distinguish *Proteus* bacilli from other closely related Gram negative enterics like *Providencia* and *Morganella* (O'Hara *et al.*, 2000). The production of hydrogen sulphide gas by *Proteus species* on certain enteric isolation media such as Deoxychocolate citrate agar (DCA) may cause the colonies of these organisms to be confused with those of the enteric pathogen of the genus *Salmonella* (Murray *et al.*, 2002). *Proteus species* differ from members of the genus *Salmonella* and other enteric bacilli by their ability to produce urease which hydrolyses urea to ammonia and carbon dioxide (Mobley, 1996). *Proteus species* are saprophytic and are found in the natural

environment. They are also found in the intestines of humans and wild and domestic animals where they are commensals. Under favourable conditions, *Proteus species* are able to cause diseases such as nosocomial infections among immunocompromised patients (Rollins et al., 2000).

2.2 CLASSIFICATION OF *PROTEUS*

According to (Penner, 1984) in Bergey's manual of systematic bacteriology, the genus *Proteus* consists of four species: *Proteus mirabilis, Proteus penneri, Proteus vulgaris* and *Proteus myxofaciens*. *Proteus* was discovered in 1885 by Hauser and classified into two species, namely *Proteus mirabilis* and *Proteus vulgaris* based on swarming characteristics exhibited by the organism. Another characteristic which divided them into the two species is based on their ability to liquefy gelatin quickly; *Proteus vulgaris* liquefies gelatin "rapidly," and *Proteus mirabilis* does so "more slowly". The ability to ferment glucose, sucrose, and maltose also served as a means to divide the organism into two groups (Hauser, 1885). *Proteus vulgaris* fermented glucose, sucrose, and maltose readily, whilst *Proteus mirabilis* fermented glucose readily and sucrose slowly and did not ferment maltose.

In 1966, a new species by name *Proteus myxofaciens* was isolated and described by Cosenza and Podgwaite. They isolated this new species which is known to produce slime from the living and dead larvae of gypsy moth. The name was derived from "myxo"

(Greek for "slime") and "faciens" (Latin for producing). *Proteus myxofaciens* is not known to have been isolated from humans (Cosenza and Podgwaite, 1966)

Until the early 1960s, bacterial classification had been based primarily on Gram stain reaction, biochemical reactions and sugar fermentation tests. (Kauffmann and Edwards, 1952). These tests are still a major line of routine identification of bacteria in spite of newer techniques such as DNA-DNA hybridization and guanine-plus-cytosine (G+C) determination (Britten and Kohne, 1965). Presently, biochemical and sugar fermentation tests and these newer techniques have enabled scientists to place new species into their correct genera (Britten and Kohne, 1965). Utilizing the technology involving DNA-DNA hybridization and guanine-plus-cytosine (G+C) determination, Brenner *et al.*, (1978) showed for the first time the genetic heterogeneity of *Proteus vulgaris*. One group of strains was indole, salicin, and esculin negative and was designated *Proteus vulgaris* biogroup 1. In 1982, Hickman *et al.*, proposed that this group be renamed *Proteus penneri* in honor of John Penner, the Canadian microbiologist who made many contributions to studies of the three genera of *Proteeae*.

Until recently, the most popular classification of the genus *Proteus* contained the species namely, *Proteus mirabilis*, *Proteus vulgaris*, *Proteus rettgeri* and *Proteus morganii*. These species are phenotypically similar in a number of respects, but many have indicated that this may be a superficial similarity which does not reflect relatedness in an evolutionary or phylogenetic sense (Penner, 1992). The DNA-DNA hybridization studies

by Brenner *et al.*, (1978) clarified these conflicting phenotypic data. *Proteus morganii* was moved to the genus Morganella because of its low relatedness to the swarming *Proteus species* (*Proteus mirabilis* and *Proteus vulgaris*). Similarly, *Proteus rettgeri* was moved to the genus *Providencia* because of closer relatedness to *Providencia stuartii* and *Providencia alcalifaciens*. The final proposal was to limit the genus *Proteus* to the four species which are closely related by DNA hybridization. These are *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri* (formerly called *Proteus vulgaris* biogroup 1) and *Proteus myxofaciens* (the species which has never been isolated from human clinical specimens). The biochemical and sugar fermentation tests and other properties that differentiate *Proteus species* from *Providencia species* and *Morganella species* is presented in Chart 1 below.

Chart 1. Biochemical reactions, sugar fermentation tests and other properties that differentiate *Proteus species* from *Providencia species* and *Morganella species*

Biochemical test or property	*Proteus species*	*Providencia species*	*Morganella species*
Urea hydrolysis	+	d	+
Hydrogen sulphide production	+	–	–
Swarming	+	–	–
Gelatin liquefaction	+	–	–
Sucrose fermentation	d	+(slow)	–
Mannose fermentation	–	+	+
Citrate utilization	+	+	–
Phenylalanine deamination	+	d	+

Key: + = positive, - = negative, d = different strains give different results

Several studies have attempted to group *Proteus* strains on the basis of O, H and K antigens, but these studies have not been sufficiently correlated to be successful as an epidemiologic tool (Thomas, 1991). The most important use of the *Proteus* antigens is in the diagnosis of rickettsial disease (Amano *et al.*, 1990). Certain *Proteus vulgaris* strains (OX-19, OX-K, OX-2) share antigens with the Rickettsia. The cross reactivity allows these antigens to serve as the antigens for the detection of rickettsial antibodies in the Weil-Felix test for typhus fever (Raoult and Dasch, 1995).

2.3 EPIDEMIOLOGY

Epidemiology is an important study which serves as a component of effective control programme for infectious diseases such as those caused by *Proteus species* (Douglas, *et al.*, 2000). Few studies have specifically investigated the epidemiology of infections caused by the genus *Proteus* including its transmission (Rollins *et al.*, 2000). According to Douglas *et al.*, (2000), the important epidemiological risk factors for infection caused by *Proteus species* include age, sex, race, socio-economic and cultural factors. Other factors are genetic, environmental, nutritional and poor hygiene (Douglas, *et al.*, 2000). The prevalence of the *Proteus species* from different parts of the world and some of the factors influencing *Proteus* infections have been presented below.

2.3.1 Prevalence of *Proteus species* isolated from different parts of the world

In Jordan, out of two hundred clinical isolates of members of the genus *Proteus* identified in a study by Na'was *et al.*, (1994), 176 (88%) were found to be *Proteus mirabilis*, 12(6%) were *Proteus vulgaris* and 12 (6%) were *Proteus penneri*. Pus yielded 62.5% of

the *Proteus species* isolated whilst 34% were isolated from urine. In a study from Poland by Reslinski *et al.*, (2005) on the prevalence of multidrug-resistant *Proteus*, 92.3% of the *Proteus species* isolated were found to be *Proteus mirabilis*. However, the majority of the bacteria were isolated from the urine of the patients studied. Another study from Thailand on the prevalence of *Proteus species* in urinary tract infections by Chung *et al.*, (1999), revealed *Proteus mirabilis* as the most frequently isolated *Proteus species* (55/74, 74.3%), followed by *Proteus vulgaris* (9/74, 12.2%). Similarly, a study from India by Abdumani *et al.*, (2005) showed that out of the 1500 uropathogens isolated from the patients, 30(2%) were found to be *Proteus mirabilis* whilst 22(1.5%) were found to be *Proteus vulgaris*. Another study from England, Wales and Northern Ireland by Jones *et al.*, (2003), reported 81% *Proteus mirabilis*, 5% *Proteus vulgaris* and 0.6% *Proteus penneri* as *Proteus* isolates obtained from the blood of the patients studied. They were unable to identify 14% of the *Proteus* isolates beyond the genus level.

A study from Nigeria by Yah *et al.*, (2007) on the prevalence of *Proteus species* in diabetic wounds of patients in the Ahmadu Bello University Hospital revealed 148 *Proteus species* of which 97 were *Proteus mirabilis* and 51 were *Proteus vulgaris*. A similar study on burn injuries from Malawi by Liwimbi and Komafele (2007) showed that *Proteus mirabilis* yielded 22.7% whilst *Proteus vulgaris* yielded 1.3% of the total number of bacteria isolated from the patients studied. In Ghana, a study by Newman *et al.*, (2006) showed that the prevalence of *Proteus species* was about 9.2%. The prevalence rates of the *Proteus species* encountered at the various clinical sites were 47.6% for wound

swabs, 4.5% for blood, 10.6% for urine, 4.8% for sputum, 4.8% for HVS and 0.8% for aspirate.

2.3.2 Distribution of *Proteus species* in gender and different age groups

A study from England, Wales and Northern Ireland by Jones *et al.*, (2003) showed that 43.92% males and 17.63% females were affected with bacteremia caused by *Proteus species*. The study also revealed an even distribution of the *Proteus species* among the different age groups studied. Another study from Thailand by Chung *et al.*, (1999) revealed that the prevalence of urinary tract infections caused by *Proteus species* was 18.5% in males and 17.4% in females showing no significant difference but most of the *Proteus species* were isolated from patients over 40 years of age. However, a similar study from India by Abdumani *et al.*, (2005) revealed more *Proteus* bacteriuria in females than in males except in the 0-10 age group where 62(4%) pathogens were isolated from males and 44(3%) from females. Another study from Nigeria by Ozumba *et al.*, (1992) showed that *Proteus species* causing urinary tract infections was common in males of 55 and above age groups whilst in females, they were more evenly distributed among the age groups.

A study from Malawi on the epidemiology of *Proteus* colonization of burn injuries by Liwimbi and Komafele (2007), showed that 27(55%) of the patients were females whilst 22(45%) were males. A similar study by Yah *et al.*, (2007) on the prevalence of *Proteus species* in diabetic wounds of patients in the Ahmadu Bello University Hospital revealed

that 156 (92.9%) males and 12(7.1%) females with a mean age of 43.86±9.22 years were infected with *Proteus species*.

2.3.3 Distribution of *Proteus species* in-inpatients and out-patients

In Pakistan, a study by Shah *et al.*, (2000) on the rate of isolation of *Proteus species* from inpatients and outpatients revealed that 40 *Proteus species* which comprised 30 *Proteus mirabilis* and 10 *Proteus vulgaris* were isolated from outpatients whereas 30 *Proteus species* which comprised 20 *Proteus mirabilis* and 10 *Proteus vulgaris* were isolated from inpatients. Another study from India by Abdumani *et al.*, (2005) to assess the prevalence of urinary tract infections due to *Proteus species*, showed that 5% of the *Proteus species* were isolated from inpatients whilst 7% were isolated from outpatients. Similarly, a study from Norway by Grude *et al.*, (1999) on the number and type of bacteria isolated from the urines of inpatients and outpatients showed that a total of 423 *Proteus species* were isolated from inpatients whilst 998 were from outpatients. However, in a related study from Thailand by Chung *et al.*, (1999) on the prevalence of *Proteus species* in urinary tract infections (UTIs), hospital-acquired UTIs accounted for more than two-thirds (51/74, 68.9%) of *Proteus species* isolates, while community-acquired UTIs accounted for approximately one-third (23/74, 31.1%) of all *Proteus* isolates. In another study from Nigeria by Ozumba *et al* (1992), the majority of the cases of urinary tract infections caused by *Proteus species* occurred in surgical department with more cases being nosocomially acquired than community acquired.

2.2.4 Transmission

Proteus infections involve many modes of transmission (Guantzel, 1994). Vehicles for the transmission of nosocomial *Proteus* infections include food, equipment, intravenous solutions, and the hands of hospital personnel (Emori and Gaynes, 1993). Patients being treated with antibiotics, severely ill patients, and probably infants are more likely to be colonized (Blumer and Toltzis, 1995). In the hospital environments, the nosocomial strains have previously been exposed to many types of antimicrobial drugs, so are probably multiple drug resistant (Blumer and Toltzis, 1995). These strains progressively colonize the intestine and pharynx of patients when there is prolonged hospitalization, resulting in an increased risk of infection (Blumer and Toltzis, 1995).

