KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SCHOOL OF GRADUATE STUDIES

COLLEGE OF HEALTH SCIENCES

FACULTY OF PHARMCY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMCEUTICS



# ANTIBIOTIC RESISTANCE PATTERNS OF STRAINS OF *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS IN THREE HOSPITALS IN KUMASI, GHANA

**BY**

**SIXTUS BIERANYE, BAYAA MARTIN SAANA**

**JUNE, 2011**

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

**BY**

**SIXTUS BIERANYE, BAYAA MARTIN SAANA**

**JUNE, 2011**

**DECLARATION**

I, Sixtus Bieranye, Bayaa Martin Saana hereby declare that this thesis "Antibiotic resistance patterns of strains of *Salmonella typhi* and *Staphylococcus aureus* isolated from patients in three hospitals in Kumasi, Ghana" consists entirely of my own work produced from research undertaken under supervision and that, no part of it has been published or presented for another degree elsewhere, except for the permissible excerpts/references from other sources, which have been duly acknowledged.



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In Ghana, *S. aureus* and *S. typhi* are common causes of human infections and are also recognized as pathogens of high public significance. Despite these evidences, isolation of bacteria and testing of their sensitivities to antibiotics are hardly practiced in our hospitals. As a result, there is very little information on the antibiotic susceptibility patterns of *S. typhi* and *S. aureus*. This study therefore sought to determine the occurrence and extent of resistance of *S. typhi* and *S. aureus* isolated from patients in three hospitals namely Kumasi South, North Suntreso and Tafo hospitals to antibiotics. A total of 1200 samples obtained from patients suspected to have bacterial infections were analyzed for the presence of *S. aureus* and *S. typhi*. Out of this number, 128 samples had *S. typhi* and 109 had *S. aureus.* The isolates were then subjected to antibiotic sensitivity testing using a modified Kirby-Bauer method. The study revealed that infection of *S. typhi* and *S. aureus* did not differ statistically between the sexes. *S. typhi* isolates were evenly distributed among the various age groups, but there were significant differences among the various age groups in relation to *S. aureus* isolates. The most susceptible age group to *S. aureus* was the 20-29 years age group. The variation in the *S. typhi* and *S. aureus* among the clinical samples from the three hospitals was not statistically significant. The antibiotic susceptibility patterns of *S. typhi* revealed that 52.8% of the isolates were resistant to Ampicillin. The resistance levels of *S. typhi* to other antibiotics were low with 17.2% being resistant to ciprofloxacin, 32.8% to co-trimoxazole, 14.8% to ceftriaxone and 25.0% to chloramphenicol. The intermediate resistance levels of the *S. typhi* isolates to these five antibiotics were very low with 6.3% exhibiting intermediate resistance to ciprofloxacin and co-trimoxazole, 12.5% to ampicillin, 14.8% to ceftriaxone and 16.4% to chloramphenicol. *S. aureus* also showed a resistance of

42.6% to gentamicin, 40.7% to ampicillin, 49.1% to erythromycin, 43.5% to ceftriaxone and 20.4% to vancomycin. The numbers of intermediate resistant *S. aureus* to the antibiotics were few except for ceftriaxone which recorded a value of 39.8%. The percentage of multiple-drug resistance among the *S. typhi* and *S. aureus* isolates were 19.5% and 32.1% respectively. The usefulness of these antibiotics will therefore depend on the effective interventions put in place by health authorities to curb the spread of resistance among bacterial strains to antibiotics

# CHAPTER ONE

## 1.0 INTRODUCTION

In Ghana, *Staphylococcus aureus* and *Salmonella typhi* are common causes of human infections and are recognized as pathogens of high public health significance (Newman *et al.,* 2006). *S. aureus* is a normal inhabitant of the human skin and the respiratory tract (Brenda and Lee, 2008). It lives as a commensal in anterior nares of over half the population of humans (Prescott, 2002). In a healthy person *S. aureus* is usually not a health concern, but an injury and/or poor hygiene can cause *S. aureus* infection (Brenda and Lee, 2008). It is also a serious opportunistic pathogen responsible for a number of infections in tissues and sites with lowered host resistance such as individuals with diabetes, old malnourished persons and children (Burnett *et al.,* 1996). *S. aureus* has emerged as one of the main important human pathogens, and has over the past decades, been a leading cause of hospital and community-acquired infections (Shittu *et al.,* 2006).

