KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICS

ANTIBIOTIC RESISTANCE PATTERNS OF *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI* ISOLATES FROM THREE HOSPITALS IN KUMASI

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF PHILOSOPHY (M.PHIL.) DEGREE IN PHARMACEUTICAL MICROBIOLOGY

BY

DUREDOH FREEMAN GEORGE

APRIL, 2011

DECLARATION

I, Duredoh F. George hereby declare that this thesis, 'Antibiotic resistance patterns of *Pseudomonas aeruginosa* and *Escherichia coli* isolates from three hospitals in Kumasi' is my own work produced under supervision towards the award of Master of Philosophy (M.Phil.) Degree in Pharmaceutical Microbiology, and that, to the best of my knowledge, it has not been published in part or whole by anyone for degree elsewhere. All references herein have been duly acknowledged.

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ABBREVIATIONS

AIDS—Acquired Immune Deficiency Syndrome

CDC---Centers for Disease Control and Prevention

CLSI---Clinical and Laboratory Standards Institute

CNS----Central Nervous System

CSF-----Cerebrospinal Fluid

EMB----Eosin Methylene Blue

HUS---Hemolytic Uremic Syndrome

ICU----Intensive Care Unit

IV------Intravenous

KNUST—Kwame Nkrumah University of Science and Technology

LPS---------Lipopolysaccharides

MOH-------Ministry of Health

MRSA------Methicillin –resistant Staphylococcus aureus

NNIS-------National Nosocomial Infections Surveillance

USAID-----United States Agency for International Development

VRE---------Vancomycin-resistant Enterococci

WHO--------World Health Organization

ABSTRACT

Nosocomial infections are worrying situations in health care delivery across the world. Every year, millions of people report of contracting one form of nosocomial infections which are difficult to treat due to the level of antibiotic resistance exhibited by these microorganisms. In this study, a total of 600 samples including swabs of door handles, benches, beds, and floors, and waste water from drainages were collected from Tafo, Kumasi-South, and Suntreso Hospitals all in the Kumasi Metropolis between January and June, 2010 and cultured. By morphological and biochemical reactions, 57 strains of *Pseudomonas aeruginosa* and 97 strains of *Escherichia coli* were isolated, identified and tested for their antibiotic sensitivities using the Kirby-Bauer agar disc diffusion assay against ampicillin (10µg), gentamicin (10µg), ceftriaxone (30µg), ciprofloxazole (5µg), and co-trimoxazole (25µg) on Mueller-Hinton agar. *E. coli* was present in all the samples while *P. aeruginosa* was mainly present in samples from the floor, beds, drainages and door handles. All the *P. aeruginosa* isolates and about 90% of the *E. coli* isolates were resistant to ampicillin. For gentamicin, 46% of the *P. aeruginosa* isolates exhibited resistance while 21% and 33% showed intermediate and sensitive responses respectively. For ciprofloxacin, 36.84% of these isolates were resistant while equal proportions (31.58%) exhibited intermediate and sensitive responses. About 40% of the *P. aeruginosa* isolates also showed intermediate response to ceftriaxone while 39% and 21% were respectively resistant and sensitive. For gentamicin, many of the resistant *P. aeruginosa* isolates (34.6%) were obtained from door handle samples while majority of the sensitive isolates (47.4%) came from drainage samples. In the case of ceftriaxone, high proportions of drainage isolates were resistant (45.5%). Almost equal proportions of the drainage sample isolates recorded the various activities to ciprofloxacin: 38.9% sensitive, 38.1% resistant and 33.3% intermediate isolates. The study has also shown that out of 52 *E. coli* isolates from hospital beddings, 53.85% were resistant to gentamicin, 25% to ceftriaxone and 61.5% to ciprofloxacin. Similarly, out of 21 *P. aeruginosa* isolates from drainage samples, 28.57% were resistant to gentamicin, 47.62% to ceftriaxone and 28.57% to ciprofloxacin. Out of 97 *E. coli* isolates, 78(80.41%) were resistant to at least three different classes of antibiotics while 32(56.14%) out of 57 *P. aeruginosa* isolates were also resistant to at least three different classes of the antibiotics.

This study has therefore highlighted the presence of antibiotic resistant pathogens in our hospital environments including more especially, hospital beddings. It has also provided data on these resistant pathogens which will be useful in health care policy planning in Ghana and the sub-region at large.

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CHAPTER ONE

1.0 INTRODUCTION

Nosocomial infections are those infections acquired as a result of treatment in a hospital or health care service providing center. These infections usually appear 48 hours or more after hospital admission or within 30 days after discharge (Benenson, 1995). Nosocomial infections have been a major problem to health care delivery. These often result in prolonged recovery of patients and even death when not treated early. Different types of bacteria, fungi and viruses have been implicated in the development of nosocomial infections (Jones *et al*., 1999).

Several species of microorganisms have been isolated from different hospitals across the world (Markovic-Denic, 2009). Even though some of these organisms were not known for causing recalcitrant nosocomial infections, they are opportunistic pathogens and hence pose a challenge to patients especially those with immunocompromised conditions. Nosocomial infections usually encountered include urinary tract infection, pneumonia, tuberculosis, gastroenteritis, legionnaire's disease and Vancomycin-resistant Enterococci infections (Shears, 2007). Microorganisms usually implicated in these infections include among others *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella species*, *Mycobacterium tuberculosis*, and *Clostridium difficile*, which are rapidly gaining resistance because of the broad spectrum antibiotics used in an attempt to control them. Most of these organisms are usually contaminants on the surfaces of most materials such as doors, beds, instruments and on care providers. They are therefore easily transmitted to patients when adequate hygienic practices are not followed

regularly. Patients at the intensive care unit are the most at risk of these hospital-acquired infections (Gunseren *et al*., 1999; Kucukates, 2005). Even though several measures have been put in place by hospital officials to prevent these infections by ensuring strict sanitation, hygienic principles and rational antibiotic use, the incidence of nosocomial infections still keep rising. This study therefore seeks to investigate the antibiotic resistance patterns of strains of some of these organisms. This will contribute to data on the susceptibility of nosocomial bacteria to antibiotics in current use.

1.1 AIM OF STUDY

- To determine the antibiotic resistance patterns of *Pseudomonas aeruginosa* and *Escherichia coli* isolated from three selected hospitals in Kumasi.

1.2 SPECIFIC AIMS

- The specific objective of this study was to isolate *P. aeruginosa* and *E. coli* from various hospitals in Kumasi.
- To determine the sensitivity of the isolates to some commonly used antibiotics (gentamicin, ampicillin, ciprofloxacin, ceftriaxone and co-trimoxazole) in Ghanaian hospitals.

1.3 JUSTIFICATION

Nosocomial organisms are responsible for causing a myriad of hospital-acquired infections in humans. These organisms may present with different antibiotic resistance patterns. The identification of pathogenic organisms and their antimicrobial susceptibility

are not usually done during bacteriological diagnosis in most of our laboratories because more materials, time, and trained personnel are required; hence little information is available on the prevalence of these pathogenic organisms and their antibiotic sensitivity patterns. Results from this study will help health care providers and planners in the control and management of nosocomial infections in hospitals and other healthcare providing facilities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Nosocomial infections

Nosocomial infections are a serious health problem resulting in an enormous burden of morbidity and mortality rates, and high health care costs. Studies have indicated that nosocomial infections occurred in 5-10% of all hospitalized patients in Europe and North America and in more than 40% in parts of Asia, Latin America, and sub-Saharan Africa (Lynch *et al*., 1997; National Nosocomial infections surveillance, 2004).

At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov, 1987). Tikhomirov, (1987 reported that the highest frequencies of nosocomial infections were recorded from hospitals in the Eastern Mediterranean(11.8%) and South-East Asia Regions (10.0%), with a prevalence of 7.7% in the European Regions and 9.0% in the Western Pacific Regions. In many West African countries, infections abound but not much study has been done to determine the proportion that is acquired from hospital and or health care providing facilities. In Ghana, Newman (2009) conducted such a study and provided data on the occurrence of nosocomial infections in Korle-Bu Teaching Hospital in Accra. These studies are therefore necessary and need to be conducted in many other parts of the country, Ghana in order to generate national data on these microorganisms, more especially on their antibiotic resistant patterns.

The various microorganisms implicated in nosocomial infections can be classified as pathogenic or normal microbial flora of the human body. Bacteria are the most common of these micro-organisms. The normal flora found on the human body can become pathogenic when the host's natural immune system is compromised. For example, cutaneous coagulase negative *Staphylococcus species* can cause intravascular line infection and intestinal *Escherichia coli* are the most common cause of urinary tract infection in hospitalized patients.

The pathogenic micro-organisms on the other hand are virulent and can cause infections irrespective of the state of the host's immune system and these include many bacterial genera such as *Clostridium, Pseudomonas, Klebsiella,* and *Legionella*. Wang *et al,* (2010), isolated *Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia* coli and *Klebsiella* species from ten teaching hospitals in China and noted that *E. coli* strains were the highest and the most frequent in all the hospitals included in the study. In a similar work done by Markovic-Denic (2009) at a Serbian University Hospital, the most dominant pathogens were *Escherichia coli* and *Pseudomonas* species, followed by *Staphylococcus* species, *Proteus mirabilis,* Klebsiella species and *Enterobacter*.

Acquisition of antimicrobial resistance is the major anticipated problem in hospitals. *P. aeruginosa, Klebsiella, Enterobacter and* other gram-negative bacilli are known to harbor plasmids that code for the production of β-lactamases, which confer resistance on these organisms. These plasmids can be transferred to other bacteria which also become resistant. The increasing rates of antibiotic resistance among nosocomial bacteria are

contributing to lots of treatment failures and death (Slaughter *et al*., 1996; Bonten *et al.*, 1996).

2.1.2 The emerging trends of nosocomial infections

Three major factors are involved in nosocomial infections. The first is long-term antimicrobial use in hospitals and other health care facilities. The increased concern about gram-negative bacilli infections in the 1970s to 1980s led to increased use of cephalosporin antibiotics. As gram-negative bacilli became resistant to earlier generations of cephalosporin antibiotics, newer generations were developed. Widespread use of cephalosporin antibiotics is often cited as a cause of the emergence of enterococci as nosocomial pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA), perhaps also in response to extensive use of cephalosporin antibiotics became a major nosocomial threat. Widespread empiric use of Vancomycin in response to concerns about MRSA and for treatment of vascular catheter associated infection by resistant coagulase-negative *Staphylococci* was the major initial selective pressure for Vancomycin-resistant *Enterococci.* Use of antimicrobial drugs in long-term care facilities such as ambulatory and extended care settings and transfer of patients between these facilities and hospitals have created a large reservoir of resistant strains in nursing homes (Taconneli *et al.*, 2008).

Many hospital personnel also fail to follow basic infection control rules such as hand washing between patient contacts. In intensive care units, asepsis is often overlooked in the rush of crisis care (Weinstein, 1991). Lastly, the long-term use of vascular or other

device-related care in immunosuppressed patients has led to higher prevalence of bloodstream infections and ventilator-associated pneumonia (Archibald *et al.*, 1997).

2.1.3 Persons mostly affected by nosocomial pathogens

Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, medical or surgical treatments. Aging of our population and increasingly aggressive medical and therapeutic interventions including implanted foreign bodies and organ transplantations have created a cohort of particularly vulnerable persons (Weinstein, 1998). As a result, the highest infection rates are among intensive care unit (ICU) patients. Nosocomial infection rates in adult and pediatric ICUs are approximately three times higher than elsewhere in hospitals. The sites of infection and the pathogens involved are directly related to treatment in ICUs. In these areas, patients with invasive vascular catheters and monitoring devices have more bloodstream infections due to coagulase-negative staphylococci. Studies have shown that cases of occult bacteremia in ICU patients are probably due to vascular access-related infections (Fridkin *et al.*, 1997).

2.2 *P. aeruginosa* as a nosocomial bacterium

Traditionally, *P. aeruginosa* is a versatile Gram-negative bacterium that grows in soil, marshes and coastal marine habitats, as well as on plant and animal tissues (Hardalo and Edberg, 1997). *P. aeruginosa* grows well on cetrimide agar and also produces colourless colonies on MacConkey agar as it does not ferment lactose. Growth of *P. aeruginosa* on cetrimide agar may express the blue-green exopigment pyocyanin and the colonies

appear flat, large, and oval. It also has a characteristic fruity smell. *P. aeruginosa* can produce catalase, oxidase, and lipase enzymes. When grown on Triple Sugar Iron (TSI) medium, it does not change the color of the medium (Mahon *et al*, 2007). Other tests such as Enzyme-link Immunosorbent Assay (ELISA) and Polymerase Chain Reactions in addition to serological reactions are useful in the detection of the presence *P. aeruginosa* in specimen (Bartosova *et al*, 2006).

2.2.1 Characteristics of *P. aeruginosa*

P. aeruginosa has very simple nutritional requirements. It is often observed growing in distilled water, which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *Pseudomonas aeruginosa* consists of acetate as a source of carbon and ammonium sulfate as a source of nitrogen (Bevec, 2010).