Community acquired *Proteus* infections also occur when the organism living in the environment gains access into humans through contaminated water, soil and occasionally food (Bronze *et al.*, 2006). Direct contact of *Proteus species* through the hands of carriers can also serve as a means of transmitting *Proteus* infections within the environment (Heinzelmann *et al.*, 2002)

2.3 PATHOGENICITY AND CLINICAL SIGNIFICANCE OF *PROTEUS SPECIES*

The virulence factors of *Proteus species* involve a number of biological features (Rozalski *et al.*, 2007). These include adhesion to epithelial surfaces, invasion or penetration of host cells and intracellular multiplication of the pathogen (Senior *et al.*,

1998). Other virulence factors are production of enzymes which damage the host defense system, and the synthesis of toxins (Johnson and Stamm, 1991; Hacker *et al.*, 1987).

Proteus species have several morphological features such as fimbriae and flagella, and biochemical features such as urease, proteases, and amino acid deaminases, and toxins such as hemolysins and endotoxin, which act individually or in concert to initiate, perpetuate and spread infection (Loomes *et al.*, 1992; Warren and Mobley, 1996).

2.3.1 Urinary tract infections (UTI)

Proteus bacilli are known to cause urinary tract infections (Sosa *et al.*, 2006: Shriniwas and Biswas, 1996). There are at least three properties that contribute to the success of *Proteus* as a uropathogen (Mobley, 1996). Evidence has been presented which suggests that fimbriae augment the virulence of *Proteus species* in producing ascending pyelonephritis, possibly by enhancing the organism's ability to adhere to and colonize the renal pelvic mucosa (Li and Mobley, 2002; Zunino *et al.*, 2003). In order to perpetuate infection, *Proteus* organisms also produce urease which alkalinize urine by hydrolyzing urea to ammonia (McLean *et al.*, 1988; Senior *et al.*, 1980). This way, it produces an environment in which it can survive. The alkaline urine leads to the precipitation of organic and inorganic compounds leading to the formation of urinary stones composed of a biochemically complex material called struvite (Mobley, 1996). The bacteria which can be found within the stones can reinitiate infection after antibiotic treatment (Klein *et al.*, 1995).The stones develop over time and may grow large enough to cause obstruction and

renal failure (Foxman, 2000). *Proteus mirabilis* is believed to be the most common cause of infection related to kidney stones, one of the most serious complications of unresolved or recurrent bacteriuria (Klein *et al.*, 1995). Kradjen *et al.*, (1984) also isolated *Proteus penneri* from the center of a stone removed from a patient with persistent bacteriuria.

Proteus species by their active motility also gain access to the urethra and enter the bladder where they multiply (Sosa *et al.*, 2006). They can confine themselves there or ascend the ureters to the kidneys (Nicolle *et al.*, 2001). The most virulent and invasive *Proteus species* can break through the single cell-thick barrier of the proximal tubules and enter the bloodstream causing systemic infection (Li & Mobley, 2002). It was suggested that flagella are responsible for the spread of *Proteus* within the urinary tract (Nicolle *et al.*, 2001).

Of all the *Proteus species*, *Proteus mirabilis* is most commonly encountered in community-acquired and hospital-acquired UTI's and may account for up to 10 percent of all uncomplicated and complicated UTI's (Foxman, 2000). Uncomplicated UTI's occur in patients with urinary tracts that are normal from both a structural and functional perspective (Nicolle *et al.*, 2001). Most women in the outpatient setting present uncomplicated UTI's and respond promptly to short term, inexpensive oral antimicrobial therapy (Miller *et al.*, 2004). Complicated UTI's occur because of anatomic, functional or pharmacologic factors that predispose the patient to persistent infection, recurrent infection or treatment failure (Foxman, 2000). These factors include enlargement of the prostate gland which occurs in elderly men, urinary tract blockages necessitating the

placement of indwelling urinary devices, and the presence of bacteria that are resistant to multiple antibiotics (Nicolle *et al.*, 2001). Major complications of UTI's include bacteremia, chronic pyelonephritis, renal abscess, and death (Bronze *et al.*, 2006). Nicolle *et al.*,(2001) showed that *Proteus species* is the third most common (after *Escherichia coli* and *Klebsiella pneumoniae*) cause of UTI causing 12% of infections and the second most common (after *Providencia stuartii*) cause of catheter-associated bacteriuria in the group of long-term catheterized patients causing 15% of infections.

2.3.2 Wound infections

Proteus species are known to cause severe infections in burns, surgical and other wounds (Kolker *et al.*, 2004). Wound infection occurs when there is deposition and multiplication of the organism in the host tissue after the organism has gained access to the tissue (Oni *et al.*, 1997). There are a number of ways in which the organism can gain access to a wound. These include transfer from equipment or the hands of carriers (direct contact), deposition of the organism on the wound from the surrounding air and physical migration of the organism from the patient's skin or gastrointestinal tract into a wound (Heinzelmann *et al.*, 2002). Whilst there is no definitive evidence to identify the most common route of entry of *Proteus species* into a wound, direct contact and poor hand-washing techniques of healthcare practitioners during pre- and post-operative phases of patient care are considered to be significant factors (Kolker *et al.*, 2004). Ultimately, the development of an infection will be influenced largely by the virulence of the organism and immunological status of the patient; for example, patients considered most at risk are those being treated with long-term steroids and those receiving chemotherapy (Kingsley,

2002). Virulence factors possessed by *Proteus species* in the cause of wound infections include release of toxins. These toxins initiate an uncontrolled proliferation of T-cells leading to cell and tissue damage. The presence of biofilms in a form of transparent sticky film covering the wound surface also causes the host cells to exhibit a decreased defence mechanism, thereby increasing the virulence of the organism (Costerton *et al.*, 1999, Zorgarni *et al.*, 2002).

2.3.3 Blood infections

Proteus species are commonly responsible for gram-negative bacteremia and septicaemia (Martin *et al.*, 2003). When these organisms invade the bloodstream, endotoxins, a component of gram-negative bacterial cell walls, apparently trigger a cascade of host inflammatory responses and leads to major detrimental effects including fever, depletion of complement, release of inflammatory mediators, lactic acidosis, hypotension, vital organ hypoperfusion, irreversible shock, and sometimes death (Oni *et al.*, 2000). *Proteus mirabilis* and *Proteus penneri* were among the bacteria isolated from patients with bacteremia by Engler *et al.*, (1990) and Watanakunakorn *et al.*, (1994). When *Proteus species* and other coliforms enter the blood stream through infected wounds, they induce inflammatory response that causes sepsis and systemic inflammatory response syndrome (a condition associated with sepsis caused all by Gram-negative bacilli) with a mortality rate of between 20 and 50 percent (Brigante *et al.*, 2005). In 1983, Williams *et al.* reported on five patients in a cardiac surgery unit with septicaemia caused by *Proteus mirabilis*.

2.3.4 Ear infections

Proteus species are known to cause of ear infections (Gates, 1996). In Nigeria, a study by Oguntibeju (2003) which aimed to identify the potential causative agents associated with ear infection, revealed *Proteus species* as the second most common bacteria isolated after *Pseudomonas aeruginosa*. In another study by Giebink (1987), *Proteus species* was one of the bacteria cultured from chronically draining ears of patients suffering from chronic suppurative otitis media (CSOM).

2.3.5 Lung infections

Proteus species, though less common, colonize the lungs as a result of infected hospital breathing equipment and causes pneumonia (Roberts, 1999). Symptoms for pneumonia include fever, chills, chest pain, rales and cough (Roberts, 1999).

2.4 LABORATORY DIAGNOSTIC CHARACTERISTICS

In order to arrive at a definitive diagnosis of the aetiological agent of a disease caused by *Proteus species*, clinical samples have to be taken from the site of infection for laboratory analysis (Weissfeld *et al.*, 2002). Gram stained smears of the specimens must be performed to distinguish between gram-positive and gram-negative bacteria and to identify contaminants and pus cells (Weissfeld *et al.*, 2002). Culture and laboratory identification are usually required (Forbes *et al.*, 2000). The organism has simple nutritional requirements and grows well on media such as MacConkey agar, cystine-lactose-electrolyte-deficient (CLED) agar and blood agar. These media are commonly

used for the isolation of the members of the Enterobacteriaceae (Weissfeld *et al.*, 2002). Specimens such as urine, purulent material from wounds or abscesses, ear swabs, sputum and blood must be collected for culture. Urine must be plated on (CLED) medium (Mahon and Manuselis, 2000). Purulent material from wounds or abscesses and sputum must be plated on blood agar and a differential medium such as MacConkey (Abbott, 2003). Blood cultures are inoculated into brain heart infusion broth and incubated overnight before plating on MacConkey agar and blood agar (Yolken, 2003). Culture requires a minimum of 18 hours of incubation at 35 to 37°C to obtain growth which is then isolated and identified (Mahon and Manuselis, 2000).

After growth occurs, various biochemical and sugar fermentation tests are then used to identify the organism further and to differentiate the genus into its species. The urease test, citrate utilization test, reaction in kligler iron agar, indole test, maltose fermentation test, and ornithine decarboxylase test are the biochemical and sugar fermentation tests commonly used (Jozefowiez and Woch, 1993). Commercial identification systems are available and consist of 'kits' or miniaturized biochemical tests which are read manually or automatically (Murray *et al.*, 2002). Other phenotyping methods commonly used in epidemiological studies include biotyping (biochemical profiles), antibiogram studies, bacteriocin and phage typing (Bronze *et al.*, 2006).

Testing for urease production is important in differentiating *Proteus species* from other enterobacteriaceae since *Proteus species* are strong urease producers (Cowan and Steel, 1995). Although genus like *Providencia* and *Morganella* are also urease producers, these

organisms unlike *Proteus species* do not produce hydrogen sulphide gas (blackening of medium) when inoculated into Kliger iron agar (Forbes *et al.*, 2002). The ability to utilize citrate by *Proteus species* is also a means of differentiating this organism from other enterobacteriaceae like *Escherichia coli* which shows a negative reaction with citrate (Forbes *et al.*, 2002).

Proteus species do not ferment lactose, so their colonies appear colourless on MacConkey agar (O'Hara *et al.*, 1997). On blood agar, *Proteus* exhibits a swarming growth characteristic. They also produce a foul odour described as 'burned chocolate' or 'fishy odour' (Weissfeld *et al.*, 2002).

Differentiation of the genus *Proteus* into species is done using biochemical and sugar fermentation tests. *Proteus vulgaris* is indole positive whilst *Proteus mirabilis, Proteus penneri and Proteus myxofaciens* are indole negative (Mahon and Manuselis, 2000). Thus, by obtaining a positive indole test on a characteristic swarming colony which is urease positive and produces hydrogen sulphide gas, a rapid identification of *Proteus vulgaris* can be made (Yolken, 2003). The inability of *Proteus mirabilis* to ferment maltose serves as a means to distinguish this organism from *Proteus penneri and Proteus myxofaciens* which ferment maltose. *Proteus penneri* is not frequently encountered in clinical laboratories (Abbott, 2003) whilst *Proteus myxofaciens* is a pathogen of gypsy moth larvae and has not been recovered from human specimens (Yolken, 2003)

Chart 2. Differentiation of *Proteus species*

Test / type of pathogen	*P. mirabilis*	*P. vulgaris*	*P. penneri*	*P. myxofaciens*
Indole production	–	+	–	–
Maltose fermentation	–	+	+	+
Ornithine decarboxylase	+	–	–	–
Human pathogen	+	+	+	–
Insect pathogen (e.g. gypsy moth)	–	–	–	+

Key: + = positive, - = negative Credit to: Hickman *et al.*, 1982

2.5 ANTIBIOTIC SUSCEPTIBILITY TESTING

Many bacteria, in particular, have unpredictable susceptibilities to antimicrobial agents commonly used for the treatment of infections they cause (Patterson *et al.*, 1999). Antimicrobial susceptibility of the isolates must be measured in vitro to help guide the selection of the most appropriate antimicrobial agent for administration to the patient

(Butler, 2001). There are several ways of doing this, but the three most common methods are broth dilution method, disk diffusion test (Kirby-Bauer method) and Stokes method.