Some infections caused by *S. aureus* include boils, scalded skin syndrome, toxic shock syndrome for menstruating women who use tampons, folliculitis, furunculosis, conjunctivitis, paronychia and mastitis. Staphylococcal pneumonia can also occur if staphylococcal infection spread to the lungs (Klodkowska-Farner *et al*., 1995).

The mode of transmission of the cocci from their normal habitats into the environment is by the hands, handkerchief, clothing and dust (Chigbu *et al.*, 2003). Most *S. aureus* infections acquired in the community are auto-infections with strains that the host has been carrying in the anterior nares, on the skin, or both. Community outbreaks are usually associated with

poor hygiene and fomite transmission to humans (Kenneth, 2004). In the hospital, *S. aureus* could be a major source of infection for newborn babies, surgical patients and hospital staff. Tuo *et al.,* (1995), observed that, sepsis of surgical wounds takes place either during operation through contaminated surgical instruments and gloves or in the ward after surgery. Hospital infections caused by strains of *S. aureus* most commonly involve patients who have undergone surgical or other invasive procedures. The source of the infection may be a patient with an overt or unapparent staphylococcal infection (e.g., decubitus ulcer) that is then spread directly to other patients on the hands of hospital personnel (Kenneth, 2004). A nasal or perineal carrier among medical, nursing, or other hospital personnel may also be the source of an outbreak, especially if carriage is heavy and numerous organisms are disseminated (Stephen and Kathleen, 2000).

Staphylococcal food poisoning can also occur in which a toxin produced by the bacteria is ingested with food. Food with a high salt or sugar content favours the growth of *S. aureus* (Tuo *et al.*, 1995). Many outbreaks of staphylococcal food poisoning result from hand contacts (Bryant *et al.*, 1998).

*S. typhi* on the other hand causes typhoid fever although *S. paratyphi*, *S. virchov, S. dublin* and *S. newport* are implicated (Mirza *et al.*, 1996). The highly adapted, human specific pathogen has evolved remarkable mechanisms for persistence in its host that help ensure its survival and transmission (Parry *et al.*, 2005).

Although typhoid fever is a rare imported infection in developed countries (Mathieu *et al.*, 1994), in developing countries where safe water supply, environmental sanitation and food hygiene are not optimal, typhoid fever is still a major problem (Mills-Robertson *et al.*, 2003), especially in Ghana where it is estimated that typhoid fever cases account for 3.2% of all infections recorded at hospitals (Mensah *et al.*, 2002). The annual global estimates for typhoid fever are 2.1 million episodes with 216 000 deaths (Crump *et al.*, 2004).



*S. typhi* is transmitted through food prepared outside the home such as iced cream (Luby *et al.*, 1998) or water that has been contaminated with faeces from either acutely infected persons, persistent excretors, or from chronic asymptomatic carriers (Mermin *et al.*, 1999). In endemic areas other risk factors identified include having a close contact with someone who has a recent typhoid fever (Luxemburger *et al.*, 2001), poor housing with inadequate facilities for personal hygiene (Gasem *et al.*, 2001) and recent use of antibiotics (Luby *et al.*, 1998). Humans are the only host for *S. typhi*; there are no environmental reservoirs (Mirza *et al.*, 1996).

In developing countries, great reliance is placed on antibiotic chemotherapy in the treatment of typhoid fever because of the difficulties in preventing typhoid fever by public health measures (Smith *et al.*, 1984). The mortality of untreated typhoid can be as high as 30%, whereas with appropriate antibiotic chemotherapy it is less than 1% (Smith *et al.*, 1984).

Attempts to control diseases caused by *S. aureus* and *S. typhi* through the use of antibiotics have resulted in increased prevalence of resistant strains of these organisms (Levy, 1998). Therefore, in order to effectively treat infections caused by these two pathogens, culture and antibiotic sensitivity tests must first be determined. Once culture and sensitivity results confirm the type of bacterial infection and sensitivity pattern, treatment may be modified (Paterson, 2000). However, most health facilities in Ghana lack adequately resourced laboratories to culture and test the antibiotic sensitivity of bacteria causing infections (Newman *et al.,* 2006). Even, where laboratory facilities are available, culture and sensitivity test results take 48 to 72 hours to be ready (Newman *et al.,* 2006). In many cases sensitivity test may not be carried out at all due to lack of microbiologists and the extra cost it constitutes for the patient (Ohene, 1997).