Generally, the *pseudomonades* are renowned for metabolic versatility. *P. aeruginosa* does not require special organic growth factors as it can use over seventy-five organic compounds for growth (Todar, 2008). *P. aeruginosa* is a highly adaptable organism that can grow on a variety of substances and alters its properties in response to changes in the environment (Lambert, 2002).

Its optimum temperature for growth is 37 degrees Celsius but can tolerate temperatures as high as 42 degrees Celsius. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics. *P. aeruginosa* has a strong liking for growth in moist environments, which is probably a reflection of its natural existence in

soil and water. These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen. These infections are difficult to manage, in part because of the natural resistance of the bacterium to antibiotics, and ultimately lead to pulmonary failure and death (Stover *et al*., 2000).

P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence (Putty, 2007). Apart from pyocyanin pigment production; some strains of *pseudomonads* also produce pyoverdin, a soluble fluorescent pigment. The pyocyanin is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. In many cases, it is a characteristic pigment of suppurative infections caused by *P. aeruginosa* (Mahon *et al*., 2007).

2.2.3 Diseases caused by *P. aeruginosa*

P. aeruginosa has been implicated in a number of infectious diseases as listed below.

2.2.3.1 Endocarditis

P. aeruginosa infects heart valves of intravenous (IV) drug users and prosthetic heart valves by direct invasion from the blood stream (Zawacki, 2004).

2.2.3.2 Respiratory tract infections

Respiratory tract infections caused by *P. aeruginosa* occur almost exclusively in individuals with a compromised lower respiratory tract or a compromised systemic defense mechanism (Qarah, 2009). Primary pneumonia occurs in patients with chronic lung disease and congestive heart failure. Bodey *et al*. (1983) also stated *P.aeruginosa* is the predominant cause of morbidity and mortality in cystic fibrosis patients whose abnormal airway epithelia are colonized.

2.2.3.3 Bacteremia and septicemia

P. aeruginosa causes bacteremia primarily in immunocompromised patients. Predisposing conditions include hematologic malignancies, immunodeficiency relating to AIDS, neutropenia, diabetes mellitus, and severe burns (Pier, 2004).

2.2.3.4 Urinary tract infections

Urinary tract infections (UTIs) caused by *P. aeruginosa* are usually hospital-acquired and related to urinary tract catheterization, instrumentation or surgery. *P. aeruginosa* is the third leading cause of hospital-acquired UTIs, accounting for about 12 percent of all infections of this type (Qarah, 2009).

2.2.3.5 Central nervous system infections

P. aeruginosa causes meningitis and brain abscesses. The organism invades the CNS from a contiguous parameningeal structure such as an ear, a mastoid paranasal sinus surgery, or is inoculated directly by means of head trauma.

(Qarah, 2009).

2.2.3.6 Ear infections including external otitis

P. aeruginosa is the predominant bacterial pathogen in some cases of inflammation of the middle ear. It inhabits wet and humid ear (Todar, 2008).

2.2.3.7 Eye infections

P. aeruginosa can cause devastating infections in the human eye. It is one of the most common causes of bacterial keratitis, and has been isolated as the etiologic agent of neonatal ophthalmia. Pseudomonas can colonize the ocular epithelium by means of a fimbrial attachment to sialic acid receptors. If the defenses of the environment are compromised in any way, the bacterium can proliferate rapidly through the production of enzymes such as elastase, alkaline protease and exotoxin A, and may cause a rapid tissue destruction and eventual blindness (Pier, 2004).

2.2.3.8 Gastrointestinal infections

P. aeruginosa can cause disease in any part of the gastrointestinal tract (GIT) from the oropharynx to the rectum. As in other forms of *Pseudomonas* disease, those involving the GIT occur primarily in immunocompromised individuals. The organism has been

implicated in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis (Todar, 2008).

2.2.4 Virulence factors in *P. aeruginosa*

The ability of *P.* aeruginosa to produce overwhelming infections is due to its arsenal of virulence factors and excreted enzymes. These extracellular products cause extensive tissue damage, bloodstream invasion, and dissemination. Some of these extracellular factors include Exotoxin A and Exoenzyme S. Exotoxin A is responsible for local tissue damage and immunosuppression. It catalyzes ADP-ribosylation and inactivation of elongation factor 2 which leads to inhibition of protein biosynthesis and cell death. Exoenzyme S is produced by the bacteria growing in burned tissues and is detectable in the blood. It is responsible for tissue destruction in lung infection and may also be important in the organism's dissemination. *P. aeruginosa* also produces some proteases (LasB elastase, LasA elastase, and alkaline protease) which are able to destroy protein elastin found in human lung tissue. This therefore interferes with the expansion and contraction functions of the lungs (Galloway, 1991).

P. aeruginosa also has the ability to do cell-to-cell signaling and this enables it to control the production of its extracellular virulent factors and cell density. This cell-to-cell singling system is called the las system, because it regulates the expression of LasB elastase. The las system also helps in the optimal production of other extracellular virulence factors like LasA protease and exotoxin A (Gambello, 1993). The importance of cell-to-cell signaling is that through the coordinated expression of virulence genes by

the entire bacterial population, they secrete extracellular factors only when they could be useful, thus allowing it to overcome hosts defense mechanisms.

Some mutants of *P. aeruginosa* produce an exopolysaccharide called alginate. This is a slime matrix that forms biofilm in which its microcolony mutants grow. The biofilm protects the organism from the actions of antibiotics and disinfectants and hence allows the bacteria to survive under harsh conditions (Iglewski, 1998).

2.2.5 Mechanism of resistance in *P. aeruginosa*

Generally, antimicrobial resistance in *P. aeruginosa* is due to a combination of factors including low permeability of the cell wall, mutation in chromosomal genes which regulate resistance and acquisition of additional resistance genes from other organisms via plasmids, transposons and bacteriophages (Lambert, 2002; Poole, 2004). The failure of antibiotics to accumulate within the organism is due to a combination of restricted permeability of outer membrane and the efficient removal of antibiotic molecules that do penetrate by the action efflux pumps (Vogne *et al.*, 2004).

2.3 *Escherichia coli* as a nosocomial bacterium

E. coli was first described in 1885 by Theodor Escherich as *Bacterium commune*, which he isolated from the feces of newborns. It was renamed *E. coli* and considered to be a commensal of the large intestine. *Escherichia* species are gram-negative bacilli that exist singly or in pairs and are commonly present in the intestines of humans and animals. *E. coli* are facultative anaerobes and can undergo both fermentative and respiratory metabolisms. They are motile by peritrichous flagella.

In some hospitals, *E. coli* ranked first and second as the most common cause of community and hospital-acquired infections respectively (Berkley *et al*, 2005*)*. A strain of the bacterium was implicated in a diarrheal outbreak among infants in 1935 (Todar, 2008). Jarvis and Martone (1992) recorded *E. coli* as the most commonly reported nosocomial pathogen in surveillance at some hospitals in the United States.

2.3.1Characteristics of *E. coli*

E. coli is nonspore-forming and beta hemolytic. On MacConkey Agar, it usually ferments lactose or produce pink colonies with surrounding areas of precipitated bile salts. It also presents with a green sheen on eosin methylene blue agar. *E. coli* strain will produce indole from tryptophan; it does not produce hydrogen sulfide, urease, and cannot use citrate as sole carbon source (Mahon *et al*., 2007).

2.3.2 Diseases caused by *E. coli*

Pathogenic strains of *E. coli* are responsible for three types of infections in humans; urinary tracts infections, neonatal meningitis, and intestinal diseases (Todar, 2008).

2.3.2.1 Urinary tract infections

Uropathogenic *E. coli* (PEC) causes 90 % of urinary tract infections in anatomically normal and unobstructed urinary tracts. The uropathogenic strains have an adherence factor called P fimbriae, or pili, which binds to the P blood group antigen and mediates the attachment of *E. coli* to uroepithelial cells. Thus, patients with intestinal carriage of this strain are at greater risk of developing UTI than the general population. Complicated

UTI and pyelonephritis caused by *E. coli* are observed in elderly patients with structural abnormalities or obstruction such as prostatic hypertrophy, neurogenic bladders or in patients with urinary catheters. The urinary tract is the most common site of *E. coli* infection and the uropathogenic strain is usually implicated in most (90 %) of all uncomplicated UTIs including uncomplicated urethritis/cystitis, symptomatic cystitis, pyelonephritis, acute prostatitis, prostatic abscess, and urosepsis (Madappa, 2010).

2.3.2.2 Neonatal meningitis

Neonatal meningitis is a life-threatening disease which affects infants. The disease is transmitted from mothers who are colonized with the K1 strain of *E. coli* during pregnancy to their infants (Madappa, 2010).

2.3.2.3. Intestinal diseases

As a cause of enteric infections, 6 different mechanisms of action of 6 different varieties of *E coli* have been reported. Enterotoxigenic *E. coli* (ETEC) causes traveller's diarrhea. Enteropathogenic *E. coli* (EPEC) is responsible for childhood diarrhea. Enteroinvasive *E coli* (EIEC) causes a *Shigella* -like dysentery. Enterohemorrhagic *E. coli* (EHEC) causes hemorrhagic colitis or hemolytic-uremic syndrome (HUS). Enteroaggregative *E. coli* (EAggEC) is primarily associated with persistent diarrhea in children in developing countries, and enteroadherent *E. coli* (EAEC) is the cause of childhood diarrhea and traveller's diarrhea in Mexico and North Africa. All the different varieties colonize the small bowel, except EIEC and EHEC which preferentially colonize the large bowel prior to causing diarrhea (Hudault *et al*., 2001).

2.3.3 Virulence factors in *E. coli*

Most *E. coli* serotypes arose as a result of horizontal gene transfer of virulence factors, and among these virulence factors are a periplasmic catalase and shiga-like toxins. Shiga-like toxins are iron regulated toxins that catalytically inactivate 60S ribosomal subunits of eukaryotic cells blocking mRNA translation and causing cell death (Reisbig *et al*., 1981). Shiga-like toxins are functionally identical to toxins produced by virulent Shigella species (Calderwood, 1987). Strains of *E. coli* that express shiga-like toxins gained this ability due to infection with a prophage containing the structural coding for the toxin, and non-producing strains may become infected and produce shiga-like toxins after incubation with shiga toxin positive strains (Strockbine *et al*., 1986).

The periplasmic catalase is encoded on the pO157 plasmid and is believed to be involved in virulence by providing additional oxidative protection when infecting the host (Brunder *et al*., 1996). It can resist phagocytic engulfment because of a component of the bacterial cell wall such as the fimbriae or capsule (Todar, 2008).

2.3.4 Mechanism of resistance in *E. coli*

Various mechanisms accounts for antibiotic-resistance in *E. coli*. Resistance to penicillins and cephalosporins is primarily due to hydrolysis of these antibiotics by the enzyme, beta-Lactamase. Also, some strains will modify their penicillin binding proteins (PBPs) through the inactivation of the dacB-encoded nonessential PBP4 which behaves as a trap target for beta-lactams (Jean, 1999).

Resistant to the Aminoglycosides is by a decreased in permeability of the cell wall due to alteration in the aminoglycosides transport system, inadequate membrane potential and modification in the Lipopolysaccharides (LPS) phenotype. *E. coli* can inactivate an antimicrobial agent through the production of enzymes which transfer acetyl or phosphate groups to the amino and hydroxyl moieties of the antibiotic (Shaw *et al*., 1993).

2.4 Prevention and control of nosocomial infections

Approximately one third of nosocomial infections are preventable. To achieve this level of prevention, certain strategies need to be pursued simultaneously (Scheckler *et al*., 1998). Aggressive infection control committee in hospitals must be formed to reduce nosocomial infections through the identification and control of predisposing factors, education and training of hospital personnel (Schwart and Stoller, 1999).

Institutional policies and practices must be developed and adhered to. In particular, optimal hand washing and glove use must be facilitated and reinforced, as transmission of organisms between patients occurs primarily on the hands of providers. There must be continuous improvement on the national surveillance of nosocomial infections for a better representative data. Surveillance of nosocomial infections, by itself, may decrease the incidence. Example, when each surgeon is provided with his/her own wound-infection rates and with other surgeons' rates for comparison, the institutional surgical-wound infection rates will decrease (Alicia *et al*., 1999).

A system must also be developed for the surveillance of nosocomial infections that occur outside the hospitals and other health care providing facilities after the patients are discharged. The successes so far achieved in controlling nosocomial infections have come from improving the design of invasive devices. The higher rates of vascular access-associated bloodstream infections, particularly in ICU patients have been drastically reduced through the use of new devices (Goldmann *et al.*, 1996).

These noninvasive monitoring devices and minimally invasive surgical techniques avoid the high risk associated with bypassing normal host defense barriers such as the skin and mucous membranes (Weinstein, 1991). Aggressive and effective antibiotic control programs are required especially in the developing nations where these drugs are abused and misused. This will help to reduce the development and spread of antibiotic resistant bacteria (Goldmann *et al*, 1996).

2.5 Antimicrobial Assay Methods

These assays determine the in-vitro susceptibility of an isolate to a range of chemotherapeutic agents (Struve *et al*., 2003). Antimicrobial susceptibility methods include Agar Diffusion Method and Broth Dilution Methods.