Broth dilution technique measures the minimum inhibitory concentration (MIC). It can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. Standardized inoculums of the test organism are then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth. By comparing the MIC value with known concentrations of the drug obtained in serum or other body fluids, the likely clinical response can be assessed. When required, the MBC can be determined by subculturing the last tube to show visible growth and all the tubes in which there is no growth (Cheesbrough, 2000).

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity. A disc blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a sensitivity testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the sensitivity of the organism. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the CLSI Kirby-Bauer disc diffusion technique. The validity of this

carefully standardized technique depends on using discs of correct antimicrobial content, an inoculum which gives confluent growth and Mueller Hinton agar. The test method must be followed exactly in every detail. After incubation at 37°C overnight, zone sizes are measured and interpreted using CLSI standards (Cheesbrough, 2003).

In the Stokes disc diffusion technique, both the test and control organisms are inoculated on the same plate. The zone sizes of the organism are compared directly with that of the control. This method is not as highly standardized as the Kirby-Bauer technique and is used in laboratories particularly when the exact amount of antimicrobial in a disc cannot be guaranteed due to difficulties in obtaining discs and storing them correctly or when the other conditions required for the Kirby- Bauer technique cannot be met (Cheesbrough, 2000).

2.6 ANTIMICROBIAL-RESISTANT *PROTEUS SPECIES*

Antimicrobial resistance has become an important public health problem associated with serious consequences for the treatment of infection (Vourli *et al.*, 2006; Stephane *et al.*, 2004). Though a global problem, antimicrobial resistance tends to be more significant in developing countries than in developed world (Tenssaie, 2001). Several studies in African countries had reported the presence of resistant strains of bacteria; all showing high levels of resistance to antimicrobial agents (Umolu *et al.*, 2002;Van Dyck *et al.*, 2001),). Most of these reports show bacterial resistance to commonly utilized and relatively cheap drugs like ampicillin, tetracycline and co-trimoxazole. High prevalence of multidrug resistant *Proteus* has been reported in many countries as indicated below.

In Ghana, Newman *et al.,* (2006) conducted a study on the prevalence of multiple drug resistance among bacterial agents. Out of a total of 397 *Proteus species* isolated, 222 were found to be multiple drug resistant isolates. This represented 55.9% and was considered to be high. The *Proteus species* isolated were particularly resistant to four commonly used antimicrobial agents: ampicillin, co-trimozaxole, tetracycline and chloramphenicol, where 50 to 70 percent were multiple drug resistant strains. Similarly, in neighbouring Nigeria, high results were obtained in Okada village in a study by Nwanze *et al.,* (2007) on the antimicrobial susceptibility pattern of urinary isolates where resistance levels of *Proteus vulgaris* were reported to be 90% to gentamicin, 95% to tobramycin, 95% to tetracycline and 95% to cefuroxime. Again in Nigeria, a study conducted by Mbata (2007) on the prevalence and antibiogram of urinary tract infections among prison inmates, the *Proteus species* isolated showed a sensitivity pattern of 9.5% to ampicillin, 51.4% to tetracycline and 85.7% to gentamicin. Ozumba *et al.,* (1992) in Nigeria also conducted a study to assess the extent of drug resistance within the genus *Proteus*. From their study, they found out that all the *Proteus* isolates were completely resistant to ampicillin, tetracycline and cotrimoxazole. Ofloxacin was the most potent antibiotic (94.4%) sensitive followed by gentamicin (83.3%) sensitive. In another study done in Nigeria by Kenechukwu *et al.*, (2005), *Proteus* isolates from urine samples of UTI patients showed a 92% resistance against cotrimoxazole, 85% resistance against tetracycline and 42% resistance against gentamicin. In another West African country, Cameroon, Gangoue *et al.,* (2000) conducted a study on antimicrobial activity against gram negative bacilli from Yaoundé Central Hospital. Their study revealed *Proteus*

mirabilis as the most sensitive species, showing only 2% resistance to cefotaxime and ceftazidime

Again in a study from Jordan by Na'was *et al.*, (1994) on the in vitro activities of antimicrobial agents against *Proteus species* from clinical specimens, the *Proteus species* isolated and tested were notably susceptible to ceftazidime and the aminoglycosides but resistant to ampicillin and tetracycline. In another study from Poland by Reslinski *et al.*, (2005) on the prevalence of multidrug-resistant *Proteus*, it was found out that all the multidrug-resistant strains were completely resistant (100%) to penicillin and cephalopsorins, 98.9% to cotrimoxazole, 63.8% to tetracycline and 38.5% to aminoglycosides

In Ghana, antimicrobial resistance of bacteria is a serious problem. Because of the absence of well equipped bacteriological laboratories, no organized surveillance exists on drug resistance patterns among common bacterial isolates and thus published data on drug resistance are few (Newman *et al.*, 2006). One of the mechanisms of antimicrobial resistance to beta-lactam antibiotics is the production of beta-lactamase which is an enzyme that hydrolyses the beta-lactam ring in these antibiotics (Koneman *et al.*, 1997). Documented report on antimicrobial resistance patterns of the various *Proteus* species is scanty and this study will form epidemiologic data and be a guide to help health planners and other health authorities to formulate plans towards successful control and treatment of *Proteus* infections at KATH and in our communities.

2.7 BETA-LACTAMASE PRODUCTION

β-lactamase is an enzyme produced by bacteria which hydrolyses the β-lactam ring of penicillins and cephalosporins (Luzzaro *et al.*, 2006). An intact β-lactam ring is necessary for antimicrobial activity. Many different β-lactamases exist and the genes that code for these enzymes are usually contained on plasmids. In gram positive bacteria, β- lactamase is secreted extracellularly; in gram negative bacteria, β-lactamase is located in the periplasmic space. This is the primary mechanism of acquired resistance (Baraniak *et al.*, 2005). Beta-lactam antibiotics account for approximately 50% of global antibiotic consumption and this heavy usage has exerted considerable selection for resistance (Livermore, 1998). Production of beta lactamase is the commonest cause of resistance to β-lactam antibacterial agents among Gram-negative bacteria (Pitout *et al.*, 1998). These enzymes undermined the utilization of ampicillin and first and second-generation cephalosporins in the chemotherapy of infections caused by Gram-negative bacteria. To overcome the problems posed by the β-lactamase enzymes, third- and fourth generation (extended-spectrum) cephalosporins were developed. Unfortunately, members of the Family Enterobacteriaceae have developed resistance to these extended-spectrum cephalosporins via production of extended-spectrum betalactamases (Georgepa-Padakou, 1993).

Several rapid methods for detecting β-lactamase have been developed, including the iodometric, acidometric and chromogenic cephalosporin procedures (O'Callaghan *et al.*, 1972). In the acidometric method, a whatman No. 1 filter paper is cut into 5 ×1cm strips

and soaked in a freshly prepared solution containing 125g/L benzyl penicillin, 0.1% (w/v) bromocresol purple and 1.25M NaOH. The strips are dried and can be stored at 4°C for 6 months with a silica gel desiccant. Such strips are available commercially. Before use, the strips must be moistened. Distilled water is recommended but it is essential that the water is not acidic. Bacteria from agar cultures are smeared on the strip and incubated for 30 minutes at 37 °C. Development of a yellow colour after incubation indicates β- lactamase production.

2.8 CONTROL AND TREATMENT OF *PROTEUS* INFCTIONS IN THE HEALTH CARE CENTRE

Prevention of *Proteus* infections, particularly those that are hospital acquired is difficult and perhaps impossible (Wenzel, 2003). Aggressive infection control committees in hospitals must be formed to reduce nosocomial infections through identification and control of predisposing factors, education and training of hospital personnel (Schwartz and Stoller, 1999). Selective decontamination of the digestive tract with a suitable non absorbable antimicrobial regimen may be useful during outbreaks caused by *Proteus species* and other coliforms in general (Raka *et al.*, 2006). Meticulous hand washing after each patient contact is a highly effective means of reducing the transmission of nosocomial pathogens by hospital personnel (Wenzel, 2003).

Localized *Proteus* infections may require drainage or other surgical processes before antimicrobial intervention is instituted. Ampicillin, sulfonamides, cephalosporins, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, and ciprofloxacin have been useful in treating non systemic infections caused by *Proteus species* and other Gram-negative bacilli. Gentamicin, amikacin, tobramycin, ticarcillin/clavulate, imipenem, aztreonam, and a variety of third-generation cephalosporins may be effective for systemic infections.

Since antibiotic resistance levels in Ghana are high and widespread, laboratory tests for drug susceptibility are essential and must be performed for all bacterial isolates (Butler *et al.*, 2001). For example, resistance of *Proteus species* to ampicillin and first generation cephalosporins is increasing rapidly to the extent that they can no longer be considered primary drugs of choice in empirical treatment of infections (Butler *et al.*, 2001).

Likewise, emergence of *Proteus species* and other Gram-negative bacilli with chromosomal or plasmid-encoded extended spectrum B-lactamase activity is causing global problems with resistance to third generation cephalosporins (Munday *et al.*, 2004). *Proteus species* may have multiple resistance due to the presence of R plasmids transmissible by conjugation, just as in other Gram-negative bacilli.

Conjugative resistance plasmids allow the transfer of resistance genes among species and genera that normally do not exchange chromosomal DNA (Dromigny *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 THE STUDY SITE AND SUBJECTS

This study was carried out at the Komfo Anokye Teaching Hospital (KATH). The subjects of the study were patients suspected of bacterial infection and were requested to undergo medical diagnosis at the Komfo Anokye Teaching Hospital microbiology laboratory. Their samples were either sent to the laboratory or they reported in person at the laboratory for diagnosis. Various clinical samples were cultured to isolate the organism. Demographic data of the patient such as age, sex, and ward were recorded during sample collection.

3.2 CULTURE AND ISOLATION

Clinical samples were taken from the site of infection and cultured on MacConkey agar, cystine-lactose-electrolyte-deficient (CLED) agar, blood agar and chocolate agar. Each of the specimens were collected and processed according to the protocol at KATH microbiology laboratory. I participated in the collection and processing of the various specimens.

3.2.1 Urine culture

Sample collection: Patients suspected of urinary tract infections who visited the laboratory for urine culture and sensitivity tests, were given sterile universal bottles and requested to provide 10-20 ml of 'clean catch' mid stream urine into it. To collect the clean catch mid-stream urine, men and women who were ambulatory patients were asked

to go to the urinal. Upon urination, they were to discard the first part of urine and to collect the mid-stream urine directly into the sterile container provided.

Bedridden patients were assisted by the nurses on duty to collect the 'clean catch' mid-stream urine into the sterile containers and the samples were sent to the laboratory by the ward staff.

In the case of infants and children, they were encouraged by their mothers or the hospital personnel to pass urine. As the child passes urine, the mother or nurse quickly collects the urine into the sterile container. The container was covered immediately and delivered to the laboratory. The container was labeled with the name and age of the patient. The ward or OPD were also written on the bottle. The laboratory number was also written and dated.

Urine sample processing: Microscopy was done immediately the urine was inoculated onto the agar plate. To do the microscopy, the remaining urine after plating was mixed well and about 10 ml was transferred to a labelled conical test tube. This was centrifuged at 1500 rev/min for 3-5 minutes. The supernatant fluid was decanted and discarded leaving the deposit or sediment behind. The sediment was remixed with the remaining fluid by tapping the bottom of the tube. One drop of the well-mixed sediment was transferred to a glass slide and covered with a cover slip. This was examined microscopically using x10 followed by x40 objective lens for bacteria, white blood cells (pus cells), epithelial cells, red blood cells, casts and crystals.

Inoculation Procedure: Using a marker, the back of a petri dish containig cystine-lactose-electrolyte-deficient (CLED) agar was divided into four segments. Each segment was labelled with the laboratory number of the patient. The urine samples were then inoculated into each segment of CLED using a sterile wire loop, calibrated to hold 0.002 ml of urine. The inoculated plates were incubated at 37°C overnight. After incubation, the plates were read. The bacterial count was calculated from the numbers of colonies growing on the inoculated area. If 200 colonies were counted, the approximate number of colony forming units per ml of urine was calculated as 1/0.002 x 200. Such a count was reported as 100,000 (10^5) bacteria/ml of urine and was considered significant bacteriuria. Less than 10,000 organisms per ml of urine (ie. less than 20 colonies) was considered non significant.