Many health personnel in these facilities therefore resort to empirical treatment for bacterial infections (Donkor *et al.,* 2008). Empirical treatment, however, has its immediate and long term problems. The immediate problem may be prescribing the wrong antibiotic leading to treatment failure and the long term result may be the evolution of antibiotic resistant bacterial strains which would make treatment of infections caused by such strains very difficult (Mills-Robertson *et al.,* 2003).

Infections caused by antibiotic resistant bacteria have led to increased hospitalization, health costs and mortality (Donkor *et al.,* 2008).

For these reasons, antibiotic resistance has become an important public health concern associated with serious consequences for the treatment of infections (Kunin, 1993).

### 1.1 Causes of antibiotic resistance

Resistance to antibiotics is a natural biological phenomenon that can be amplified or accelerated by a variety of factors, including human practices (Kenneth, 2008). Human practices that have exacerbated the problem of antibiotic resistance include unnecessary prescription of expensive broad-spectrum agents, and not following established recommendations for using chemo-prophylaxis (Lalitha, 2004). The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antibiotics (Prescott, 2002). Furthermore, the incorporation of antibiotics into herbal or "folk" remedies also increases inappropriate use of these agents (Lalitha, 2004). Much evidence therefore supports the view that the total consumption of antibiotics is the critical factor in selecting resistance (Donkor *et al.*, 2008). Paradoxically, underuse through lack of access, inadequate dosing, poor adherence, and substandard antibiotics may play as important a role as overuse (Kunin, 1993).

### 1.2 Bacterial mechanisms of antibiotic resistance

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that the cell does not recognize the antibiotic (Kenneth, 2008). The most common mode of bacterial resistance to antibiotics is enzymatic inactivation such that an existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site (Levy, 1998)

### 1.3 Mechanisms of *S. typhi* resistance to antibiotics

In developing countries the antibiotics most readily available for typhoid treatment are penicillin, cephalosporins, chloramphenicol and trimethoprim-sulfamethoxazole (co-trimoxazole) (Mermin *et al.*, 1999). Unfortunately, strains of *S. typhi* resistant to all the above mentioned agents have emerged (Islam *et al.*, 1993). According to Mills-Robertson *et al* (2003), ten out of fifty eight *S. typhi* isolated from patients in Accra, were resistant to ampicillin, chloramphenicol and co-trimoxazole (trimethoprim-sulfamethoxazole) which are first line antibiotics. There are also reports of resistance of *S. typhi* to cephalosporins (Newman *et al.*, 2006).

The production of β-lactamases which hydrolyze the β-lactam ring of penicillin and cephalosporins is one of the mechanisms of resistance by *S. typhi* (Prescott, 2002). Resistance to chloramphenicol by bacteria has been found to be caused by the enzyme, acetyltransferase which inactivates the antibiotic (Park and Arthur, 2002). The overproduction of the target of co-trimoxazole (dihydrofolate transferase) and the development of efflux pumps which expel ciprofloxacin are some of the mechanisms which make bacteria resistant to these antibiotics (Julian and Vera, 2004).

### 1.4 Mechanisms of *S. aureus* resistance to antibiotics

A significant number of staphylococcal species that infect humans and domestic animals are known to exhibit some degree of antibiotic resistance (Chigbu *et al.*, 2003). Chigbu *et al.*, (2003) in a study on antibiotic resistant *S. aureus* in Abia State of Nigeria revealed that all the isolates were resistant to a variety of different antibiotics. Resistance of *S. aureus* to macrolides, cephalosporins, penicillins, glycopeptides and fluoroquinolones has also been

reported (Newman *et al.,* 2006). In the clinical setting, methicillin resistance in *S. aureus* (MRSA) strains has contributed to the scope of antibiotic resistance since the early 1960s (Morris *et al.,* 2006). According to Aubry-Damon *et al* (1998), strains of *S. aureus* are resistant to all clinically useful antibiotics except vancomycin. However, some workers have reported the presence of vancomycin resistant strains (Shakibaie *et al.*, 2002).



Staphylococcal resistance to penicillin and cephalosporins is by β-lactamase production. *S. aureus* also produces aminoglycoside modifying enzymes (adenyltransferase), and efflux pumps which confer resistance to aminoglycosides (Julian and Vera, 2004). glycopeptide resistance exhibited by *S. aureus* is as a result of the synthesis of a type of peptidoglycan which vancomycin will not bind (Kenneth, 2008). The mechanism of macrolide resistance is enzymatic modification of the ribosome which is the target of macrolides (Prescott, 2002).