2.5.1 Agar diffusion methods

The antibiotic diffuses from a paper disc or small cylinder into an agar medium that contains test organisms. A common application of these methods is the Kirby-Bauer test; where paper discs containing known concentrations of antibiotics are applied to the surface of seeded Mueller-Hinton agar and the plate incubated. After overnight

incubation, zone of inhibition sizes are measured in millimetres (Cheesbrough, 2000). Zone of inhibition is observed as a failure of the organism to grow in the region of the antibiotic (Baron *et al*., 1994).

2.5.2 Broth Dilution Method

This method depends upon inoculation of broth containing antibiotics at varying levels; usually, doubling dilutions are used. This method is used to determine Inhibitory Concentration (MIC) or breakpoint of an antimicrobial agent required to inhibit the growth of a bacterial isolate (Mahon *et al*, 2007). 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 (Cheesbrough, 2000).

To determine the MBC, a 0.01ml aliquot of each clear tube or well from the MIC determination is subcultured to an agar medium and incubated at 37^0C for 24 hours. After overnight incubation, the numbers of colonies that grow on subculture are compared with the actual number or organisms inoculated into the MIC test tubes (Mahon *et al*., 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites and sampling

The study was done at Tafo, Suntreso and Kumasi South Hospitals all in the Kumasi Metropolis of Ghana. These facilities were chosen for the study because many people visit them.

A total of 600 swab samples of beds, floors, benches, door handles, and waste water from the drainages were collected between January and June, 2010.

The samples were obtained by rubbing sterile cotton wool swabs on these materials and or areas. The swabs were then put into sterile test tubes, closed tightly and labeled appropriately. Similarly, sterile swabs were dipped into drainages and treated as above. All of the samples were then transported to the laboratory for studies.

3.2 Coding of Sample

A combination of alphabets and numerals were used to indicate the name of the hospital and type of sample.

Table 3.1 Interpretation of codes used to label samples

Code	Interpretation
TFL	Tafo Hospital floor (OPD)
TB	Tafo Hospital bench (OPD)
TFW	Tafo Hospital females ward bed
TMW	Tafo Hospital males ward bed
TPW	Tafo Hospital pediatrics ward bed
TD	Tafo Hospital drainage
TDH	Tafo Hospital door handle
SFL	Suntreso Hospital floor (OPD)
SB	Suntreso Hospital bench (OPD)
SFW	Suntreso Hospital females ward bed
SMW	Suntreso Hospital males ward bed
SPW	Suntreso Hospital pediatrics ward bed
SD	Suntreso Hospital drainage
SDH	Suntreso Hospital door handle
KSFL	Kumasi South Hospital floor (OPD)
KSB	Kumasi South Hospital bench (OPD)
KSFW	Kumasi South Hospital females ward bed
KSMW	Kumasi South Hospital males ward bed
KSPW	Kumasi South Hospital pediatrics ward bed
KSD	Kumasi South Hospital drainage
KSDH	Kumasi South Hospital door handle

3.3 Cultivation and Isolation of organisms

The various samples collected were separately inoculated into 10 ml of nutrient broths (Oxoid, Basingstoke, UK) and incubated at 37^{o}C for 24 hours. Tubes of 20 ml each MacConkey Agar, eosin methylene blue Agar and *Pseudomonas* Cetrimide Agar (Oxoid,

Basingstoke, UK) were melted, stabilized in a thermostatically controlled water bath (New Brunswick Scientific, New Jersey, USA) at 45^{o}C for 15 minutes, poured into separate sterile Petri dishes and allowed to solidify. Using a sterile platinum loop, each culture was separately streaked onto the surface of MacConkey Agar plates, labeled and incubated at 37^{o}C for 48 hours and observed for signs of growth and colony appearance.

Colonies that appeared pink on the MacConkey agar plates were fished out with sterile inoculating wire and separately streaked onto the surface of eosin methylene Blue Agar plates. These were also incubated at 37^{o}C for 24 hours. Separated black coloured colonies with metallic sheen were again fished out into nutrient broths and incubated at 37^{o}C for 24 hours.

White and golden to brown colonies on the MacConkey Agar plates were also picked with sterile inoculating wire and streaked on the surface of *Pseudomonas* cetrimide agar plates, which were then incubated at 37^{o}C for 48 hours. The yellowish-green colonies observed were fished out into 10 ml tubes of nutrient broth, labeled appropriately and incubated at 37^{o}C for 24 hours. The various sub-cultures were streaked onto nutrient agar slants, incubated at 37^{o}C for 48 hours and then kept (in the refrigerator at -20^{o}C) for further identification and antibiotic sensitivity studies.

3.4 Identification of organisms

3.4.1 Gram Stain

A clean grease free slide was obtained by cleaning with hot water and soap. After which it was rinsed and the excess water blotted out with a lint-free cloth. A drop of distilled water was placed on the slide and with the help of an inoculating needle; the isolate picked from the surface of the agar plate was gently and thoroughly emulsified in the drop of water and thinly spread on the glass slide. The smear was dried in air and passed through a Bunsen flame to fix. It was then flooded with ammonium oxalate crystal violet solution for about one minute and rinsed off with water. Gram's iodine solution (mordant) was then applied for about one minute and washed off. The excess water was blotted off with bibulous paper and 95% ethyl alcohol was applied and allowed to drain off until no more colour was seen leaving the smear. It was again washed with water and Safranin was added and left for about 30 seconds. The contrast stain was then washed off and the smear dried in air (Harley and Prescott, 1990). The slides were examined with the aid of a light microscope (Olympus, Tokyo, Japan).

3.4.2 Biochemical Tests

Various biochemical tests were performed on the isolates to confirm their identities. These included indole, oxidase and arginine dehydrolase production, citrate utilization, nitrite reduction, fermentation of carbohydrates (such as xylose, maltose, arabinose, glycerol and starch), Methyl Red - Voges Proskauer test and growth at 42^{o}C and on

Triple Sugar Iron Agar. The results are as recorded in tables 1 to 6 (Appendix II). *E. coli* (TCC 11229) and *P. aeruginosa* (NCTC 7244) were the control microorganisms used.

3.4.2.1 Indole production test

The Indole test was performed by growing the isolates in 10 ml sterile Tryptone Water (Oxoid, Basingstoke, UK) for 24 hours at 37°C. Kovacs' reagent (0.5 ml) was then added to the culture using a pipette. The test tube was shaken and examined after one minute (Harley and Prescott, 1990). The presence of Indole was detected by the appearance of a red layer in the medium while its absence was denoted by a yellow layer.

3.4.2.2 MR-VP test

The methyl red test was done by inoculating the isolate into a labeled Methyl Red- Voges Proskauer (MR-VP) broth by means of a sterile loop. The test tubes were then incubated at 37^{o}C for 72 hours. After incubation, the content of each tube was divided into two equal portions; one of which was used for the methyl red test and the other for the Voges-Proskauer's test. Two drops of methyl red indicator were added to the portion meant for MR test (Harley and Prescott, 1990). Appearance of red color in the medium was recorded as a positive reaction.

Barritt's method was employed in the VP test. 0.6 ml of α-naphthol and 0.2 ml of 40 % potassium hydroxide solutions were added to the second portion. The appearance of red color denoted a positive test (Harley and Prescott, 1990).

3.4.2.3 Citrate Utilization test

Using a straight platinum wire, the isolate was inoculated into Koser's Citrate medium and incubated at 37°C for 48 hours. Citrate utilization was denoted by turbidity and colour change in the medium from light green to blue (Brown, 2001). Citrate negative cultures showed neither growth nor color change in the medium.

3.4.2.4 Reaction in Triple Sugar Iron Agar

The isolates grown on nutrient agar slants were picked with a sterile platinum wire and then inoculated into triple sugar iron agar. The tubes were capped and incubated at 37°C for 24 hours. A control of TSI Agar which was not inoculated was also incubated alongside the tests (Mahon *et al*, 2007). After incubation, the tubes were examined for the presence of sugar fermentation, gas and hydrogen sulfide production (Harley and Prescott, 1990). A yellow butt and yellow slant due to the fermentation of lactose, sucrose and glucose was recorded as a positive test.

3.4.2.5 Oxidase production test

This test was done to identify *P. aeruginosa*. The oxidase reagent was prepared by dissolving 0.1 g of tetramethyl-p-phenylenediamine in 10 ml of sterile distilled water. A loopful of the isolate was streaked on nutrient agar and incubated at 37°C for 24 hours. Clean Whatman No.2 filter paper was placed in Petri dish and three drops of the freshly prepared oxidase reagent added to it. The culture of the isolate was smeared across the impregnated paper with a platinum loop (Harley and Prescott, 1990). A positive reaction was indicated by the appearance of a dark purple colour on the paper within 10 seconds.

3.4.2.6 Nitrate Reduction Test

Two loopfuls of the isolates were inoculated into nitrate broth and incubated at 37°C for five days. A drop each of 0.8% sulfanilic acid and 0.5% N, N-dimethyl-α-naphthylamine solutions were added. Upon observation, the appearance of red colour in the medium after 3 minutes indicated a positive test. Negative tests were confirmed by the addition of about 10 mg of powdered zinc. Red colouration was a confirmation of the negative result.

3.4.2.7 Arginine dehydrolase production

About 1ml of the isolate was inoculated into 5 ml arginine broth and incubated at 37oC for 24 hours. The Nessler's reagent (0.25 ml) was then added and observed (Barrow and Felthan, 2003). arginine hydrolysis was detected by the development of brown colour in the medium.

3.4.2.8 Carbohydrate Fermentation test

The Fermentation medium (containing 0.5% of the chosen carbohydrate) was inoculated with two loopfuls of the isolate suspension. The tubes containing inverted Durham tubes were then incubated at 37^{o}C and observed 24 hourly for two days. Growth, acid and or gas production were observed and recorded. The various carbohydrates tested were arabinose, maltose, starch, glycerol and xylose.

3.4.2.9 Growth at 42^{o}C

The suspected *Pseudomonas* isolates were inoculated into 10 ml nutrient broths and incubated at 42^{o}C for 24 hours and observed for signs of growth (Hsueh *et al*., 1998).

3.5 Antibiotic Sensitivity test

The Kirby-Bauer Disc Diffusion Method (Struve *et al*, 2003) was used to test the *in vitro* susceptibility of the identified isolates to gentamicin (GM) 10µg, ciprofloxacin (CIP) 5µg, ceftriaxone (CRO) 30µg, ampicillin (AMP) 10µg, and co-trimoxazole (SXT) 25µg.

A sterile platinum loop was used to pick colonies of *E. coli* from the culture plate and emulsified in 4 ml of sterile peptone water to match with 0.5 McFarland turbidity standards ($1.5x10^{8}$ cfu/ml). Using a sterile swab, the surface of Mueller Hinton Agar (Oxoid, Basingstoke, UK) in a Petri dish was evenly inoculated with the suspension. With the Petri dish lid in place, about 10 minutes was allowed for the surface of the agar to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at 37°C for 18 hours. The diameters of the zones of inhibition were measured in millimetres.

The exercise was replicated and the results compared with chart (Table 3.2) provided by the Clinical and Laboratories Standards Institute (Gloria *et al*., 2003). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as control. The above procedure was repeated for *P. aeruginosa* isolates.

Table 3.2: Acceptable susceptible zone of inhibition values for antibiotics used based on CLSI

Antibiotic	Resistant	Intermediate	Susceptible
Ciprofloxacin(5µg)	≤15	16-20	≥21
Ampicillin(10µg)	≤13	14-16	≥17
Gentamicin(10µg)	≤12	13-14	≥15
Ceftriaxone(30µg)	≤13	14-20	≥21
Co-trimoxazole (25µg)	≤10	11-15	≥16

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation and identification of *P. aeruginosa* and *E. coli*

A total of 150 (lactose fermenter) and 100 (non-lactose fermenter) isolates recovered on MacConkey agar were respectively suspected as *E. coli* and *P. aeruginosa*. These were screened through the various microscopic examination and biochemical reactions. *P. aeruginosa* and *E. coli* isolates were successfully identified from the various locations (benches, floor, door handles and drainages, male, female and pediatrics wards) in the three hospitals. A total of 97 isolates of *E. coli* and of 57 *P. aeruginosa* were obtained from the three hospitals.