Proteus is a non-lactose fermenter, hence, colonies which appeared colourless on CLED after overnight incubation were further taken through biochemical and sugar fermentation tests to identify and differentiate them.

3.2.2Culture of Wounds, Pus and Abscesses

Pus from abscesses were collected into a sterile leak-proof container by the medical officer and submitted to the laboratory by a nurse. When the pus was scanty, a sterile-cotton wool swab was used to collect it from the infected site. The swab was then immersed in a bottle containing Stuart transport medium (Appendix A). The specimen was labeled and delivered as soon as possible with a completed request form to the laboratory. Specimens from wounds were brought in from the wards. Some too were

collected in the laboratory using sterile cotton wool swabs. After removing the top debris on the surface of the wound using a cotton wool swab, a fresh swab was used to collect the sample from deep parts of the wound to avoid collecting surface debris which might contain contaminants. The swab was then immersed in a bottle containing Stuart transport medium. The specimen was labeled and delivered as soon as possible with a completed request form to the laboratory.

A smear of the specimen was prepared on a clean glass slide and stained by Gram technique (Appendix B). The smears were examined under the microscope for pus cells and the presence of organisms (especially Gram-negative rods).

The specimens were also inoculated on MacConkey agar and blood agar plates using cooled sterile bacteriological loop. The inoculated plates were incubated overnight at 37°C. After overnight incubation, the plates were read. If no growth occurred, they were reincubated for another 24 hours. If still no growth occured, they were discarded.

On blood agar, when there was spreading of the organism across the surface of the blood agar, a phenomenon known as swarming, *Proteus* was suspected. On MacConkey agar, colonies which were non-lactose fermenting and therefore appeared colourless after overnight incubation were suspected to be *Proteus* and were further taken through biochemical and sugar fermentation tests to identify and differentiate them. The growth characteristic of *Proteus* isolated from wound sample is illustrated in plates 1 and 2.

Plate 1. Proteus from wound sample showing typical swarming on blood agar.

Plate 2. Non lactose fermenting proteus growing on MacConkey agar.

3.2.3 Blood culture

The blood samples were collected by a phlebotomist. To collect the samples, a tourniquet was applied to the upper arm in order to locate a suitable vein. About 50mm diameter of the venepunture site was then disinfected with 70% ethanol and mopped to dry. Using a 5ml sterile syringe and a 21G x $^{1}/_{2}$ inches needle, about 5ml of blood were drawn from the patients' vein and inoculated into brain heart infusion broth (Appendix A). To inoculate the brain heart infusion broth with the blood, the top of the culture bottle cap was disinfected with 70% ethanol. The needle used for blood collection was replaced with a fresh needle. The fresh needle was then inserted through the cap to dispense the blood into the culture bottle containing 20ml of brain heart infusion broth (Appendix A). The top of the culture bottle was disinfected again with 70% ethanol. The bottle was

labeled with the name, date and number of the patient and immediately placed in the 37°C incubator.

The following day, the culture bottles were examined for growth and subcultured onto blood agar and MacConkey agar using a cooled sterile bacteriological loop. The agar plates were incubated at 37°C for 18-24 hours or overnight. The blood culture bottles were incubated at 37°C for up to 7 days, examined for growth daily but subcultured every other day onto blood agar and MacConkey agar.

When there was spreading of the organism across the surface of the blood agar plate (a phenomenon known as swarming), *Proteus* was suspected. On MacConkey agar, growth of colourless colonies after overnight incubation was further taken through biochemical and sugar fermentation tests to identify and differentiate them.

3.2.4 Ear discharge culture

Sterile cotton wool swabs were used to collect pus from individuals with ear discharge. The samples were immersed into a bottle containing Stuart transport medium and delivered as soon as possible with a completed request form to the laboratory.

A thin smear of the specimen was prepared and stained by Gram technique (Appendix B). The smears were examined under the microscope for pus cells and the presence of organisms, especially Gram-negative rods. The swabs were inoculated onto MacConkey agar and blood agar plates using a cooled sterile bacteriological loop. The plates were then incubated at 37°C for 18-24 hours or overnight and inspected for growth. If there

were no growths on the agar plates, they were reincubated for another 24 hours. If still no growth occured, the agar plates were discarded. When growth occured, they were taken through biochemical and sugar fermentation tests to identify and differentiate them.

3.2.5 Sputum culture

A clean, wide-mouthed monowax container with a close-fitting lid was given to patients who had cough and were requested by the physician to do sputum culture and sensitivity tests. The patient was asked to go to a quiet place away from public to cough up sputum and to deposit it into the container and to submit it to the laboratory. The container was then labeled with the patient's name, sex, age and ward.

Two thin smears of the specimen were prepared. One was stained by Gram technique (Appendix B) and the other by Ziehl-Neelson (ZN) technique (Appendix B). The Gram stained smear was examined under the microscope for pus cells and presence of organisms. The slide stained by ZN method was examined for Acid fast bacilli (AFB) to rule out tuberculosis. Sputum samples negative for AFB's were cultured for other bacteria. The sample was plated on blood agar, chocolate agar and MacConkey agar using a sterile loop. The plates were then incubated overnight at 37°C and inspected for growth. The growths were further taken through biochemical and sugar fermentation tests to identify and differentiate them.

3.2.6 Ascitic, pleural fluid and synovial fluid (joint aspirate) culture

These samples were collected from patient by the requesting medical officer. The aspirates were collected aseptically into sterile screw-capped containers and submitted to the laboratory with a completed or filled laboratory request form.

A thin smear of the specimen was prepared and stained by Gram technique (Appendix B). The smears were examined under the microscope for pus cells and organisms.

The samples were plated on blood agar and MacConkey agar using a sterile bacteriological loop. The plates were then incubated overnight at 37°C and inspected for growth. The growths were further taken through biochemical and sugar fermentation tests to identify and differentiate them.

3.3 BIOCHEMICAL TESTS

After the preliminary identification from MacConkey, CLED, blood agar or chocolate agar plates, colonies suspected to be *Proteus* were identified and differentiated using biochemical and sugar fermentation tests. The biochemical and sugar fermentation tests were performed to identify and distinguish between the various organisms. Identification tests performed included urease production, citrate utilization, reaction on Kligler iron agar (KIA), indole production, and fermentation of maltose. Before these tests were performed, colonies suspected to be *Proteus* were streaked onto nutrient agar to obtain purity plates. The colonies from the purity plates were used to perform the tests. The

principles, composition, mode of action of the various tests performed are presented in Appendices A and B.

3.3.1 Urease Production Test

To perform urea hydrolysis test, few colonies of the test organism were picked from the nutrient agar plate using a sterile straight inoculating wire and inoculated on the urea agar slope in a test tube. This was incubated overnnight at 37°C. Urease production was detected by the colour change of the medium from yellow to red or pink, which *Proteus* does, so the test was read positive after incubation. Uninoculated urea tube was used as a control.

3.3.2. Citrate Utilization Test

To perform this test, few colonies of the test organism were picked from the nutrient agar plate (purity plate) using a sterile straight inoculating wire. The colonies were used to streak the slope and stab the butt of a Simmon's citrate agar in a test tube and incubated at 37°C overnight. Citrate utililization was detected by the colour change of the medium from green to deep blue accompanied by visible growth, so the test was read positive after incubation. Uninoculated citrate tube was used as a control.

3.3.3 Reaction in Kligler Iron Agar with production of Hydrogen sulphide

To perform the test, few colonies of the test organism were picked from the nutrient agar plate using a sterile straight inoculating wire, and stabbed into the butt and then streaked

on the slope of a kligler iron agar in a test tube and incubated at 37°C overnight. The test was considered positive and suggestive of *Proteus* when after incubation, the medium gave rise to red slope, yellow butt, blackening of the medium due to hydrogen sulphide production and crack in the medium due to gas production. The red slope and yellow butt was usually not seen when a lot of H_2S was produced.

3.3.4 Indole Production Test

The organism to be tested was picked from a nutrient agar plate using a sterile straight inoculating wire and inoculated into peptone water. The peptone water was incubated at 37°C overnight. Two to three drops of kovac's reagent (Appendix A) were then added to the overnight culture. The formation of a red ring in the surface layer within 3 minutes was read as positive. Yellow colour indicated a negative result. This test is positive for *Proteus vulgaris* but negative for *Proteus mirabilis*, *Proteus penneri* and *Proteus myxofaciens*.

3.3.5 Maltose Fermentation Test

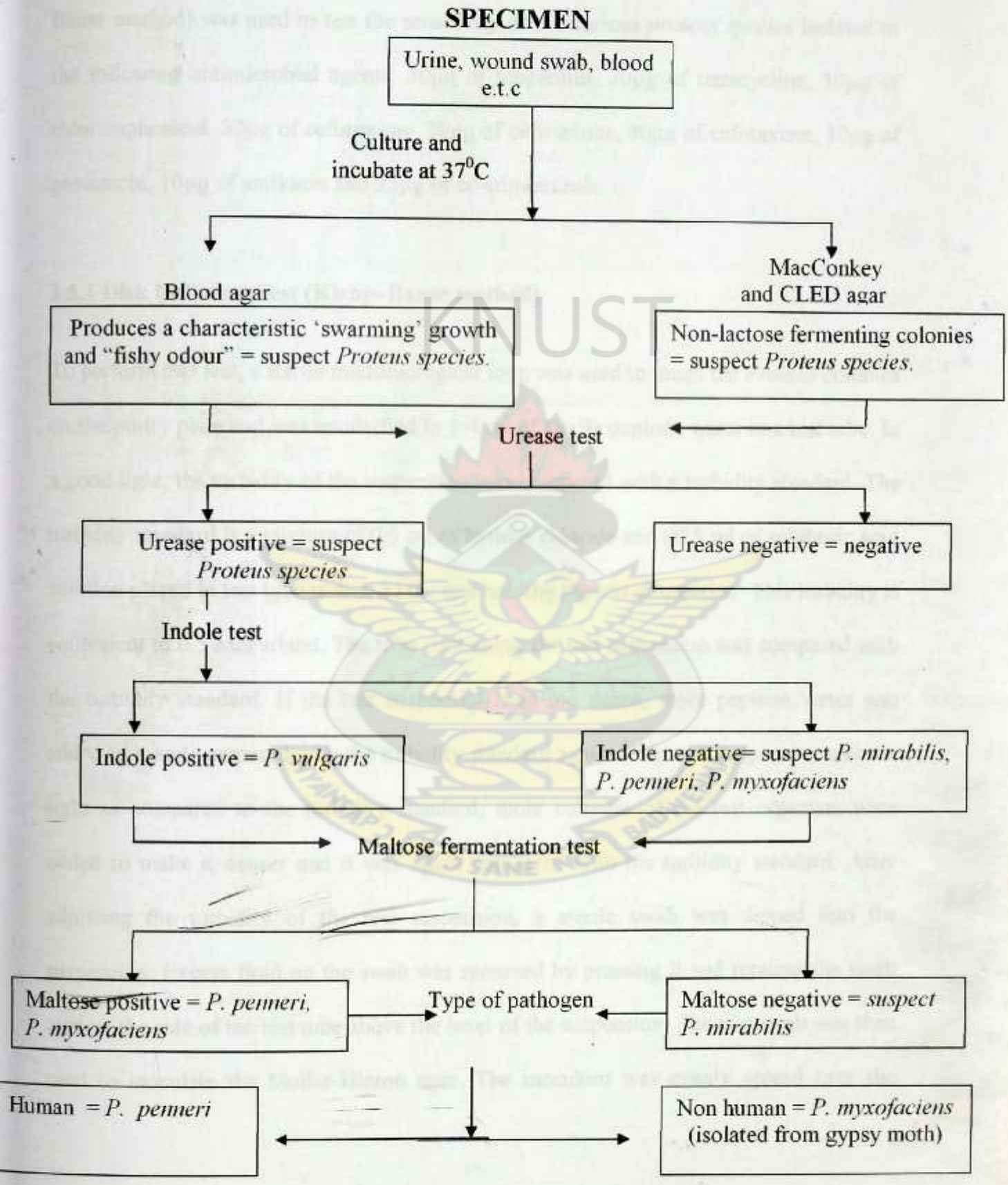
To perform the test, a maltose fermentation broth was inoculated with the test organism and incubated for 24-48 hours at 37°C. Fermentation of maltose by the organism led to the formation of an acid which was demonstrated by a change in colour of the maltose broth from orange to yellow and production of gas which was collected in a Durham tube placed in the broth. When there was no fermentation, the maltose medium remained

orange. The test is positive for *Proteus vulgaris, Proteus penneri* and *Proteus myxofaciens* but negative for *Proteus mirabilis*.

3.4 MOTILITY TEST

A sterile loop was used to collect a drop of peptone water culture of the isolate and placed on a clean glass slide. A cover slip was placed over the slide making sure no air bubbles were trapped. The slide was then viewed under the microscope with low power (x10 objective), with the condenser sufficiently closed to allow contrast and then later viewed with high power (x40) objective. *Proteus species* are actively motile. Care was taken to observe true locomotion but not passive drifting and Brownian motion. *Escherichia coli* was used as positive control.

Chart 3. Flowchart for isolation and identification of *Proteus species*



3.5 ANTIMICROBIAL SUSCEPTIBILITY TESTING

After identifying the various *proteus species*, the disk diffusion test (modified Kirby-Bauer method) was used to test the sensitivity of the various *proteus species* isolated to the following antimicrobial agents: 10µg of ampicillin, 30µg of tetracycline, 30µg of chloramphenicol, 30µg of cefuroxime, 30µg of ceftriaxone, 30µg of cefotaxime, 10µg of gentamcin, 10µg of amikacin and 25µg of co-trimoxazole.

3.5.1 Disk Diffusion Test (Kirby- Bauer method)

To perform this test, a sterile microbiological loop was used to touch the *Proteus* colonies on the purity plate and was emulsified in 3-4 ml of sterile peptone water in a test tube. In a good light, the turbidity of the suspension was compared with a turbidity standard. The turbidity standard is a mixture of 0.5 ml of barium chloride and 99.5 ml of sulphuric acid solution placed in test tube similar to the one holding the test suspension. This turbidity is equivalent to 0.5 McFarland. The tube containing the test suspension was compared with the turbidity standard. If the test suspension was too dense, more peptone water was added to it and compared with the turbidity standard again. If the test suspension was too light as compared to the turbidity standard, more colonies of the test organism were added to make it denser and it was again compared with the turbidity standard. After adjusting the turbidity of the test suspension, a sterile swab was dipped into the suspension. Excess fluid on the swab was removed by pressing it and rotating the swab against the side of the test tube above the level of the suspension. The wet swab was then used to inoculate the Muller-Hinton agar. The inoculum was evenly spread over the

surface of the medium to ensure even distribution. The agar was left for 3-5 minutes for the surface moisture to dry.

Antibiotic discs (multidisc) were applied to the surface of the inoculated agar plate using a pair of forceps and incubated overnight at 37°C. The zones of inhibition for each antibiotic was measured in millimeters and compared with values provided by the Clinical and Laboratory Standards Institute (CLSI) presented in chart 4. The result for the antibiotic was reported for the organism as resistant, intermediate resistant or sensitive (CLSI, 2003). *Escherichia coli* (ATCC 25922) was used for quality control.

Chart 4. The interpretative chart for the zone of inhibition of the antibiotics used

Antibiotics	Diameter of zone of inhibition (mm)		
	Susceptible	Intermediate resistant	Resistant
Ampicillin	≥ 17	14-16	≤13
Cefotaxime	≥23	15-22	≤14
Ceftriaxone	≥23	15-22	≤14
Cefuroxime	≥23	15-22	≤14
Chloramphenicol	≥18	13-17	≤12
Tetracycline	≥19	15-18	≤14
Gentamicin	≥15	13-14	≤12
Amikacine	≥15	13-14	≤12
Co-trimoxazole	≥16	11-15	≤10

3.6 BETA-LACTAMASE PRODUCTION TEST

To perform the beta-lactamase production test, a strip of Whatman No. 1 filter paper was placed in the bottom of a petri dish and a few drops of buffered crystalline penicillin bromocresol purple solution was added until the paper is almost saturated. Using a sterile wire loop, 10-20 colonies of the test organism were spread on the filter paper, covering an area approximately 5mm in diameter. The lid of the petri dish was replaced and the test was incubated at 37°C for up to 30 minutes. The filter paper spots were examined for a colour change. Beta-lactamase production was indicated by colour change from purple-blue to yellow. Non-beta lactamase producing organisms produced no colour change.

CHAPTER FOUR

4.0 RESULTS

4.1 TYPES OF *PROTEUS SPECIES* ISOLATED FROM CLINICAL SPECIMENS OF THE PATIENTS STUDIED

From November 2006 through July 2007, a total of 2369 clinical samples were tested. Out of this number, two hundred (200) *Proteus species* were isolated. Clinical samples tested included blood, wound swab, ear swab, urine, sputum and aspirates (joint aspirate, pleural fluid, ascitic fluid, pus). Three different *Proteus species* were identified. These were *Proteus mirabilis*, *Proteus vulgaris* and *Proteus penneri*. They were distributed among patients from the outpatient department (OPD), mother baby unit (MBU), pediatric emergency unit (PEU), block A (mostly obstetrics and gynaecology), block B (unit for children which handles cases including surgery, accident and tumour), block C (accident/surgical unit) and block D (medical unit including ear, nose, throat and burns units)

The 200 *Proteus species* were isolated from 86(43%) males and 114(57%) females. The ages of the patients ranged between 1 day and 90 years. *Proteus mirabilis* was the most commonly isolated *Proteus species* in all the age groups. The highest *Proteus* infection was detected in individuals whose ages ranged between 1-9 years.

Proteus mirabilis was the most frequently isolated *Proteus species* accounting for 61.5% (123/200) of all the *Proteus species* followed by *Proteus vulgaris* 30.5% (61/200), with

Proteus penneri 8.0% (16/200) being the least of all the *Proteus species* isolated as shown in figure 1 below

Figure 1. Distribution of the various *Proteus species* isolated

4.2 DISTRIBUTION OF THE *PROTEUS SPECIES* IN IN-PATIENTS AND OUT - PATIENTS

The *Proteus species* were isolated from 118 (59%) out-patients and 82 (41%) in-patients. In the case of in-patients, block D (medical unit) had the highest number of *Proteus* isolates registering 11.5% whilst Pediatric Emergency Unit (PEU) had the least (2.0%). Both blocks B (childrens unit) and C (accident/surgical unit) had 10% *Proteus* isolates each. Block A (obstetrics and gynaecology unit) had 3.5% whilst Mother Baby Unit (MBU) had 4.0% of the *Proteus* isolates. These values are shown in table 1.

Table 1: Distribution of the *Proteus species* in the various blocks, units and OPD

Place	Species			Total	
	P.mirabilis	*P. vulgaris*	*P. penneri*	Number	Percentage
Block A	3	3	1	7	3.5
Block B	9	8	3	20	10
Block C	11	5	4	20	10
Block D	19	3	1	23	11.5
MBU	6	2	0	8	4.0
PEU	3	1	0	4	2.0
OPD	72	39	7	118	59
TOTAL	123	61	16	200	100

4.3 DISTRIBUTION OF THE *PROTEUS SPECIES* IN CLINICAL SPECIMENS

The distribution of *Proteus species* in the clinical samples is shown in table 2. Most isolates of *Proteus species* (129) were obtained from wound swabs. Out of the 129 *Proteus* isolates, 38.5% were *Proteus mirabilis*, 20.0% were *Proteus vulgaris* and 6.0 % were *Proteus penneri*. The over all prevalence in wound was 64.5%.

Ear swab was the second sample yielding the most frequent *Proteus* isolates, followed by blood, pus and urine in this order. Prevalence of the *Proteus species* in ear infections was

13.5%. Distribution of the *Proteus species* in ear swab samples revealed that percentage distribution of both *Proteus mirabilis* and *Proteus vulgaris* were similar registering 6% and 6.5% respectively. Though 6% of *Proteus mirabilis* were isolated from blood only 1.5% of *Proteus vulgaris* were isolated.

Samples such as urine, joint aspirate, sputum, ascitic fluid and pleural fluid did not yield any *Proteus penneri*. The other samples also registered very low numbers for the organism. Other clinical samples such as sputum and the aspirates yielded very low numbers for all the *Proteus species* isolated (table 2).

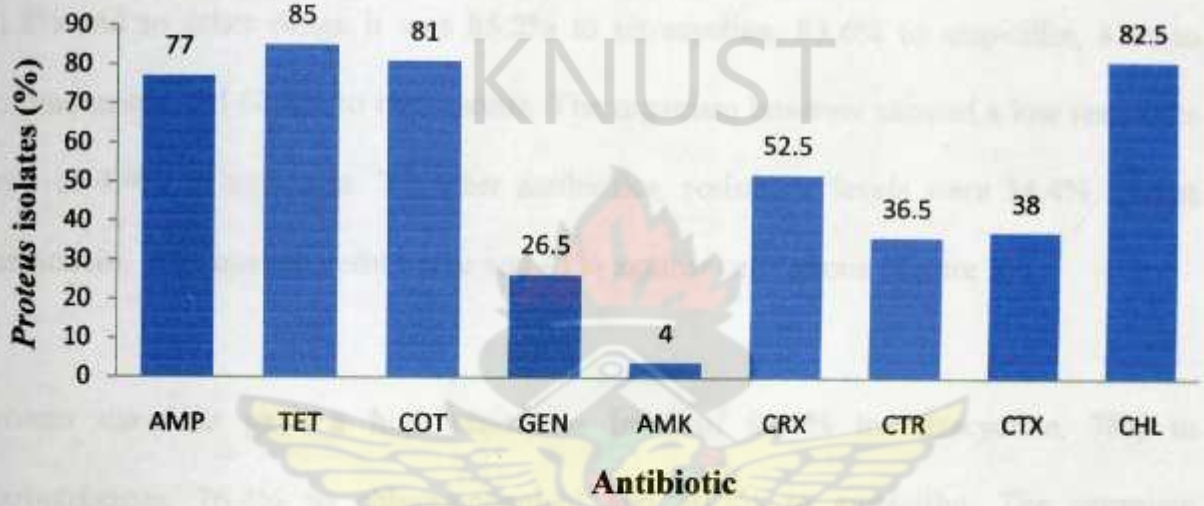
Table 2: Distribution of the *Proteus species* in the clinical specimens

Specimen	*P.mirabilis*		*P. vulgaris*		*P. penneri*		Total	
	Number	%	Number	%	Number	%	Number	%
Wound Swab	77	38.5	40	20.0	12	6.0	129	64.5
Ear swab	12	6	13	6.5	2	1	27	13.5
Urine	7	3.5	0	0	0	0	7	3.5
Blood	12	6	3	1.5	1	0.5	16	8.0
Pus	6	3	2	1	1	0.5	9	4.5
Joint aspirate	4	2	1	0.5	0	0	5	2.5
Sputum	3	1.5	1	0.5	0	0	4	2.0
Ascitic fluid	2	1	0	0	0	0	2	1.0
Pleural fluid	0	0	1	0.5	0	0	1	0.5
Total	123	61.5	61	30.5	16	8.0	200	100

4.4 ANTIBIOTIC RESISTANCE AMONG THE VARIOUS *PROTEUS SPECIES*

All the *Proteus* isolates showed a high resistance of 85% to tetracycline, 82.5% to chloramphenicol, 81% to cotrimoxazole and 77% to ampicillin. They showed a very low resistance of 4% to amikacin. Their resistance levels to other antibiotics were 26.5% to gentamicin, 36.5% to ceftriaxone, 38% to cefotaxime and 52.5% to cefuroxime as shown in figure 2 below.