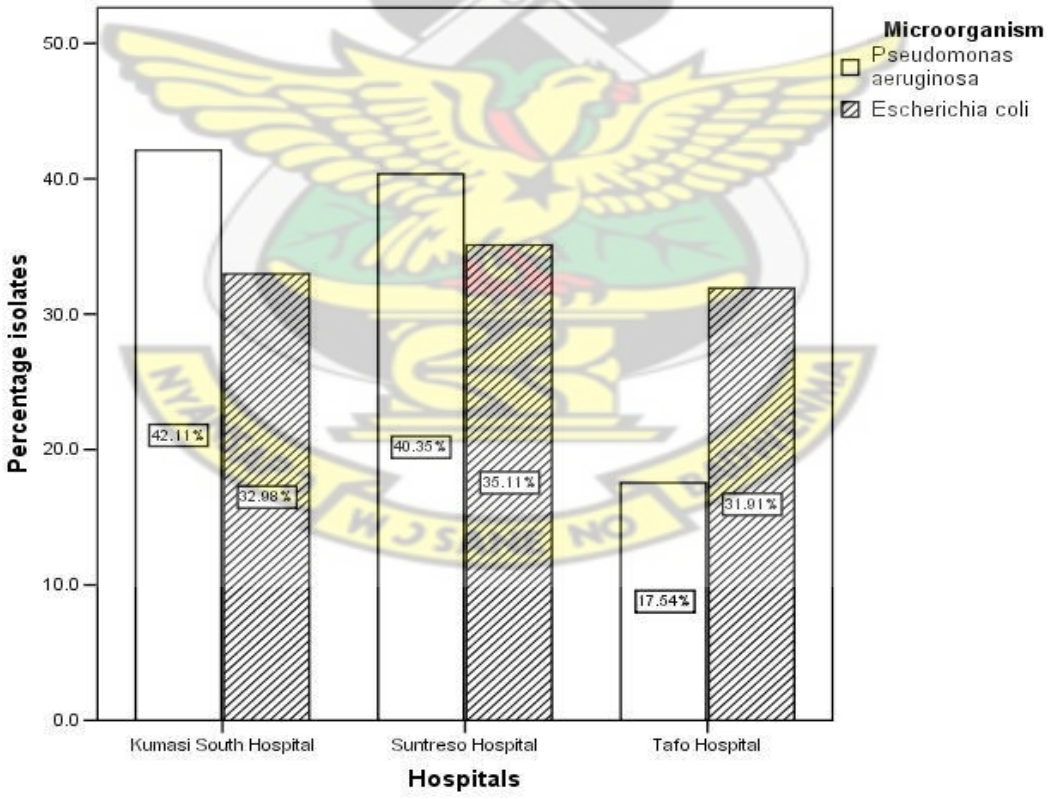


Fig.4.1: Distribution of isolates within the three hospitals.

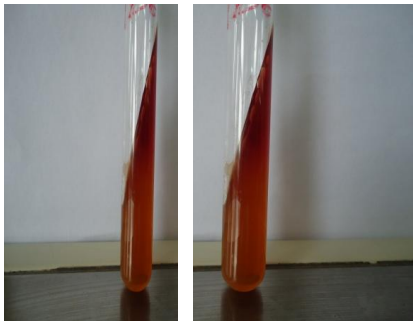
4.2 Biochemical reactions and microscopic examinations that confirmed the presence of *P. aeruginosa* and *E. coli* in the cultures.

Suspected *E. coli* isolates appeared pink on MacConkey agar and on EMB agar, these colonies were black in colour with metallic sheens. They fermented arabinose, glycerol, maltose and xylose but were not able to do same with starch (Tables 1 to 6 in Appendix II). They also produced indole from tryptophan and tested positive for acid production in the MR-VP test (while VP negative) but could not utilize citrate for growth. They were Oxidase negative but tested positive for nitrite reduction. On TSI agar the suspected *E. coli* isolates exhibited lactose fermentation resulting in yellow colouration and cracking of the medium (indications of acid and gas production). Microscopically, these isolates appeared rod shaped and stained gram-negative (Figure 4.2).

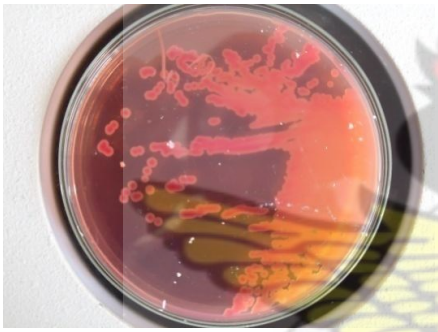
Suspected *P. aeruginosa* isolates on the other hand, grew and produced greenish-yellow colonies on cetrimide agar and appeared as gram negative rods under the microscope. These isolates could produce oxidase and arginine dihydroxylase enzymes and were also able to utilize citrate for growth. They were however, not able to produce Indole, nor acid in MR-VP medium but tested positive for VP and nitrite reduction tests. Starch hydrolysis test was also negative for the *P. aeruginosa* suspected isolates.

The isolates that tested otherwise to these microscopic and biochemical features were not considered in the subsequent studies since they were not the targeted organisms.

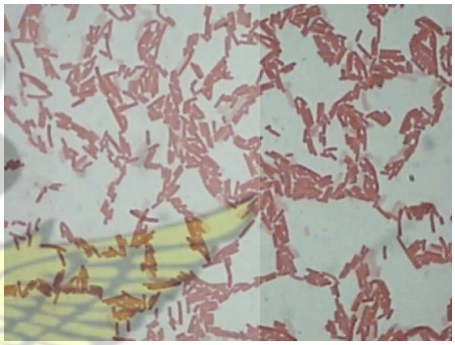
A: *Escherichia coli* on TSI agar



B: *Pseudomonas aeruginosa* on TSI agar

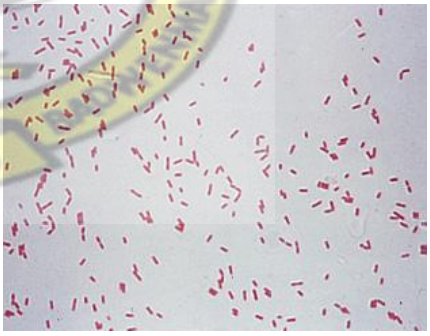


C: *E. coli* on MacConkey Agar



C: Gram-stain appearance of *E. coli* cells

E: *P. aeruginosa* on Cetrimide Agar



F: Gram stain appearance of *P. aeruginosa* cells

Figure 4.2: Some biochemical reactions and microscopic features of the isolates.

Most of the samples collected from Kumasi South Hospital, with the exception of male and female wards, contained both *P. aeruginosa* and *E. coli*. These organisms were also identified in three of the samples collected from Suntreso Hospital, namely floor, drainage and door handles. Across the hospitals recruited in the study, *E. coli* emerged as the predominant microorganism in the various samples collected from different locations (Tables 4.1 and 4.2).

Table 4.1: Number of samples containing *E. coli* isolates

	Tafo hospital (n)	Kumasi South hospital (n)	Suntreso hospital (n)
Beds	17(90)	17(90)	18(90)
Floors	7(30)	3(30)	10(30)
Benches	5(30)	6(30)	6(30)
Door handles	0(30)	5(30)	0(30)
Drainages	0(20)	3(20)	0(20)

Key: n = total number

Table 4.2: Number of samples containing *P.aeruginosa* isolates

	Tafo hospital (n)	Kumasi South hospital (n)	Suntreso hospital (n)
Beds	0(90)	5(90)	0(90)
Floors	2(30)	5(30)	4(30)
Benches	0(30)	5(30)	0(30)
Door handles	3(30)	5(30)	7(30)
Drainages	5(20)	4(20)	12(20)

Key: n = total number

4.3 Effect of antibiotics on *P. aeruginosa* isolated from the hospitals in Kumasi.

All the *P. aeruginosa* isolates were (100%) resistant to ampicillin as indicated in Fig.4.3. For gentamicin, 46% of the *P aeruginosa* isolates exhibited resistance while 21% and 33% showed respectively intermediate and sensitive responses. For ciprofloxacin, 36.84% of these isolates were resistant while equal proportions (31.58%) exhibited intermediate and sensitive responses. About 40% of the *P. aeruginosa* isolates also showed intermediate response to ceftriaxone while 39 and 21% were respectively resistant and sensitive.

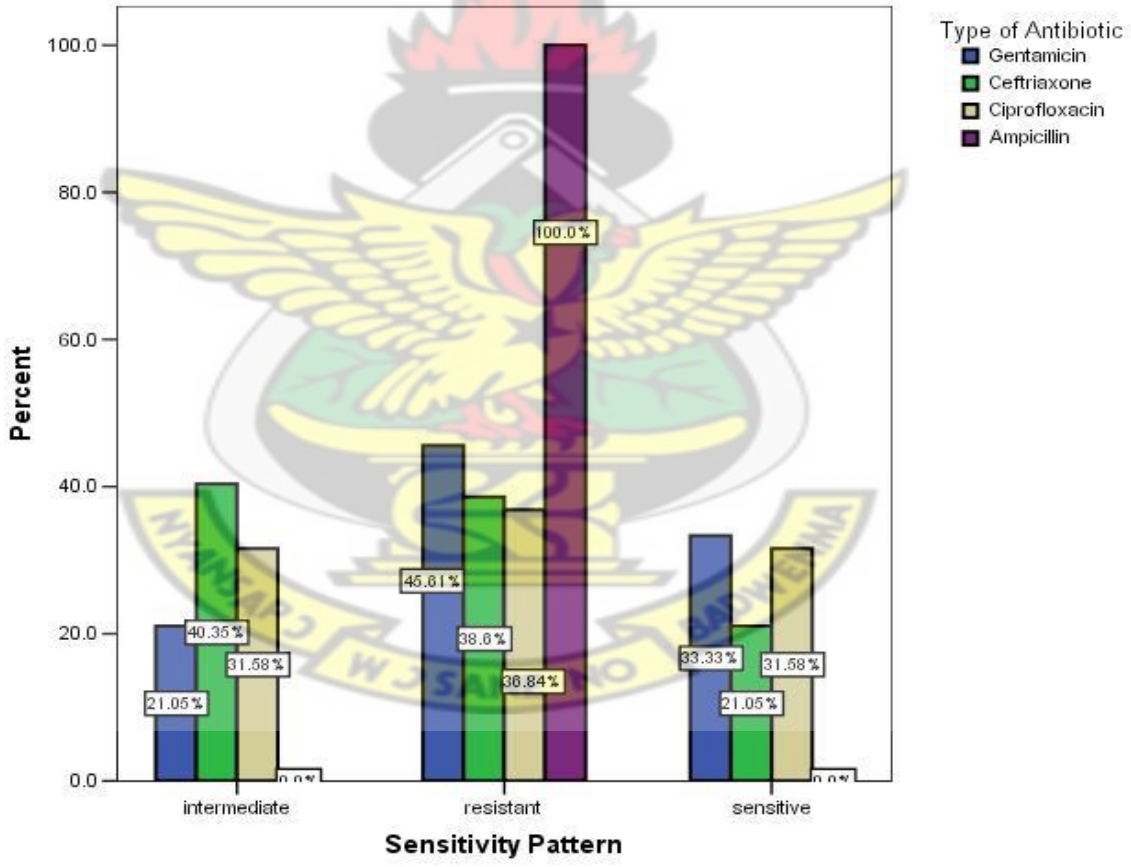


Figure 4.3: General sensitivity pattern of *P. aeruginosa* to some antibiotics tested

4.4 Effect of antibiotics on *P. aeruginosa* from each of the three hospitals

The sensitivity patterns of *P. aeruginosa* isolates from the 3 hospitals under study are shown in Fig.4.4. Resistance to ampicillin was highest at Kumasi-South Hospital (42.11%), followed by Suntreso Hospital (40.35%) with Tafo Hospital being the place with the least recorded resistance of 17.54%. Out of the 45.61% gentamicin resistant *P. aeruginosa* isolates from the three hospitals, 28.07% were found in Kumasi South Hospital. The remaining proportion was equally shared between Tafo and Suntreso Hospitals. Out of the total (36.84%) of *P. aeruginosa* isolates resistant to ciprofloxacin, almost half (17.58%) were isolated from Kumasi South Hospital while 12.28% and 7.02% came from Suntreso and Tafo Hospitals respectively.

At Suntreso hospital, intermediate sensitivity responses 14.04, 17.54 and 10.53 % of the *P. aeruginosa* isolates were recorded for gentamicin, ceftriaxone and ciprofloxacin respectively. Each of Tafo and Kumasi South Hospitals recorded 3.51% of the isolates with intermediate response to gentamicin. Approximately 5% and 18% of the isolates from Tafo and Kumasi South Hospitals respectively, gave intermediate response to ceftriaxone. Intermediate response of the *P. aeruginosa* isolates to ciprofloxacin was about 14% in Kumasi South and 7% in Tafo Hospitals.

In the case of gentamicin sensitive *P.* aeruginosa isolates, more than half (17.54%) were isolated from Suntreso Hospital while 10.53% and 5.28% were from the Kumasi South and Tafo Hospitals respectively. Similar proportions of ciprofloxacin sensitive isolates were also observed at the hospitals except Tafo were the proportion was slightly less (3.51%). ceftriaxone sensitive isolates at the hospitals ranged between 5 and 9 %.

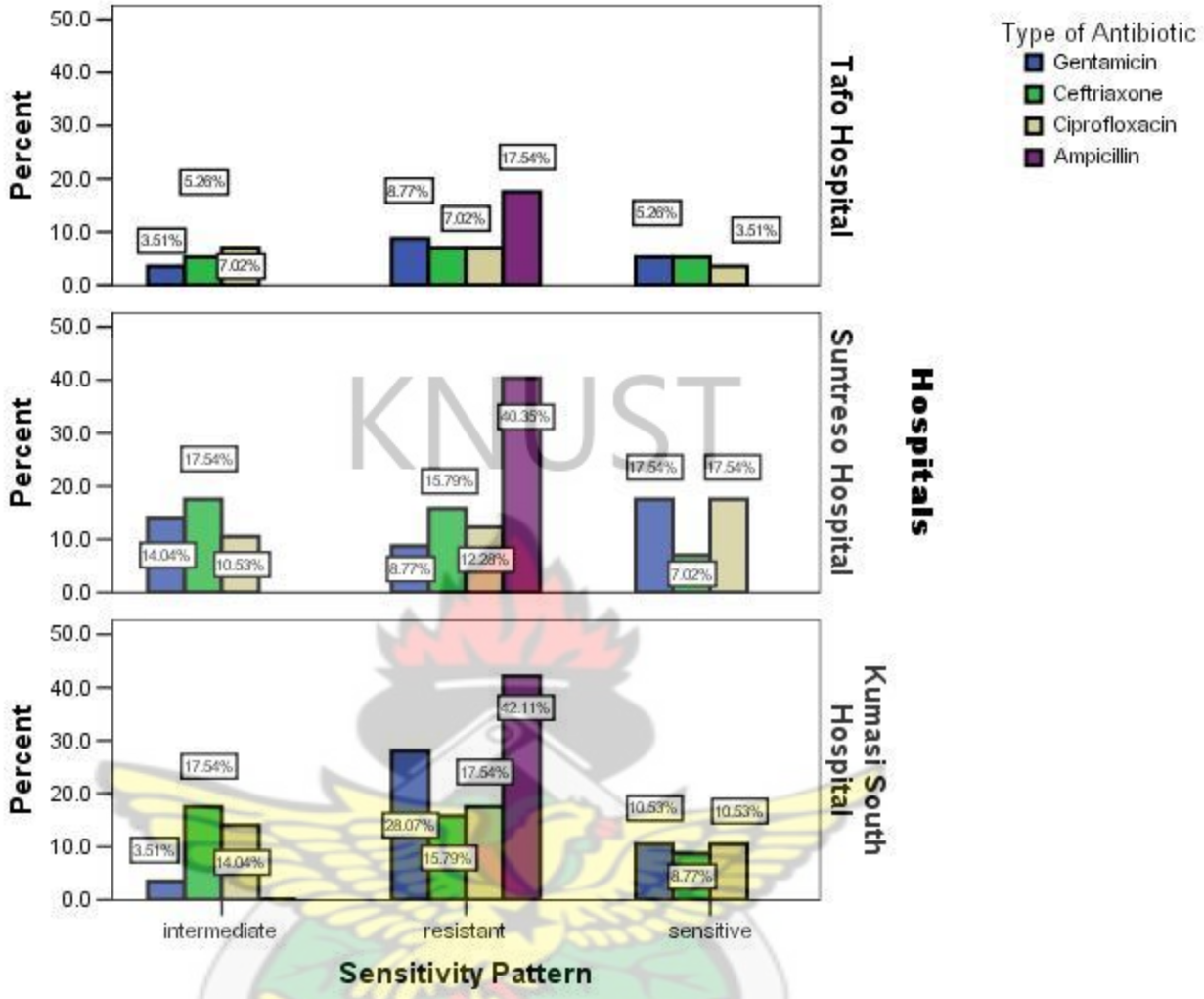


Fig. 4.4: Sensitivity pattern of *P. aeruginosa* to some antibiotics tested

4.5 Sources of *P. aeruginosa* isolates in relation to sensitivity patterns

For gentamicin, the most sensitive *P. aeruginosa* isolates (47.4%) were obtained from drainage samples and those from door handle samples were the most resistant (34.6%). However, 50% of drainage isolates exhibited intermediate activity (Table 4. 3).

In the case of ceftriaxone, high proportions of drainage isolates were resistant (45.5%) as well as sensitive (41.7%) as compared to the other sample types.

Almost equal proportions of the drainage sample isolates recorded the various activities to ciprofloxacin: 38.9% sensitive, 38.1% resistant and 33.3% intermediate isolates. However, all the *P. aeruginosa* isolates were resistant to ampicillin.

Table 4.3: Sources of *P. aeruginosa* isolates in relation to sensitivity patterns

Type of Antibiotic			Source of sample					Total
			Floor	Drainage	Door handle	Bench	Female ward	
gentamicin	intermediate	Count (%)	2 (16.7)	6 (50.0)	3 (25.0)	0 (0)	1 (8.3)	12 (100)
	Resistant	Count (%)	6 (23.1)	6 (23.1)	9 (34.6)	3 (11.5)	2 (7.7)	26 (100)
	sensitive	Count (%)	3 (15.8)	9 (47.4)	3 (15.8)	2 (10.5)	2 (10.2)	19 (100)
ceftriaxone	intermediate	Count (%)	7 (30.4)	6 (26.1)	6 (26.1)	3 (13.0)	1(4.3)	23 (100)
	Resistant	Count (%)	2 (9.1)	10 (45.5)	6 (27.3)	1(4.5)	3 (13.6)	22 (100)
	sensitive	Count (%)	2 (16.7)	5 (41.7)	3 (25.0)	1(8.3)	1 (8.3)	12 (100)
ciprofloxacin	intermediate	Count (%)	4 (22.2)	6 (33.3)	4 (22.2)	2 (11.1)	2 (11.1)	18 (100)
	Resistant	Count (%)	3 (14.3)	8 (38.1)	7 (33.3)	1 (4.8)	2 (9.5)	21 (100)
	sensitive	Count (%)	4 (22.2)	7 (38.9)	4 (22.2)	2 (11.1)	1 (5.6)	18 (100)
ampicillin	Resistant	Count (%)	11(19.3)	21 (36.8)	15 (26.3)	5 (8.8)	5 (8.8)	57 (100)

4.6 Effect of antibiotics on *E. coli* isolates from hospitals in Kumasi

About 91% of the *E. coli* isolates exhibited resistance to ampicillin while 6 and 3.09% respectively showed intermediate and sensitive (4.5). For co-trimoxazole, 78.35% of the isolates were resistant while 9.28 and 12.37% intermediate and sensitive responses. Also, between 28.6 to 46.39% of the *E. coli* isolates showed resistance responses to gentamicin, ciprofloxacin and ceftriaxone while 14.43 to 47.42% gave intermediate responses. ceftriaxone, ciprofloxacin and gentamicin sensitive isolates are also in the range of 21.28 to 38.3% (Fig.4.5).

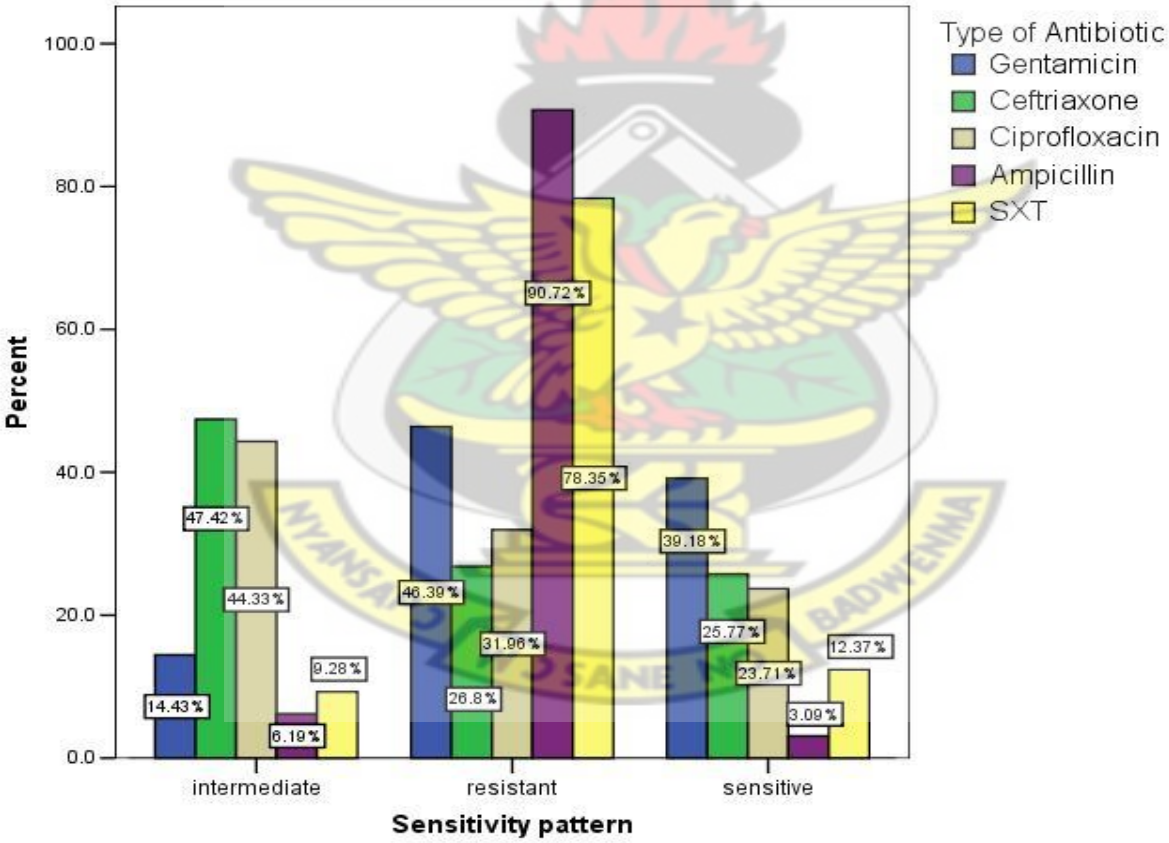


Fig.4. 5: General sensitivity pattern of *E. coli* to some antibiotics tested

4.7 Effect of antibiotics on *E. coli* isolates from the three hospitals

At Suntreso Hospital, about 30.93% of the *E. coli* isolates showed resistance to ampicillin. Each of Kumasi South and Tafo Hospitals recorded 30.93 and 28.87% ampicillin-sensitive isolates. However, no ampicillin-intermediate *E. coli* isolates were recovered from Tafo Hospital (Fig.4.6). For gentamicin, 9.28, 6.19 and 18.56% of the Kumasi South Hospital isolates showed resistance, intermediate and sensitive responses respectively.

Co-trimoxazole-resistant isolates were the second highest apart from ampicillin; 29.9, 25.77 and 22.68% of these resistant isolates were recovered from Kumasi-South, Tafo and Suntreso hospitals respectively. The *E. coli* isolates that exhibited intermediate activity against co-trimoxazole in all the hospitals were below 7%. Approximately 3.9 and 9.28% of the *E. coli* isolates were sensitive to co-trimoxazole in Suntreso and Kumasi South Hospitals while none of them gave sensitive response in Tafo Hospital. *E. coli* isolates from Kumasi South did not give sensitive response to ampicillin. At Suntreso Hospital most (11.34%) of these isolates gave sensitivity to gentamicin.

Ceftriaxone-resistant isolates from all the hospitals studied were below 11% while the intermediate isolates lies between 12.37 and 21.65%. Suntreso hospital recorded the highest ciprofloxacin-resistant *E. coli* isolates (about 13%) and the least was obtained from Tafo hospital (about 8%). For ciprofloxacin-Intermediate response, Suntreso hospital had the highest isolates (about 16%) as shown in Fig.4.6.