Figure 2. Antibiotic resistance levels of the *Proteus* isolates



Legend: AMP-Ampicillin, TET-Tetracycline, COT-Co-trimoxazole, GEN-Gentamicin, AMK-Amikacin, CRX-Cefuroxime, CTR-Ceftriaxone, CTX-Cefotaxime, CHL-Chloramphenicol.

The various species of *Proteus* were also tested and their antimicrobial sensitivity levels differ among the species identified, which were *Proteus mirabilis, Proteus vulgaris* and *Proteus penneri*.

All the *Proteus penneri* isolates were resistant to ampicillin, tetracycline and cotrimoxazole, with none at all being sensitive to those antibiotics. To other antibiotics, *Proteus penneri* resistance levels were 93.8% to both chloramphenicol and cefuroxime, 68.8% to cefotaxime, 56.3% to both gentamicin and ceftriaxone and finally 12.5% resistance to amikacin (Figure 3).

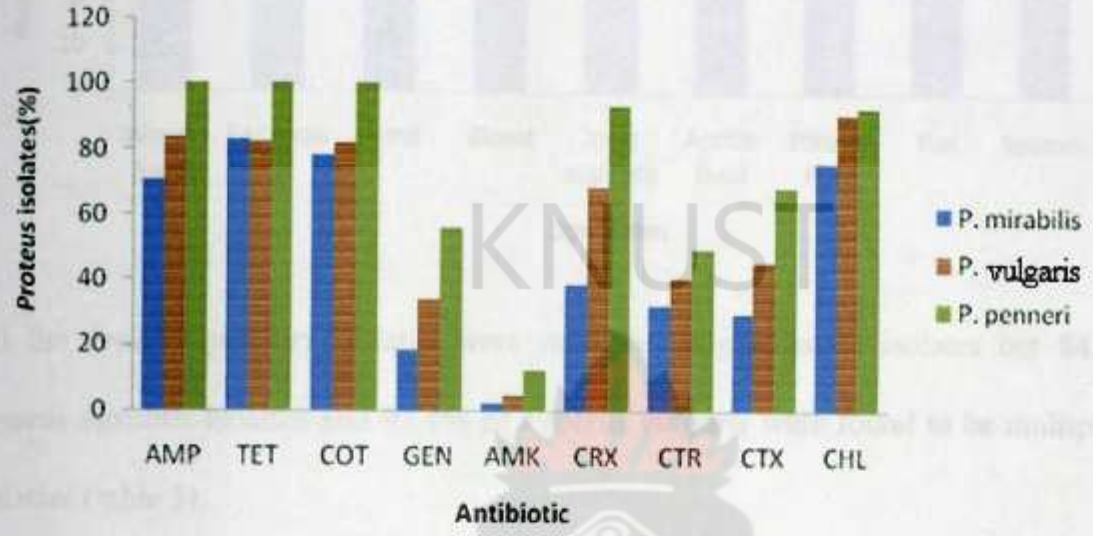
Proteus vulgaris resistance levels were high to chloramphenicol, where it registered 91.8% and to other drugs it was 85.2% to tetracycline, 83.6% to ampicillin, 82% to cotrimoxazole and 68.8% to cefuroxime. The organism however showed a low resistance level of 4.9% to amikacin. To other antibiotics, resistance levels were 34.4% against gentamicin, 46% against cefotaxime and 41% against ceftriaxone (Figure 3).

Proteus mirabilis gave a high resistance level of 82.9% to tetracycline, 78% to cotrimoxazole, 76.4% to chloramphenicol and 70.7% to ampicillin. The organism however showed lower resistance level of 37.5% to cefuroxime, 32.5% to ceftriaxone and 30% to cefotaxime, and a low resistance of 18.6% and 2.4% to gentamicin and amikacin respectively (Figure 3).

Antimicrobial resistance comparisons among all the *Proteus species* identified (*P. mirabilis, P. vulgaris* and *P. penneri)* was found to be statistically insignificant ($P > 0.05$), so also antimicrobial resistance comparison between two species was also statistically non significant ($P > 0.05$). The excel output for the calculation of ANOVA is presented in

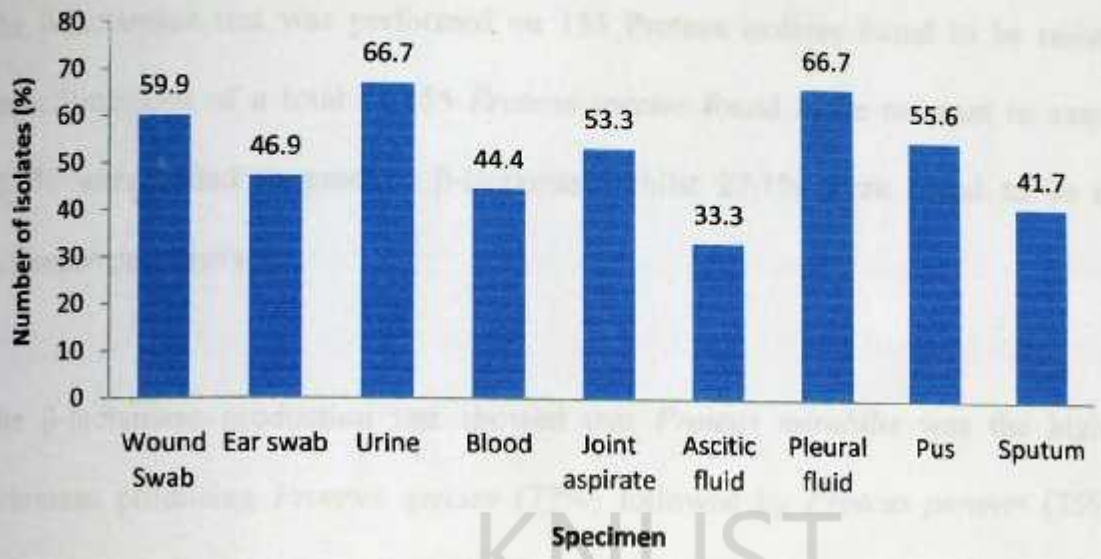
appendix D. The antimicrobial resistance among the species identified is presented in figure 3.

Figure 3. Antimicrobial resistance among the species of proteus isolates



Level of antibiotic resistance of the isolates to the antibiotics tested (ampicillin, tetracycline, chloramphenicol, cefuroxime, ceftriaxone, cefotaxime, gentamcin, amikacin and co-trimoxazole) among the different clinical specimens is presented in figure 4 below. The highest resistance levels were found among urine and pleural aspirates where 66.7% resistance was registered. Wound isolates was the second highest with resistance level of 59.9%. The resistance levels within other specimens were pus (55.6%), joint aspirate (53.3%), ear swab (46.9%), blood (44.4%), sputum (41.7%) and ascitic fluid (33.3%).

Figure 4. Antibiotic resistance level amongst specimens



All the *Proteus penneri* isolates were multiple drug resistant isolates but 84.6% of *Proteus mirablis* isolates and 93.4% of *Proteus vulgaris* were found to be multiple drug resistant (table 3).

Table 3: Multiple drug resistant (MDR) *Proteus* isolates

Organisms isolated	Total isolates	No. of isolates found to be MDR	% MDR isolates
P.mirabilis	123	104	84.6
P. vulgaris	61	57	93.4
P. penneri	16	16	100
Total	200	177	88.5

NOTE: "Multiple-drug resistance" was defined as resistance to at least 3 antibiotics

4.5 BETA-LACTAMASE PRODUCING *PROTEUS SPECIES*

The β-lactamase test was performed on 155 Proteus isolates found to be resistant to ampicillin. Out of a total of 155 *Proteus species* found to be resistant to ampicillin, 72.9% were found to produce β-lactamase whilst 27.1% were found to be non β-lactamase producers.

The β-lactamase production test showed that *Proteus mirabilis* was the highest β-lactamase producing *Proteus species* (77%) followed by *Proteus penneri* (75%) and *Proteus vulgaris* (64.5%) as shown in table 4 below.

Table 4: β-lactamase production by the various *Proteus species*

Species	No. tested for β-lactamase production	No. positive	% positive	No. negative	% negative
P.mirabilis	87	67	77	20	23
P. vulgaris	52	34	65.4	18	34.6
P. penneri	16	12	75	4	25
Total	155	113	72.9	42	27.1

CHAPTER FIVE

5.1 DISCUSSION

Species identification and surveillance of antimicrobial resistance are essential in management and control of infections. These practices are usually not done in most of our hospitals mainly due to limited resources, time and labour. This problem of infection control can be addressed by carrying out frequent studies in that direction and making the findings available to health planners and other health authorities, for them to adopt strategies towards the control and management of infections in our communities. This study aims at investigating the species of *Proteus* responsible for infections and their antimicrobial susceptibilities at the Komfo Anokye Teaching Hospital (KATH).

5.1.1 *Proteus species* isolated from the specimens tested

Three *Proteus species* were identified to be responsible for causing infections in various specimens such as wound, blood, urine, ear, sputum and aspirates at KATH. These isolates were *Proteus mirabilis*, *Proteus vulgaris* and *Proteus penneri*. *Proteus mirabilis* was the most common species isolated, accounting for 61.5% of all the *Proteus species*, followed by *Proteus vulgaris* (30.5%) with the least being *Proteus penneri* (8.0%). This result is similar to a report by Jones *et al.*, (2003) in a study carried out in England, Wales and Northern Ireland, where 81% of *Proteus mirabilis*, 18.4% of *Proteus vulgaris* and 0.6% of *Proteus penneri* were isolated from the patients studied. The high frequency of isolation of *Proteus mirabilis* as compared to the other species shows that the organism is responsible for majority of *Proteus* infections as reported by Chow *et al.*, (2005).

The highest percentage of *Proteus* isolates (64.5%) was obtained from wound. This result is consistent with the findings of Newman *et al.*, (2006) who also isolated the majority of *Proteus species* (47.6%) from wounds of patients in Ghana. Another study from Nigeria by Yah *et al.*, (2007) also found *Proteus species* as the most common bacteria isolated from wounds at a rate of 41.9%. These findings show that *Proteus* is a common cause of wound infections in Ghana and other parts of Africa and therefore must be given the necessary attention.

Results from other parts of the world, especially Europe (Reslinski *et al.*, 2005) and Asia (Chung *et al.*, 1999) have shown that *Proteus species* are more commonly encountered in urine than all other clinical specimens, however, my findings at KATH showed that urine yielded only 3.5% of the total number of *Proteus species* isolated from the patients studied as compared to the 64.5% obtained from wounds.

Proteus mirabilis was the only *Proteus species* encountered in urine. Other *Proteus species* (*vulgaris* and *penneri*) were not isolated from urine. This finding agrees with other reports (Nawa's *et al.*, 1994; Chung *et al.*, 1999; Reslinski *et al.*, 2005) which indicated that *Proteus mirabilis* is the most commonly encountered *Proteus species* responsible for urinary tract infections caused by *Proteus species* and that *Proteus vulgaris* and *Proreus penneri* infections in urine are rare (Foxman, 2000). Also, according to Mobley (1996), *Proteus mirabilis* has a higher propensity for colonizing the

urinary tract than *Proteus vulgaris* and *Proteus penneri* due to difference in its pathogenicity.

Clinical specimens such as sputum and pleural fluid had very low number of *Proteus* isolates. This is an indication that *Proteus species* do not commonly cause infection in such clinical sites, a finding which was reported by Roberts (1999) that *Proteus species* are not a common cause of respiratory infections.

5.1.2 Distribution of the *Proteus species* among the patients studied

Distribution of the *Proteus species* among the patients studied indicated that the *Proteus species* were isolated more from out-patients (59%) than from in-patients (41%). This trend is similar to the finding of Chung *et al.*, (1999) who isolated 68.9% *Proteus species* from out-patients and 31.1% *Proteus species* from in-patients.

Blocks B (childrens unit), C (accident/surgical unit) and D (medical unit) had more patients with wound infections than the other blocks. This was not unexpected, as these are blocks with burns, accident and surgical units of the hospital. *Proteus* is known to be a common cause of post operative wound and burns infections (Kolker *et al.*, 2004). These infections can be acquired nosocomially as it can be transferred from equipment or the hands of hospital staff (Kolker *et al.*, 2004).

Mother baby unit (MBU) which accommodates babies, expectedly had most of the isolates obtained from blood (7/16, 43.8%) since babies are more likely prone to sepsis (Martin *et al.*, 2003).

5.1.3 Distribution of the *Proteus species* in gender and different age groups

Two hundred (200) *Proteus species* were isolated with 86(43%) obtained from males and 114(57%) from females. There was no significant difference ($p > 0.05$) between the males and females infected with *Proteus* in this study.

The *Proteus* infections were detected in all age groups with 1-9 years registering as the highest group infected (17.5%). It was detected in this study that most of the individuals in the 1-9 years group were below age two, supporting the assertion that infants are more prone to infections in general, as shown by *Proteus species* due to their weak immune system (Blummer *et al.*, 1994). The weakness of the immune system of infants also explains the reason why more blood isolates (7/16, 43.8%) were obtained in the mother baby unit.

5.1.4 Antibiotic resistance pattern of the *Proteus species* isolated

The *Proteus species* isolated were found to have high antimicrobial resistance. The antimicrobial resistance were against four antimicrobial agents, namely tetracycline (85%), chloramphenicol (82.5%), cotrimoxazole (81%) and ampicillin (77%). Similar results were obtained in Ghana by Newman *et al.*, (2006), where they had 82% of the

isolates being resistant to tetracycline, 76% resistant to ampicillin, 75% resistant to chloramphenicol and 73% resistant to co-trimoxazole. The resistance levels which are consistently obtained put ampicillin, tetracycline and co-trimoxazole in a category where they may no more be suitable for empirical treatment of *Proteus* infections and perhaps other infections too. Though chloramphenicol is not a drug of choice for the treatment of *Proteus* infections, its low activity against the *Proteus* isolates is an indication of what the resistance level to it may be among the entrobacteriaceae, and perhaps among the *Salmonellae*. This study and the study of Newman *et al.*, 2006 have shown that there is rapid increase in the prevalence of resistance in *Proteus species* to most of the older, less expensive antimicrobial drugs used in the management of infections in Ghana. These inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (Newman *et al.*, 2006). Indiscriminate ingestion of antibiotics is known to provide selective pressure leading to a higher prevalence of resistant bacteria (Levy *et al.*, 1999). Not only are these species potential causes of infections but they are also potential reservoirs of resistance genes that could be transferred to pathogens. For this reason, the trend seen with *Proteus species* may also occur with other pathogenic organisms. According to Hodge *et al.*, 1998, enteric pathogens are showing increasing trend in antimicrobial resistance.

The *Proteus species* isolated showed resistance of 4% to amikacin, 26.5% to gentamicin, 36.5% to ceftriaxone and 38% to cefotaxime. These are injectable antibiotics and with the

exception of gentamicin, have been on the Ghanaian market for a relatively shorter period of time as compared to ampicillin and chloramphenicol. Again in addition to being injectables, amikacin, cefotaxime and ceftriaxone are very expensive and are usually more difficult to administer as compared to ampicillin and chloramphenicol which are cheap capsules. These might be some of the reasons for the relatively low levels of resistance seen in this study to these injectables antibiotics. According to Newman *et al.*, (2006) in Ghana, Ozumba *et al.*, (1992) in Nigeria, Tenssaie (2001) in Ethiopia and Reslinski *et al.*, (2005) in Poland, the suitable antibiotics for treating *Proteus* infections are amikacin, gentamicin, ceftriaxone and cefotaxime. The usefulness of these drugs will, however depend on effective interventions to halt the spread of reistance species among enteric bacterial pathogens to these drugs (Levy, 1999).

Proteus penneri was the isolate that showed the highest resistance to the antimicrobial agents tested. This was followed by *Proteus vulgaris*, with *Proteus mirabilis being* the species with the least resistance. Vourli *et al.*,(2006) similarly reported that among the various *Proteus species*, *Proteus mirabilis* are generally more susceptible to antimicrobial agents than are *Proteus vulgaris* and *Proteus penneri*. Gangoue *et al.*, (2000) in a similar study also reported *Proteus mirabilis* as the most sensitive *Proteus species,* showing only 2% resistance to both cefotaxime and ceftazidime.

5.1.5 Levels of antibiotic resistance in the various specimens

Proteus species isolated from urine produced the highest resistance level of 66.7% followed by wound (59.9%) and pus (55.6%). The relatively high resistance of *Proteus* isolated from urine agrees with reports from many parts of the world, where in all cases *Proteus species* from urine showed very high resistance against various antimicrobial agents tested. For instance, Nwanze *et al.*, (2007) reported *Proteus vulgaris* isolated from urine to be 90% resistant to gentamicin, 95% to tobramycin, 95% to tetracycline and 95% to cefuroxime. In the case of wound infections, since most of the cases were out-patient cases as indicated in table 5 (Appendix C) , it may be due to the reason that patients usually undergo self medication before they report to the hospital because of the subtle nature by which these infections start (Kolker *et al.*, 1999). This practice could result in an increase in the antibiotics resistance level of the pathogen, since the usage of the appropriate antibiotics in their correct dosage is always a problem (Ohene, 1997).

5.1.6 Multiple drug resistant (MDR) *Proteus* isolated

Many of the *Proteus species* isolated were found to be multiple drug resistant. Out of the 200 *Proteus species* isolated, 177 representing 88.5% were found to be multiple drug resistant (resistance to at least three antibiotics). This value is a little higher than the finding of Newman *et al*, (2006), who reported the multiple drug resistant *Proteus* isolates in Ghana at 55.9%. The high level of MDR *Proteus species* isolated in this study is an indication that a very large proportion of the *Proteus species* have probably been exposed to several antibiotics, as KATH is a tertiary hospital and patients usually report

with cases of infections referred from other hospitals who might have been treated with antibiotics elsewhere before being transferred to KATH, but the study by Newman *et al.*, (2006), covered almost the whole of Ghana including both district and regional hospitals. This might be one of the reasons for the relatively high level of MDR at KATH, as compared to that for almost the whole country.

All the *Proteus penneri* isolates were found to be multiple drug resistant whilst 84.6% of *Proteus mirabilis* and 93.4% of *Proteus vulgaris* were multiple drug resistant strains respectively. The alarmingly high prevalence of MDR obtained for the various species is a reflection of inappropriate use of antimicrobials, probably leading to widespread resistant plasmid genes among the species. Thus, rational use of drugs should be practiced (Yah *et al.*, 2007)

5.1.7 β-lactamase production by the various *Proteus species*

It was found that about 72.9% of the *Proteus species* isolated produced β-lactamase. The β-lactamase test showed that about 77% of the *Proteus mirabilis* isolates produced β-lactamase followed by *Proteus penneri* (75%) and *Proteus vulgaris* (64.5%) indicating that β-lactamase production is common among the *Proteus* isolates. A similar study from Italy by Pagani *et al.*, (2002) on beta-lactamase production by *Proteus mirabilis* using nitrocefin test revealed that out of the 147 (52%) isolates found to be resistant to ampicillin, all were beta-lactamase producers, showing results higher than what was obtained in this study. Another study by Opferkuch and Cullmann (2005), on beta-

lactamase production by *Proteus vulgaris* resistant to ampicillin, showed that 87.9% of the *Proteus vulgaris* resistant to ampicillin were beta-lactamase producers, also indicating high beta-lactamase production. These results and the results from this study show that beta-lactamase production by *Proteus species* correlates with clinical failure of ampicillin therapy (Pagani *et al.*, 2002).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

This study identified *Proteus mirabilis*, *Proteus vulgaris* and *Proteus penneri* as the *Proteus species* responsible for the various *Proteus* infections at KATH. *Proteus mirabilis* was the commonest species isolated followed by *Proteus vulgaris*, with *Proteus penneri* being the least species isolated. The isolates were found in both the inpatients and the cases from the community, but there was no significant difference (P> 0.05) between them. Similarly, the isolates were common among both sexes. The isolates were found among all age groups with 1-9 years age group registering the highest number of isolates. Blocks D (medical unit), B (childrens unit) and C (accident/surgical unit) were the most *Proteus* infection prone blocks at KATH, recording the highest cases of *Proteus* infections in that order.

This study also identified wound as the most common specimen from which the *Proteus* isolates were obtained. The various species were widespread among the clinical specimens tested, however *Proteus vulgaris* and *Proteus penneri* were not detected in urine samples. Again, *Proteus penneri* was not isolated from sputum, joint aspirate as well as pleural and ascitic fluids.

Proteus penneri was identified as the species with the highest resistance to the antibiotics tested followed by *Proteus vulgaris* with the least being *Proteus mirabilis*. It is of interest to note that the commonest species responsible for infections is more susceptible to

antibiotics than the less common ones, as *Proteus penneri* was found to be the most drug resistant species of *Proteus* responsible for human infections.

The study also revealed that, aminoglycosides such as amikacin and gentamicin, and third generation cephalosporins such as ceftriaxone and cefotaxime were the antibiotics to which the *Proteus* isolates were sensitive whilst ampicillin, tetracycline, chloramphenicol and co-trimoxazole had very high percentages of the *Proteus* isolates being resistant to them.

A large percentage (88.5%) of the *Proteus species* isolated were found to be multiple drug resistant strains and also produced beta-lactamase as one of the mechanisms for acquiring resistance to ampicillin. The highest beta-lactamase producing species was found to be *Proteus mirabilis* though the multidrug resistant strains were found among *Proteus penneri*. This study has demonstrated that antimicrobial resistance is widespread among the *Proteus species* isolated. Also, multidrug resistant species are also common and widespread among all clinical samples tested.

In order to generate national data on the prevalence of antimicrobial resistant isolates, it is necessary to equip regional and district laboratories with culture and sensitivity facilities as well as providing them with well trained personnel to help generate reliable resistance data in the country. In addition, it will be necessary to set up quality control mechanism before one could get reliable resistance data in the country.

It is also recommended that national surveillance programme on antimicrobial resistance of bacterial isolates must be instituted. Information from these surveillance programmes would serve as epidemiological data for health planners. It will also be a reference guide for clinicians and other prescribers when they choose antibiotics for emperical treatment and also for researchers. For the meantime, laboratory generated antimicrobial sensitivity should be relied upon to guide prescribers in the choice of antibiotics for the treatment of bacterial infections including infections caused by *Proteus species*.

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APPENDIX A

Liquid Media

I. Peptone Water

Peptone water may be used as a growth medium or as the basis of carbohydrate fermentation media, whilst pure culture peptone water is a convenient inoculum for a series of fermentation tubes or other diagnostic media.

Mode of Action

The amino acid tryptophan is found in nearly all proteins. Bacteria that contain the enzyme tryptophanase can hydrolyse tryptophan to its metabolic products namely; indole, pyruvic acid and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs. Indole is not used and accumulates in the medium. The presence of indole can be detected by the addition of Kovacs' reagent. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium. Bacteria producing a red layer following addition of Kovacs' reagent are indole positive, the absence of a red colour indicates tryptophan was not hydrolyzed, and the bacteria are indole negative (Downes and Ito, 2001).

II. Kovac's Reagent

This reagent is used for the detection of indole. It is prepared by dissolving 10 grams of 4-dimethylamino-benzaldehyde in 150ml of iso-amyl alcohol. After dissolution 50ml of concentrated hydrochloric acid is added to it. It is then stored in a refrigerator in an amber bottle.

III. Maltose Fermentation Broth

This is a medium for the study of fermentation reactions of microorganisms which serve as a basis for identifying and differentiating between various microorganisms.

Mode of Action

This medium demonstrates the ability of micro-organisms to utilize maltose. Degradation of maltose results in the production of acid, the presence of which can be detected by the colour change of the pH indicator (methyl orange) in the medium from orange to yellow and formation of gas which is detected by bubble formation in a Durham tube placed in the medium. When there is no fermentation, the pH indicator remains orange but there can still be growth due to the use of amino acids as sources of energy usually by respiration. When there is fermentation with the production of acid (yellow colour) but no gas, a slight amount of acid is seen in the tube, but fermentation is still recorded for this tube. Fermentation with the production of acid (yellow colour) and insoluble gas (bubble in Durham tube) is simply due to deamination of amino acids whose alkaline reaction has not been over-neutralized by the acid diffusing through the tube from fermentation.