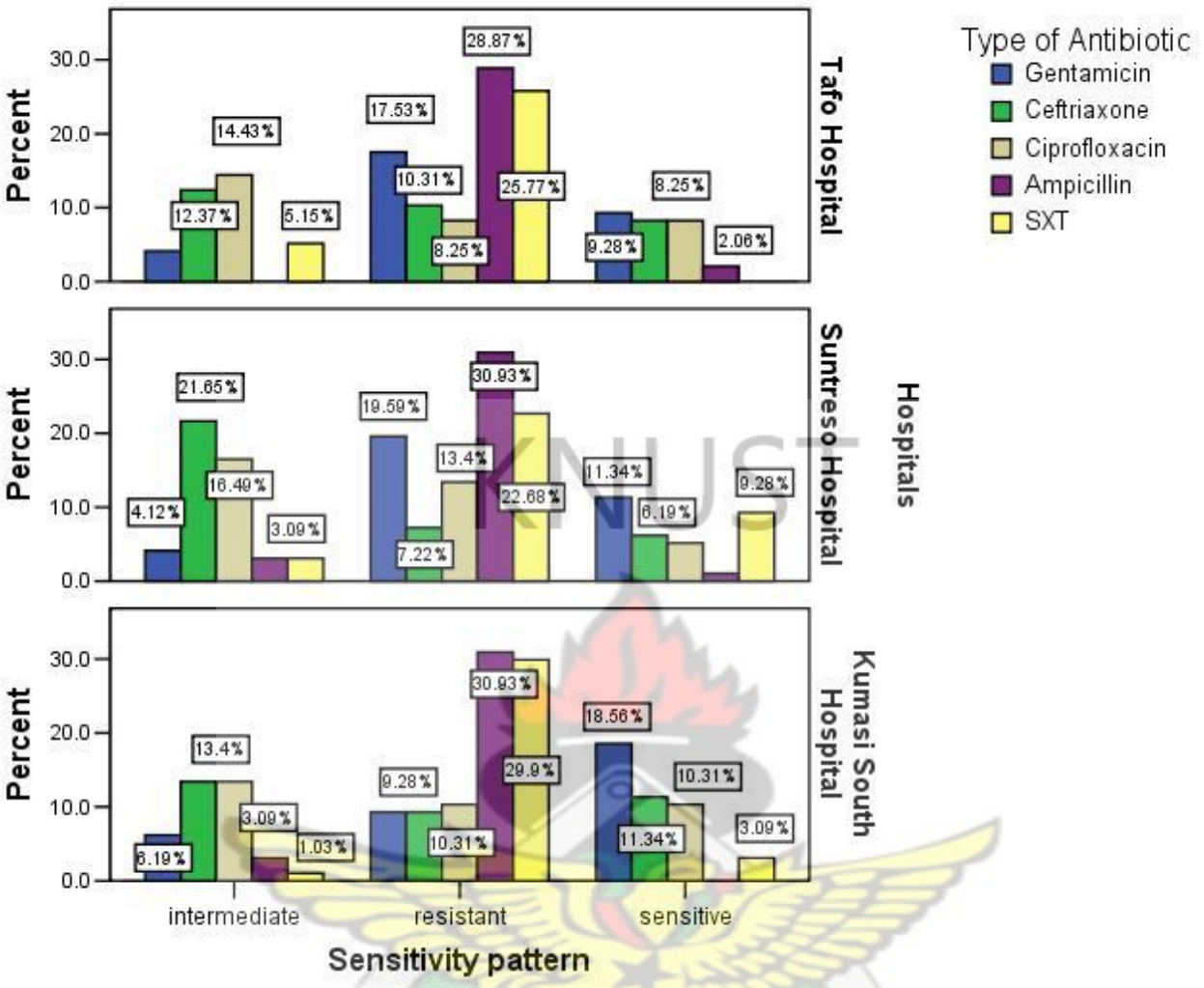


Fig.4. 6: Sensitivity pattern of *E. coli* to some antibiotics tested

4.8 Sources of *E. coli* isolates in relation to sensitivity patterns

Majority of the gentamicin sensitive *E. coli* isolates (28.9%) were from the male wards followed by floor samples (21.1%) as shown in Table 4.4. None of the drainage samples were resistant to gentamicin while 20% each from the floor and female wards proved resistant. 26.9% isolates from bench samples showed gentamicin intermediate activity. Out of the total 46 *E. coli* isolates that were ceftriaxone intermediate, approximately 30% were from the male wards, 21.4% from floor and 2.2% from drainage samples. 26.9% of

the resistant isolates were from the benches and 19.2% were from male wards while no resistant isolates were recovered from door handles (Table 4.4).

Ciprofloxacin resistant *E. coli* isolates were recovered from floor samples were the highest (29%) followed by those from Paediatric wards (19.4%). Approximately 30% of ciprofloxacin intermediate *E. coli* was from male ward samples and none from door handles. Majority of the sensitive isolates were from female wards (30.4%), followed by male and pediatric wards samples (17.4%). Floors and benches samples showed equal ciprofloxacin sensitivity of 13% each.

The distributions of ampicillin resistant *E. coli* isolates were 22.7, 20.5 and 19.3% for male wards, floors and benches respectively.

Table 4.4: sources of *E. coli* isolates in relation to sensitivity patterns

Type of Antibiotic			Source of sample							Total
			Floors	Drainages	Door handles	Benches	Female wards	Male wards	Paediatric wards	
Gentamicin	intermediate	Count (%)	3 (21.4)	1 (7.1)	0 (0)	4 (28.6)	3 (21.4)	1 (7.1)	2 (14.3)	14 (100)
	resistant	Count (%)	9 (20.0)	0 (0)	1(2.2)	6 (13.3)	9 (20.0)	11 (24.4)	9 (20.0)	45 (100)
	sensitive	Count (%)	8 (21.1)	2 (5.3)	1 (2.6)	7 (18.4)	6 (15.8)	11 (28.9)	3 (7.9)	38 (100)
Ceftriaxone	intermediate	Count (%)	12 (26.1)	1 (2.2)	2 (4.3)	4 (8.7)	8 (17.4)	14 (30.4)	5 (10.9)	46 (100)
	resistant	Count (%)	3 (11.5)	2 (7.7)	0 (0)	7 (26.9)	4 (15.4)	5 (19.2)	5 (19.2)	26 (100)
	sensitive	Count (%)	5 (20.0)	0 (0)	0 (0)	6 (24.0)	6 (24.0)	4 (16.0)	4 (16.0)	25 (100)
Ciprofloxacin	intermediate	Count (%)	8 (18.6)	1 (2.3)	0 (0)	10 (23.3)	5 (11.6)	15 (34.9)	4 (9.3)	43 (100)
	resistant	Count (%)	9 (29.0)	1 (3.2)	1 (3.2)	4 (12.9)	6 (19.4)	4 (12.9)	6 (19.4)	31 (100)
	sensitive	Count (%)	3 (13.0)	1 (4.3)	1 (4.3)	3 (13.0)	7 (30.4)	4 (17.4)	4 (17.4)	23 (100)
Ampicillin	intermediate	Count (%)	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (16.7)	2 (33.3)	2 (33.3)	6 (100)
	resistant	Count (%)	18 (20.5)	2 (2.3)	2 (2.3)	17 (19.3)	17 (19.3)	20 (22.7)	12 (13.6)	88 (100)
	sensitive	Count (%)	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	3 (100)
Co-trimoxazole	intermediate	Count (%)	2 (22.2)	1 (11.1)	0 (0)	1 (11.1)	2 (22.2)	2 (22.2)	1 (11.1)	9 (100)
	resistant	Count (%)	14 (18.4)	1 (1.3)	2 (2.6)	15 (19.7)	14 (18.4)	17 (22.4)	13 (17.1)	76 (100)
	sensitive	Count (%)	4 (33.3)	1 (8.3)	0 (0)	1 (8.3)	2 (16.7)	4 (33.3)	0 (0)	12 (100)

Many of the isolates obtained were found to be resistant to more than two different classes antibiotics as shown in Appendix II (Tables 6 to 12). The numbers of the multi-drug resistant (MDR) strains from the various hospitals are as shown in Table 4.5 below.

Table 4.5: Number of *E. coli* isolates that exhibited multidrug resistance

HOSPITAL	Total No. of MDR *E. coli* isolates	Total no. *E.coli* isolates	Percent of MDR
Tafo	27	30	90
Suntreso	28	34	82.4
Kumasi-South	23	33	69.7
Total	78	97	80.4

Table 4.6: Number of *P. aeruginosa* isolates that exhibited multidrug resistance

HOSPITAL	Total No. of MDR *P. aeruginosa* isolates	Total No. of *P. aeruginosa* isolates	Percent of MDR
Tafo	6	10	60
Suntreso	11	23	47.8
Kumasi-South	15	24	62.5
Total	32	57	56.1

CHAPTER FIVE

DISCUSSION

According to Archibald (2004), extensive use of antibiotics creates selective advantage for microorganisms and that transmission of resistant strains from patient to patient within hospitals is possible. In the three hospitals studied, *P. aeruginosa* and *E. coli* were isolated from floor, beds, door handles, female, male and paediatric wards and waste water from drainages (Tables1and 2).

The two bacteria studied in this project, *P. aeruginosa* and *E. coli*, are important bacteria commonly implicated in many nosocomial diseases (Archibald, 2004; Blanc *et al*, 1998; Kiffer *et al*, 2005; Shrestha, *et al*, 2009; Strateva *et al*, 2007). The presence of resistant strains of these two organisms in hospitals and environment should be of much concern because both patients and hospital staff are exposed to these microorganisms.

All the *P. aeruginosa* isolates obtained in this study were resistant to ampicillin (Fig.4.4). This result is similar to a study conducted by Strateva *et al*. (2007) in Europe where more than 90% of *P. aeruginosa* isolates were resistant to ampicillin. Resistance to ampicillin is largely due to the production of extended spectrum β-lactamase (ESBL) enzymes by the bacteria. Resistance to ampicillin by *P. aeruginosa* could also be due to a combination of mechanisms such as the expression of chromosomal AmpC cephalosporinases and over expression of active efflux systems (McGowan, 2006).

Gentamicin, cumulatively, was the most potent antibiotic against the *P. aeruginosa* isolates within the hospitals with 33.29% susceptibility. A similar observation has been reported in Brazil where *P. aeruginosa* isolates were found to be more sensitive to

gentamicin (53.2%) compared to ciprofloxacin (Kiffer *et al.*, 2005). The percentage resistance of *P. aeruginosa* isolates to gentamicin in this study was found to be similar to that of Newman *et al., (*2006) where a total of 42% of the isolates were resistant to gentamicin. Bacterial resistance to gentamicin is mainly due to an enzymatic modification of the antibiotic (Poole, 2005): For example, adenyl or acetyl groups are added respectively to the hydroxyl and amino moieties of the antibiotic. This could be the reason why *P. aeruginosa* resistance (38.6%) to gentamicin was high, even though its use in Ghanaian hospitals is limited (Ghana National Drugs Programme, 2004).

The level of resistance (38.6%) of the *P. aeruginosa* isolates to ceftriaxone registered in this study was close to results obtained by Newman *et al.* (2006) who registered resistance of 29% by *P. aeruginosa* isolates obtained from nine regions of Ghana to ceftriaxone. According to Ariffin *et al*, (2004), resistance of gram negative bacteria is due to the production of extended spectrum of beta-lactamases. The gene (OXA-10) responsible for producing ESBL is widespread among various bacterial populations and is acquired more quickly in population of P.aeruginosa (Tanez *et al*., 2010).

P. aeruginosa thrives very well at habitats with adequate amount of moisture (Hardalo *et al.*, 1997). This was evident in this study in that majority of the *P. aeruginosa* isolates (36.8%) were from drainage samples collected from the hospitals (Table 4.3). Door handle samples gave the next highest proportion of isolates (26.3%). In hospitals and all other places of human habitation, door handles are the most common article of contact by the people. It is therefore not surprising that it also gave very high *P. aeruginosa* isolates since people with wet hands (water or sweat) may easily come into contact with it. The

places with least number of isolates were the benches and wards and these are places that are likely to be dry most of the time in the hospitals.

In general, the relatively higher proportion of intermediate responses of *P. aeruginosa* isolates to all the antibiotics suggests the gradual migration to development of resistance of the microorganism to these antibiotics.

More *E. coli* strains (97) were isolated from the three hospitals than *P. aeruginosa* strains (57). A similar observation was made by Jarvis and Martone (1992) who recorded *E. coli* as the most common nosocomial pathogen in some hospitals in the US. Also, *E. coli* has been reported to be among the most frequent isolates in hospitals in Ethiopia (Yismaw *et al*., 2010). In this study, majority of the *E. coli* isolates (53.6%) were recovered from the hospital beddings while about 21% were from floor samples and most of the *E. coli* isolates (91 to 78%) were resistant to ampicillin and co-trimoxazole (Table 4.1). The high occurrence of *E. coli* isolates in these samples could be attributed to poor hygienic conditions in the hospitals studied and the conditions in other hospitals are not different as the country lacks adequate number of health care facilities. These result in over-crowding in the few hospitals available and hence the unhygienic conditions.

A total of 46.4, 31.95, and 26.81% of the *E. coli* isolates exhibited resistance to gentamicin, ciprofloxacin and ceftriaxone respectively. Yismaw (2010) has also reported resistance of *E. coli* to gentamicin (47%), ciprofloxacin (33%) and ceftriaxone (26%). According to Namboodiri *et al.* (2011), these antibiotics have been subjected to widespread abuse resulting in the high rates of resistance.

Antibiotic resistance develops when microorganisms are exposed to effective doses of an antibiotic within a shorter period or when the microorganisms are exposed to smaller concentrations or residues of the antibiotic over a longer period of time (Todar, 2008). Any of these theories may support the results of this study as people with this resistant mutants end up in the hospitals due to antibiotic treatment failures. Resistance of *E. coli* strains to ampicillin is mainly due to the production of β-lactamases. In Ghana, many people employ ampicillin for a wide range of infectious diseases and even as growth promoter in animal husbandry (Namboodiri *et al.*, 2011; Danishta *et al.*, 2010). These excessive uses and abuses could have exerted the high selection pressure on microorganisms including *E. coli* to develop resistance as observed in this study. Co-trimoxazole is also largely misused in the country and hence it is not surprising that many of the *E coli* strains isolated in the study were resistant to it.

Out of 97 *E. coli* isolates, 78 (80.41%) were resistant to at least three different classes of antibiotics (Tables 4.5) while among the *P. aeruginosa* isolates 32 (56.14 %) out of 57 exhibited this kind of multi-drug resistance (Table 4.6) as defined by Obritsch *et al.* (2005).

These high numbers of resistant *E. coli* and *P. aeruginosa* isolates in the environment are potential reservoirs of resistant genes which can easily be transferred to other pathogens.

CONCLUSION

The study has shown the presence of resistant nosocomial *E. coli* and *P. aeruginosa*, in the three hospitals studied in the Kumasi Metropolis: Tafo, Kumasi-South and Suntreso Hospitals. All the *P. aeruginosa* isolates were resistant to ampicillin while less than 10% of the *E. coli* isolates exhibited sensitive and intermediate activities to the antibiotic. Also, about 77% of the isolates were also found to be resistant to co-trimoxazole. The potencies of gentamicin, ceftriaxone and ciprofloxacin against these isolated pathogens are also on the decrease as less than 34% of *P.* aeruginosa isolates and less than 38.4% of the *E. coli* isolates were found to be sensitive to these antibiotics.

The study has also shown that out of 52 *E. coli* isolates from hospital beddings, 53.85% were resistant to gentamicin, 25% to ceftriaxone and 61.5% to ciprofloxacin. Similarly, the has shown that out of 21 *P. aeruginosa* isolates from drainage samples, 28.57% were resistant to gentamicin, 47.62% to ceftriaxone and 28.57% to ciprofloxacin.

Some strains of the *E. coli* and *P. aeruginosa* isolates obtained from the three hospitals in the study exhibited resistance against at least three different classes of the antibiotics tested. Out of 97 *E. coli* isolates, 46(47.2%) were resistant to at least three antibiotics while 20(35.08%) out of 57 *P. aeruginosa* isolates were resistant to at least three antibiotics. The prevalence of multidrug resistance obtained for strains of these two organisms suggests that proper hygienic practices are not adhered to.

This study has therefore showed the need for the hospital management to be concerned about the potential of hospitalized patients becoming infected while receiving treatments.

RECOMMENDATION

1. Health workers must be made aware of the increasing resistance of nosocomial microorganisms and the proper administration of antibiotics in treating such infections.
2. Other hospitals in the metropolis and the country at large must also be studied in order to generate enough data which will help in the development of a holistic control programme in dealing with the threat posed by resistant nosocomial pathogens.
3. More antibiotics currently administered in our hospitals should be included in the study to determine the level of resistance of microorganisms to them.

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APPENDICES

APPENDIX I PREPARATION OF NUTRIENT MEDIA AND REAGENTS

A. MacConkey Agar No. 2 (Oxoid CM109)

Composition	Grams/litre
Peptone	20.0
Lactose	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

pH 6.9-7.3

The powder (51.5 g) was suspended in 1 litre of distilled water. It was then boiled shortly to dissolve completely. It was distributed into test tubes in 20ml quantities, corked firmly and then sterilized by autoclaving at 121^0C for 15 minutes.

B. Mueller-Hinton Agar (Oxoid CM337)

Composition	Grams/litre
Beef infusion	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0

pH 7.2-7.4

35 grams of the powder was dissolved in 1 litre of distilled water. It was then boiled shortly to dissolve the medium completely and distributed into test tubes in 20ml quantities. The tubes were corked firmly and then sterilized by autoclaving at 121^0C for 15 minutes.

C. Cetrimide agar (Oxoid CM0579)

Composition	**Grams/litre**
Gelatin Peptone	20
Magnesium Chloride	1.4
Potassium Sulphate	10
Cetrimide	0.3
Agar	13.6

45.3 grams was suspended in 1 litre of distilled water. 10ml of glycerol was added and brought to the boil to dissolve completely and distributed into test tubes in 20ml quantities, corked firmly and autoclaved at 121^oC for 15 minutes.

D. Eosin Methyl Blue Agar (Oxoid CM01 – 068)

Composition	**Grams/litre**
Peptone	10
Lactose	10
DiPotassium Phosphate	2
Yellowish Eosin	0.40
Methylene Blue	0.065

37.5 grams of the powder was suspended in 1 litre distilled water and brought to the boil to dissolve completely. It was distributed into test tubes in 20ml quantities and sterilized by autoclaving at 121^{O}C for 15 minutes.

E. MRVP Medium (Oxoid CM43)

Composition	**Grams/litre**
Peptone	5
Dextrose	5
Phosphate buffer	5

15 grams of the powder was dissolved in distilled water, mixed well and distributed into test tubes in 10ml quantities. It was sterilized by autoclaving at 121^{o}C for 15 minutes.

F. Tryptone Water (Oxoid CM 87)

Composition	**Grams/litre**
Tryptone(Oxoid L42)	10
Sodium Chloride	5

Fifteen (15) grams of the powder was dissolved in distilled water, mixed well and distributed into test tubes. It was autoclaved at 121oC for 15 minutes.

G. Triple Sugar Iron Agar (Oxoid CM277)

Composition	**Grams/litre**
Lab-Lemco Powder (Oxoid L29)	3
Yeast Extract (Oxoid L20)	3

Peptone (Oxoid L37)	20
Sodium Chloride	5
Lactose	10
Sucrose	10
Dextrose	1
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	q.s
Agar No. 3 (Oxoid L13)	12.0

65 grams of the powder suspended in 1 litre of distilled water and brought to the boil to dissolve completely. It was mixed well and distributed into test tubes in 10ml quantities and sterilized by autoclaving at 121oC for 15 minutes and then allowed to set in sloped forms.

H. Arginine Broth

Composition	**grams/l**
Peptone	5
Yeast extract	3
Glucose	1
Bromocresol purple, 0.2% solution	10ml

The solids were dissolved in 1 liter of distilled water, pH adjusted to 6.7 and then 10ml Bromocresol indicator (0.2%) was added. It was distributed 10ml of quantities into test tubes containing inverted Durham's tubes and sterilized at 115^0C for 20 minutes.

I. Fermentation Medium

Composition	**grams/l**
Peptone	20
Sodium Chloride	5
Sugar (e.g. Maltose)	1%
Bromocresol	0.2%

pH of the medium was 6.7±0.1

The solids were dissolved in 1 liter of distilled water, and Bromocresol indicator (0.2%) was added. (The various carbohydrates: arabinose, maltose, Xylose, starch and glycerol were respectively substituted as the sugar component). The solution was distributed in 10 ml quantities into test tubes containing inverted Durham's tubes and sterilized at 115^oC for 20 minutes.

J. Nitrate Broth

Composition	**Grams/l**
Potassium nitrate	1
Nutrient broth	1000ml

The Potassium nitrate was dissolved in 1 liter of Nutrient broth and distributed in 10ml of quantities into test tubes containing inverted Durham's tubes. It was then sterilized at 115^0C for 20 minutes.

K. Kovac's Reagent

Composition	**Quantity**
P-dimethylaminobenzaldehde	5 grams
Amyl alcohol	75ml
Concentrated hydrochloric acid	25ml

5 grams of p-dimethylaminobenzaldehde was dissolved in 75ml of Amyl alcohol, mixed gently by shaking. 25ml of concentrated hydrochloric acid slowly added to the solution

APPENDIX II: RAW DATA ON BIOCHEMICAL REACTIONS AND ANTIMICROBIAL ACTIVITY SCREENING

Table 1: Work Sheet for the Identification of *E. coli* Isolates from Suntreso Hospital

Specimen no	Characteristics on Agar Media			Biochemical Tests														Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	arabinose	Starch Hydrolysis	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	Slant														
SUHP2	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHP3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHP5	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM2	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM4	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM5	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM6	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHM8	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW1	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW2	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW4	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW7	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW8	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFW9	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHB3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHB4	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHB8	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHB9	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL7	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL11	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL12	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL13	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
SUHFL20	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*

Table2: Work Sheet for the Identification of *Pseudomonas aeruginosa* Isolates from Suntreso Hospital

Specimen no.	Characteristics on Agar Media			Biochemical Tests															Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	Arabinose	Starch Hydrolysis	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	slant														
SUHD1	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD2	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD3	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD4	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD11	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD12	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD15	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD16	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD18	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHD20	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH1	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH2	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH3	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH5	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH7	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH8	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHDH11	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHFL1	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHFL2	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHFL27	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
SUHFL30	B/D	Y/G	C/L	Red	Red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*

Key: SUH=Suntreso Hospital, D=Drainage, DH=Door handle, FL=floor, Mac=MacConkey, Cet=Cetrimide, EMB=Eosin Methylene blue
B/D=brown with dark center,Y/G=yellow-green/L=colourless, (-)=negative, +=positive , Ind=Indole, MR=Methyl Red, VP=Voges-Proskauer

Table 3: work sheet for the identification of *E. coli* isolates from Tafo Hospital

Specimen no	Characteristics on Agar Media			Biochemical Tests															Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	arabinose	Starch Hydrolysis	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	Slant														
TFFL1	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL2	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL3	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL5	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL6	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL7	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFL9	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFB4	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFB7	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFB8	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFB12	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFB13	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFW2	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFW3	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFW4	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFW8	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFFW13	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW5	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW7	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW8	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW9	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW10	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW15	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW16	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFMW17	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFPW3	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFPW4	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFPW5	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFPW8	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*
TFPW9	Pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	-	*E. coli*

Key: TF=Tafo Hospital, FL=floor, B=benches, FW=female ward, MW=male ward, PW=pediatric ward, Mac=MacConkey, Cet=cetrimide, EMB=eosin methylene blue, N/G=no growth, B/M=metallic sheen, +=positive, (-)=negative

Table 4: Work Sheet for the Identification of *Pseudomonas aeruginosa* Isolates from Tafo Hospital

Specimen no	Characteristics on Agar Media			Biochemical Tests														Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	Arabinose	Starch Hydrolysis	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	slant														
TFFL10	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFFL15	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFD2	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFD3	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFD4	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFD5	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFD8	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFDH3	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFDH8	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
TFDH10	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*

Key: TF=Tafo Hospital, FL=floor, D=drainage, DH=door handle, Mac=MacConkey, Cet=Cetrimide, EMB=Eosin Methylene blue, TSI=Triple Sugar Iron, Ind=Indole, MR=Methyl Red, VP=Voges-Proskauer

Table 5: Work Sheet for the Identification of *E. coli* Isolates from Kumasi South Hospital

Specimen no	Characteristics on Agar Media			Biochemical Tests															Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	Arabinose	Starch Hydroly	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	slant														
KSDH2	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSDH8	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSD2	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSD4	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSD9	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW4	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW5	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW10	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW15	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSPW18	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB5	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB13	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB19	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB28	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSB30	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW1	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW3	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW7	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW10	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW14	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSMW17	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFL10	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFL13	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFL16	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFW10	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFW21	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFW24	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFW25	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*
KSFW26	pink	N/G	B/M	acid	acid	+	+	-	-	-	+	-	+	+	+	-	+	+	*E. coli*

Key: KS= Kumasi South Hospital, FL=floor, B=bench, FW=female ward, MW=male ward, PW=paediatric ward, N/G=no growth, B/M=black with metallic sheen, Mac=MacConkey, Cet.=Cetrimide, EMB=Eosin Methylene blue, TSI=Triple Sugar Iron, Ind=Indole, MR=Methyl Red, VP=Voges-Proskauer. (-)=negative, +=positive

Table 6: Work Sheet for the Identification of *Pseudomonas aeruginosa* Isolates from Kumasi South Hospital

Specimen no	Characteristics on Agar Media			Biochemical Tests															Identification
				TSI Agar		Ind	MR	VP	Citrate	Oxidase	Nitrate	Arginine	Xylose	Maltose	arabinose	Starch Hydrolysis	glycerol	Growth at 42^0 C	
	Mac	Cet	EMB	Butt	slant														
KSB4	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSB13	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSB16	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSB20	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSB23	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
HSD1	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSD3	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSD4	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSD10	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFW3	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFW6	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFW8	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFW11	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFW12	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFL3	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFL13	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFL16	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*
KSFL21	B/D	Y/G	C/L	red	red	-	-	+	+	+	+	+	-	-	-	-	-	+	*P.aeruginosa*

Key: KS=Kumasi South Hospital, Mac=MacConkey, Cet=Cetrimide, EMB=Eosin Methylene blue, TSI=Triple Sugar Iron, B/D=brown with dark center, Y/G=yellow-green. C/L=colourless, (-)=negative, +=positive

Table 7: Zones of inhibition for *Pseudomonas aeruginosa* isolates from Tafo **Hospital**

Isolate	ANTIBIOTICS Measured in millimeter			
	GM(10µg)	CRO(30µ)	CIP(5µg)	AMP(10µ)
TFL10	10 (r)	21 (s)	20 (i)	6 (r)
TFL15	17(s)	6 (r)	21(s)	6(r)
TD2	20(s)	22 (s)	7 (r)	6(r)
TD3	9 ((r))	8 (r)	16 (i)	6(r)
TD4	7 (r)	6(r)	12(r)	6(r)
TD5	14 (i)	23 (s)	19 (i)	6(r)
TD8	13 (i)	20 (i)	20 (i)	6(r)
TDH3	10(r)	20 (i)	21 (s)	6(r)
TDH8	6 (r)	6 (r)	6 (r)	6(r)
TDH10	16 (s)	19 (i)	12 (r)	3(r)

KEY: T=Tafo Hospital, FL=floor, D=drainage¸ DH=door handle, i=intermediate, R=resistant, s= sensitive