IV. Brain Heart Infusion Broth

A highly nutritious infusion medium recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. Suitable for blood culture work.

Mode of Action

Brain Heart Infusion is essentially a buffered infusion broth giving similar results to the brain dextrose broths originally employed for cultivation of streptococci, and for the cultivation of dental pathogens.

V. Stuart Transport Medium

This is a transport medium for fastidious pathogenic organisms.

Mode of Action

This is a non-nutritional semi solid substrate for the preservation of *Neisserria* species and other fastidious organisms during their transport from clinic to laboratory. It may also be used fr transport of other bacteriological specimens.

VI. Turbidity Standard Solution

This is a barium sulphate standard against which the turbidity of the test and control inocula can be compared. When matched with standard, the inocula should give confluent or almost confluent growth.

Preparation

One percent solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water. Also, 1% solution of barium chloride was prepared by dissolving 0.5 grams of dehydrated barium chloride ($BaCl_2.2H_2O$) in 50ml of distilled water. Zero point six millitres of the barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed. Small volume was then transferred to a capped tube same as the type used for preparing the test and control inocula.

VII. Sample Storage

Glycerol broth was used to store the isolates.

Preparation

The broth was prepared by weighing 20% of brain-heart infusion broth. Distilled water and glycerol was added using the ratio of 4:1 distilled water to glycerol. The mixture was stirred until a uniform solution was obtained.A micropipette was used to pipette 1ml of the solution into Eppendorf tubes.The broth was then sterilized at 121°C for 15 minutes.

VIII. Buffured crystalline bromocresol purple broth

This medium is used to test for beta-lactamase production by acidometric method.

Composition	**Quantity**
Benzyl penicillin	125 g/L
NaOH.	1.25M
Bromocresol purple	0.1% (w/v)

Preparation

A whatman No. 1 filter paper was cut into 5 ×1cm strips and soaked in a freshly prepared solution containing 125g/L benzyl penicillin, 0.1% (w/v) bromocresol purple and 1.25M NaOH. The strips were dried and could be stored at 4°C for 6months with a silica gel desiccant. Such strips are available commercially. Before use, the strips must be moistened. Distilled water is recommended but it is essential that the water is not acidic.

Mode of Action

Bacteria from agar cultures were smeared on the strip and development of a yellow colour within 5 minutes indicates β- lactamase activity.

APPENDIX B

Solid Media, Gram staining and ZN staining techniques

I. Urea Agar

This medium is a composite one containing urea. Some micro-organisms are able to hydrolyze urea by producing hydrolyzing enzymes while others are not. It is therefore for the differentiation and isolation of micro-organisms that decompose urea.

Composition

The medium is prepared from urea agar base formulated by Oxoid Ltd. Basingstoke Hampshire, England to contain the following in grams per litre.

Mode of Action

When inoculated with organisms that decompose urea, the organism produces enzyme urease. Ths enzyme hydrolyses the urea to form carbon dioxide and ammonia (Handbook of microbiology, 1980). The ammonia produces an alkaline reaction in the medium which is indicated by a colour change of the p^H indicator, phenol red from yellow to purple.

II. MacConkey Agar

This is a differential medium for the differentiation and isolation of the *Enterobacteriaceae*. This medium also supports the growth of *Staphylococci* and *Enterococci*, but inhibits the growth of *Streptococci* and the *Haemophilus*. Swarming of *Proteus* is prevented.

Mode of Action

This medium is used as a differentiated medium, differentiating organisms into lactose and non-lactose fermenting organisms. The lactose-fermenting organisms, by the

fermenting of lactose, produce acids which act upon the bile salt and absorb the neutral red, giving red colonies. The non-lactose fermenting colonies give an alkaline reaction, do not absorb the neutral red and produce colourless colonies.

III. Cystine-Lactose-Electrolyte Deficient (CLED)

This medium is recommended for diagnostic urinary bacteriology. The medium supports the growth of all urinary potential pathogens giving good colonial differentiation and clear diagnostic characteristics.

Mode of Action

This medium supports the growth of all urinary pathogens and gives good colonial differentiations and clear diagnostic characteristics. The presence of important contaminants such as diphtheroids, lactobacilli and micrococci is also clearly elicited, giving an indication of the degree of contamination. In the laboratory CLED medium provides a valuable non-inhibitory diagnostic agar for plate culture of urinary organisms. It is electrolyte deficient to prevent the swarming of *Proteus* species.

IV. Blood Agar

This is an enriched medium used to culture most bacteria of medical importance.

Mode of Action

This is an enriched general purpose medium widely employed for the growth of pathogenic and non-pathogenic bacteria. Most bacteria of medical importance grow on this medium and the presence of intact red blood cells allows the haemolytic properties of organisms to be recognized.

V. Mueller-Hinton Agar

This is an antimicrobial susceptibility testing medium which may be used in internationally recognized standard procedures.

Mode of Action

Mueller-Hinton Agar was designed to be a reproducible culture medium for the isolation of pathogenic *Neisseria* species. The inclusion of starch ensures that toxic factors found during growth will be absorbed and its presence is often essential to establish growth from very small inocula.

VI. Nutrient Agar

This is a general purpose medium which may be enriched with 10% or other biological fluid.

Mode of Action

This is basic culture medium used to sub-culture organisms for maintenance purpose or to check the purity of subcultures from isolation plates prior to biochemical or serological tests.

VII Kligler Iron agar

Thi is a medium whose reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

Mode of Action

A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only. The slope is red-pink due to the reversion of the acid reaction under aerobic conditions. This reaction is seen with *Salmonella* and *Shigella* species and other enteric

pathogens Cracks and bubbles in the medium indicate gas production from glucose fermentation. Gas is produced by *S. paratyphi* and some faecal commensals. A yellow slope and yellow butt indicate fermentation of lactose and possibly glucose. This occurs *E. coli* and other Enterobacteriaceae. A red pink slope and butt indicate no fermentation of glucose or lactose. This is seen with most strains of *P. aeruginosa*. Blackening along the stab line or throughout the medium indicate hydrogen sulphide production, e.g. *S. typhi* produces a small amount of blackening whereas *S. typhimurium* causes extensive blackening.

Viii Simmon's Citrate Agar

This test is perfomed to see if certain bacteria will use citrate as their carbon source for their metabolic processes.

Mode of Action

The citrate test uses an agar medium with citrate and indicator Bromothymol blue present. At the pH of the un-inoculated medium, the colour is green. If the organisn can utilize citrate as a carbon source, the breakdown of citrate releases bicarbonate HCO3-) into the medium. The bicarbonate ions raise the pH of the medium above 7.4. This causes the Bromothymol blue indicator to turn dark blue in color.

Gram staining technique

The gram staining reaction is used to help identify bacteria in specimens and cultures by their gram reaction (gram-negative or gram-positive) and morphology. Pus cells can also be identified in gram smears.

Gram staining protocol

1. Make a smear of the specimen that is to be stained on a slide. Heat the slide for few seconds until it becomes hot to the touch so that bacteria are firmly mounted to the slide.

2. Add the primary stain crystal violet and wait 1 minute. Rinse gently with water. This step colors all cells violet.

3. Add Gram's iodine, for 30 seconds. It is not a stain; it is a mordant. It doesn't give color directly to the bacteria but it fixes the crystal violet to the bacterial cell wall. All cells remain violet.

4. Wash with ethanol and acetone, the decolorizer. If the bacterium is Gram-positive it will retain the primary stain. If it is Gram-negative it will lose the primary stain and appear colorless.

5. Add the secondary stain, safranin, and wait 1 min, and then wash with water for a maximum of 5 seconds. If the bacterium is Gram-positive then the cell will retain the primary stain, will not take the secondary stain, and will appear black-violet. If the bacteria is Gram-negative then the cell will lose the primary stain, take secondary stain, and will appear red-pink.

6. Examine the smear microscopically, first with the x40 objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

Ziehl-Neelson staining protocol

1. Heat-fixed the dried sputum smear on a glass slide.

2. Cover the smear with carbol fuchsin stain.

3. Heat the stain until vapour just begins to rise (ie. about 60°C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes.

4. Wash off the stain with clean water.

5. Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decoulorized.

6. Wash well with clean water.

7. Cover the smear with malachite green stain for 1-2 minutes, using the longer time when the smear is thin.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.

10. Examine the smear microcopically, using the x100 oil immersion objective.

Appendix C

Table 5. Distribution of *Proteus species* in various wards, outpatient department and specimen types

	Blood (n=853)			Wound (n=541)			Urine (n=516)			Ear swab (n=76)			Sputum (n=205)			Aspirates (n=170)			Total (n=2369)			
ace	P.m	P.v	P.p	P.m	P.v	P.p	P.m	P.v	P.p	P.m	P.v	P.p	P.m	P.v	P.p	P.m	P.v	P.p	P.m	P.v	P.p	No.
ock A	0	2	0	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	3	3	1	7
ock B	2	0	0	6	7	1	0	0	0	0	1	1	0	0	0	1	0	1	9	8	3	20
ock C	0	0	1	7	4	3	0	0	0	3	0	0	0	0	0	1	1	0	11	5	4	20
ock D	0	0	0	10	3	1	1	0	0	1	0	0	0	0	0	7	0	0	19	3	1	23
CU	2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	1	0	4
BU	6	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	2	0	8
PD	2	0	0	50	24	6	6	0	0	8	11	1	3	1	0	3	3	0	72	39	7	118
otal	12	3	1	77	40	12	7	0	0	12	13	2	3	1	0	12	4	1	123	61	16	200

Key: P.m = *Proteus mirabilis*; P.v = *Proteus vulgaris*; P.p = *Proteus penneri*, MBU = mother baby unit; PEU = pediatric emergency unit; OPD = outpatient department; n = number of clinical specimens cultured

APPENDIX D : EXCEL ANALYSIS

Anova calculation to compare the distributin of the *Proteus species* between male and female sexes

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	43	14.33333	145.3333
Column 2	3	57	19	219.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32.66667	1	32.66667	0.1792	0.693831	7.708647
Within Groups	729.1667	4	182.2917			
Total	761.8333	5				

Anova calculation to compare the distribution of *Proteus species* in In-patents and out-patients

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	100	33.33333	756.3333
Column 2	3	99.8	33.26667	681.6133

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006667	1	0.006667	9.27E-06	0.997716	7.708647
Within Groups	2875.893	4	718.9733			
Total	2875.9	5				

APPENDIX D : EXCEL ANALYSIS

Anova calculation on the comparison of antimicrobial resistance between all three *Proteus* species

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	430.5	47.83333	878.5725
Column 2	9	535	59.44444	860.1853
Column 3	9	675.1	75.01111	938.1261

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3347.312	2	1673.656	1.875676	0.175035	3.402826
Within Groups	21415.07	24	892.2946			
Total	24762.38	26				

Anova calculation on comparison of antimicrobial resistance between *P. mirabilis* and *P. penneri*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	430.5	47.83333	878.5725
Column 2	9	675.1	75.01111	938.1261

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3323.842	1	3323.842	3.659211	0.07383	4.493998
Within Groups	14533.59	16	908.3493			
Total	17857.43	17				

APPENDIX D : EXCEL ANALYSIS

Anova calculation on the comparison of antimicrobial resistance between *P. mirabilis* and *P. vulgaris*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	430.5	47.83333	878.5725
Column 2	9	535	59.44444	860.1853

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	606.6806	1	606.6806	0.697832	0.415814	4.493998
Within Groups	13910.06	16	869.3789			
Total	14516.74	17				

Anova calculation on the comparison of antimicrobial resistance between *P. vulgaris* and *P. penneri*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	535	59.44444	860.1853
Column 2	9	675.1	75.01111	938.1261

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1090.445	1	1090.445	1.212743	0.287081	4.493998
Within Groups	14386.49	16	899.1557			
Total	15476.94	17				