Table 8: Zones of inhibition for *E.coli* isolates from Tafo Hospital

Isolate No.	ANTIBIOTICS Zones of inhibition (millimeter)				
	GM(10µg)	CRO(30µg)	CIP(5µg)	SXT(25µg)	AMP(10µg)
TFL1	16 (s)	21 (s)	23 (s)	6 (r)	6 (r)
TFL2	13(i)	26 (s)	23 (s)	6 (r)	6 (r)
TFL3	10(r)	18 (i)	20 (i)	6 (r)	20 (s)
TFL5	15(s)	21 (s)	21 (s)	15 (i)	18 (s)
TFL6	6 (r)	6 (r)	6 (r)	6 (r)	7 (r)
TFL7	12 (r)	20 (i)	18 (i)	6 (r)	12 (r)
TFL9	17(s)	17 (i)	20 (i)	13(i)	12 (r)
TB4	13(i)	12 (r)	12 (r)	10(r)	8 (r)
TB7	15 (s)	18 (i)	18 (i)	10 (r)	5 (r)
TB8	13 (i)	27 (s)	21 (s)	9 (r)	3 (r)
TB12	10 (r)	13 (r)	11 (r)	9(r)	13 (r)
TB13	6 (r)	6 (r)	6 (r)	6 (r)	6 (r)
TFW2	16 (s)	21 (s)	19 (r)	6 (r)	6 (r)
TFW3	13 (i)	20 (i)	21 (s)	6 (r)	9(r)
TFW4	8 (r)	13 (r)	19 (i)	12(i)	10(r)
TFW8	3 (r)	12 (r)	15 (i)	9 (r)	5 (r)
TFW13	6 (r)	24 (s)	21 (s)	6 (r)	5(r)
TMW5	15 (s)	18 (i)	18 (i)	6 (r)	8(r)
TMW7	10 (r)	19 (i)	22 (s)	9 (r)	9(r)
TM W8	10 (r)	18 (i)	20 (i)	10 (r)	8(r)
TMW9	6 (r)	20 (i)	17 (i)	12 (i)	6(r)
TMW10	7 (r)	7 (r)	8 (r)	8 (r)	6(r)
TMW15	16 (s)	14 (i)	20 (i)	6 (r)	6(r)
TMW16	7 (r)	21 (s)	20(i)	6 (r)	2(r)
TMW17	4 (r)	6 (r)	9 (r)	6 (r)	3(r)
TPW3	17 (s)	14 (i)	21 (s)	6 (r)	6(r)
TPW4	12 (r)	6 (r)	9 (r)	11 (i)	6(r)
TPW5	8 (r)	15 (i)	8 (r)	10 (r)	6(r)
TPW8	19 (s)	21 (s)	6 (r)	10 (r)	6(r)
TPW9	6 (r)	9 (r)	8 (r)	9 (r)	6(r)

KEY: T=Tafo Hospital, FL=floor, B=bench, FW=female ward, MW=male ward, PW=pediatrics ward, I=intermediate, r=resistant, s=sensitivity

Table 9: Zones of inhibition for *E. coli* isolates from Suntreso Hospital

Isolate	GM(10µg)	CRO(30µg)	CIP(5µg)	SXT(25µg)	AMP(10µg)
SPW2	6 (r)	10 (r)	25 (s)	6 (r)	6 (r)
SPW3	7 (r)	21 (s)	22 (s)	6(r)	6 (r)
SPW5	6 (r)	17 (i)	15 (r)	6(r)	6 (r)
SMW2	17 (s)	20 (i)	25 (s)	24(s)	22 (s)
SMW3	27 (s)	28 (s)	20 (i)	20 (s)	16 (i)
SMW4	17 (s)	17 (i)	27 (s)	24 (s)	6 (r)
SMW5	6 (r)	18 (i)	18 (i)	6 (r)	15 (i)
SMW6	5 (r)	12 (r)	19 (i)	17 (s)	6 (r)
SMW8	10 (r)	19 (i)	20 (i)	6 (r)	6(r)
SMW19	12 (r)	20 (i)	17 (i)	10 (r)	7(r)
SMW23	13 (i)	16 (i)	20(i)	12 (i)	6(r)
SMW26	18 (s)	21 (s)	18 (i)	9 (r)	6(r)
SFW1	6 (r)	20 (i)	6 (r)	5 (r)	6(r)
SFW2	6 (r)	19 (i)	21 (s)	12 (i)	11(r)
SFW4	7 (r)	10 (r)	8 (r)	22(s)	5(r)
SFW7	15 (s)	22 (s)	20 (i)	19 (s)	6(r)
SFW9	6 (r)	18 (i)	18 (i)	6 (r)	15(i)
SFW10	14(i)	16 (i)	13(r)	6 (r)	12(r)
SB3	16 (s)	25 (i)	13 (r)	17 (s)	10(r)
SB4	13 (i)	6 (r)	6 (r)	6 (r)	6(r)
SB8	21 (s)	7 (r)	18 (i)	6 (r)	6(r)
SB9	7 (r)	22 (s)	19 (i)	8 (r)	6(r)
SB20	6 (r)	9 (r)	16 (i)	9(r)	10(r)
SB22	12 (r)	19 (i)	20 (i)	12 (i)	9(r)
SFL3	12 (r)	19 (i)	9 (r)	6 (r)	6(r)
SFL7	15 (s)	20 (i)	11 (r)	7 (r)	6(r)
SFL11	15 (s)	19 (i)	16 (i)	6 (r)	6(r)
SFL12	17(s)	21(s)	16 (i)	5 (r)	6(r)
SFL13	6 (r)	17(i)	20 (i)	17 (s)	5(r)
SFL20	6 (r)	9 (r)	11(r)	17 (s)	10(r)
SFL23	12 (r)	20 (i)	13 (r)	10 (r)	7(r)
SFL26	17 (s)	16(i)	14(r)	6 (r)	6(r)
SFL28	14 (i)	19(i)	14 (r)	10(r)	8(r)
SFL30	9 (r)	20(i)	8 (r)	5(r)	7(r)

KEY: S=Suntreso Hospital, MW=male ward, FW=female ward, B=bench, FL=floor, DH=door handle

D=drainage, PW=pediatrics wards, i=intermediate, r=resistant, s=sensitive.

Table 10: Zone of inhibition for *P. aeruginosa* isolates from Suntreso Hospital

isolate	ANTIOBIOTICS Zones of inhibition measured in millimetres			
	GM(10µg)	CRO(30µg)	CIP(5µg)	AMP(10µg)
SFL1	15 (s)	20 (i)	21 (s)	6(r)
SFL2	18 (s)	18(i)	22 (s)	6(r)
SFL27	13 (i)	20(i)	20 (i)	6(r)
SFL30	13(i)	19(i)	21 (s)	6(r)
SD1	19 (s)	21 (s)	23 (s)	6(r)
SD2	6 (r)	6 (r)	6 (r)	6(r)
SD3	17 (s)	12 (r)	21 (s)	6(r)
SD4	13 (i)	10 (r)	7 (r)	6(r)
SD5	17 (s)	21 (s)	21 (s)	6(r)
SD10	15 (s)	20(i)	21 (s)	6(r)
SD12	18 (s)	18(i)	22 (s)	6(r)
SD15	13 (i)	10 (r)	8 (r)	6(r)
SD16	14 (i)	11 (r)	11 (r)	6(r)
SD17	13 (i)	6 (r)	14 (r)	6(r)
SD18	20 (s)	20(i)	21 (s)	10(r)
SD20	11 (r)	20(i)	20 (i)	6(r)
SDH1	14 (i)	21 (s)	19 (i)	6(r)
SDH2	14 (i)	6 (r)	20 (i)	6(r)
SDH3	16 (s)	18(i)	18 (i)	12(r)
SDH5	11 (r)	20(i)	21 (s)	6(r)
SDH7	15 (s)	21 (s)	20 (i)	6(r)
SDH8	6 (r)	6 (r)	6 (r)	6(r)
SDH11	7 (r)	13 (r)	11 (r)	5(r)

Key: SFL=floor, SD=drainage, i=intermediate, r=resistant, S=sensitive

Table 11: Zone of inhibition for *E. coli* isolates from Kumasi South Hospital

Isolate No.	Zones of inhibition measured millimetres				
	GM(10µg)	CRO(30µg)	CIP(5µg)	AMP(10µg)	SXT(25µg)
KSDH2	18 (s)	15 (i)	23 (s)	9 (r)	6(r)
KSDH8	8 (r)	16 (i)	6 (r)	12 (r)	6(r)
KSD9	17 (s)	15 (i)	26 (s)	8 (r)	18 (s)
KSD2	14 (i)	13(r)	13 (r)	15 (i)	15 (i)
KSD4	15 (s)	13 (r)	20 (i)	11 (r)	6(r)
KSPW3	10 (r)	18 (i)	21 (s)	15 (i)	6(r)
KSPW4	13 (i)	21 (s)	18 (i)	12 (r)	5(r)
KSPW5	11 (r)	13 (r)	20 (i)	8 (r)	7(r)
KSPW10	16 (s)	21 (s)	17 (i)	14 (i)	6(r)
KSPW15	14 (i)	10 (r)	17 (i)	9 (r)	6(r)
KSPW18	12 (r)	20 (i)	13 (r)	12(r)	9(r)
KSB3	20 (s)	21 (s)	25 (s)	9(r)	10(r)
KSB5	6 (r)	27 (s)	9 (r)	7(r)	6(r)
KSB13	15 (s)	10 (r)	20 (i)	8(r)	6(r)
KSB19	17 (s)	25 (s)	20 (i)	7(r)	6(r)
KSB28	13 (i)	18 (i)	16 (i)	6(r)	6(r)
KSB30	20 (s)	21 (s)	25 (s)	9 (r)	10(r)
KSMW1	17 (s)	26 (s)	6 (r)	7(r)	6(r)
KSMW3	8 (r)	14 (i)	14 (r)	8(r)	6(r)
KSMW7	15 (s)	9 (r)	16 (i)	12(r)	7(r)
KSMW10	9 (s)	16 (i)	22 (s)	6(r)	6(r)
KSMW14	20 (s)	19 (i)	17 (i)	5(r)	6(r)
KSMW17	17 (s)	10 (r)	20 (i)	7(r)	30 (s)
KSFL10	13 (i)	21 (s)	17 (i)	6(r)	6(r)
KSFL13	17 (s)	10 (r)	20 (i)	7(r)	30(s)
KSFL16	12 (r)	20 (i)	13(r)	7(r)	10(r)
KSFW10	6 (r)	13 (r)	22 (s)	15(r)	6(r)
KSFW21	6 (r)	20 (i)	6 (r)	5(r)	6(r)
KSFW24	17 (s)	16 (i)	9 (r)	6(r)	6(r)
KSEW25	20 (s)	21 (s)	23 (s)	6(r)	6(r)
KSFW26	14 (i)	19 (i)	14 (r)	8(r)	10(r)
KSFW28	17(s)	21(s)	23(s)	6(r)	9(r)
KSFW30	17(s)	21(s)	23(s)	7(r)	9(r)

KEY: KS=Kumasi South Hospital, FL=floor, B=bench, DH=door handle, D=drainage,

FW=female ward MW=male ward, Pw=Pediatrics ward, i=intermediate, r=resistant, S=sensitive

Table 12: zones of inhibition for *P. aeruginosa* isolates from Kumasi South Hospital

Isolate no.	Zones of inhibition of antibiotics measured (mm)			
	GM(10µg)	CRO(30µg)	CIP(5µg)	AMP(10µg)
KSB4	17 (s)	25 (s)	29 (s)	6 (r)
KSB13	10 (r)	20(i)	18 (i)	6(r)
KSB16	15 (s)	18(i)	21 (s)	6(r)
KSB20	12 (r)	20 (i)	17 (i)	6(r)
KSB23	10 (r)	9 (r)	13(r)	6(r)
KSD1	17 (s)	23 (s)	30 (s)	6(r)
KSD3	8 (r)	8 (r)	20 (i)	4(r)
KSD4	6 (r)	11 (r)	19 (i)	6(r)
KSD10	15 (s)	18 (i)	13 (r)	6(r)
KSFW3	18 (s)	23(s)	30 (s)	9(r)
KSFW6	6 (r)	6 (r)	18 (i)	6(r)
KSFW8	13 (i)	6 (r)	6 (r)	6(r)
KSFW11	15 (s)	16 (i)	20 (i)	6(r)
KSFW12	6 (r)	12 (r)	15 (r)	5(r)
KSFL3	11 (r)	21 (s)	16 (i)	6(r)
KSFL13	6(r)	13 (r)	11 (r)	6(r)
KSFL16	7(r)	20(i)	20 (i)	3(r)
KSFL21	7(r)	20 (i)	8 (r)	9(r)
KSFL22	10(r)	15 (i)	6 (r)	6(r)
KSDH6	13(i)	21 (s)	21 (s)	6(r)
KSDH20	9 (r)	16 (i)	12 (r)	6(r)
KSDH21	11(r)	10 (r)	9 (r)	6(r)
KSDH23	6 (r)	20 (i)	22 (s)	6(r)
KSDH25	12 (r)	8 (r)	11 (r)	6(r)

KEY: KS=Kumasi South Hospital, B= bench, D=drainage, DH=door handle, FL=floor, MW=male ward, FW=female ward, i=intermediate, r=resistant, s=sensitive