**1.5 Justification**

*S. typhi* persists in patients who just recovered from typhoid fever. These patients therefore carry the bacterium asymptomatically for long periods. Other asymptomatic carriers may never have the disease and it is the belief that these silent carriers contribute to continued episodes of infection. *S. aureus* on the other hand is responsible for various infections especially in immunocompromised patients. *S. typhi* and *S. aureus* may also exhibit varied antibiotic susceptibility patterns. Isolation and identification in our hospitals is however hardly practiced probably due to time constraint and the cost involved (Newman *et al.,* 2006). For these reasons, there is very little information on the antimicrobial susceptibility patterns of these two organisms in Ghana.

As a result our hospitals usually administer antibiotics to treat suspected typhoid fever and staphylococcal infections without considering whether these antibiotics are effective or not. This study is intended to isolate *S. typhi* and *S. aureus* from patients in certain hospitals and determine their susceptibility patterns to antibiotics commonly used to treat infections caused by these two organisms. The results of this study could therefore serve as guide for empirical treatment by health care personnel in these hospitals.



### 1.6 Main Objective

- This study is to determine the occurrence and extent of resistance of *S. typhi* and *S. aureus* isolated from patients in Kumasi to some antibiotics.

#### 1.6.1 Specific Objectives

- To randomly collect samples from various hospitals in Kumasi.
- To isolate and characterize *S. aureus* and *S. typhi* from the samples collected.
- To determine their susceptibility patterns to antibiotics.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 General Characteristics of the Genus *Staphylococcus*

The genus *Staphylococcus* consist of spherical cells arranged primarily in irregular clusters and occasionally in short chains and pairs (Park and Arthur, 2002). Staphylococci have a typical Gram-positive cell wall structure. Like all medically important cocci, they are nonflagellated, nonmotile and non-spore forming (Kenneth, 2004). Staphylococci grow best aerobically but are facultatively anaerobic and are catalase positive (Prescott, 2002). Staphylococci ferment glucose anaerobically and this is one distinguishing characteristic between this genus and micrococci (Cowan and Steel, 1970).

### 2.2 Classification and identification of *Staphylococcus aureus*

The genus Staphylococcus belongs to the family Micrococcaceae (Betty *et al.,* 2007). Within this family Staphylococci are closely related in structure to another group of bacteria; Micrococci and the view was held that Micrococci could not be differentiated from Staphylococci (Shaw *et al.,* 1951). However, various proposals were made with such distinguishing criteria as the ability to utilize glucose anaerobically in a complex medium (Evans *et al.*, 1955), utilization of Mannitol anaerobically (Mossel, 1962) and the ability to produce acetylmethylcarbinol (Kocur and Martinec, 1962). It was then agreed that catalase positive and Gram positive aerobic cocci that ferment glucose be classified as Staphylococci and those that oxidize or do not attack glucose classified as Micrococci (Cowan and Steel, 1970).

Staphylococci can be divided into pathogenic and relatively nonpathogenic strains based on the synthesis of the enzyme coagulase. Coagulase-positive strains, such as *S. aureus* often produce a yellow carotenoid pigment which has led to their being commonly called 'golden staph' and cause severe chronic infections (Brenda and Lee, 2008). Coagulase-negative Staphylococci such as *S. epidermidis* do not produce coagulase, are nonpigmented, and are generally less invasive but have increasingly been associated (as opportunistic pathogens) with serious nosocomial infections (Betty *et al.,* 2007).



Staphylococci are further classified into slime producers and non-slime producers. The ability to produce slime has been proposed as a marker for pathogenic strains of Staphylococci (Prescott, 2002). Clinical tests for Staphylococci are directed toward separation of species within the genus (Harley and Prescott, 1990).

Currently thirty-one (31) species have been placed in the genus *Staphylococcus,* but the most important human pathogens are *S. aureus, S. epidermidis, S. capitis, S. hominis* and *S. saprophyticus.* Of these, *S. aureus* is considered the most serious pathogen, although the other species have become increasingly associated with opportunistic infections and can no longer be regarded as harmless commensals (Park and Arthur, 2002). According to Betty *et al.,* (2007), the first method of preliminary identification of *S. aureus* is Mannitol fermentation. However, they observed that *S. saprophyticus* can also ferment Mannitol and thus resemble *S. aureus* on Mannitol Salt Agar. One key technique for separating *S. aureus* from other species is the coagulase test. By definition any member of the genus

*Staphylococcus* that coagulates plasma is *S. aureus* (Betty *et al.*, 2007). This conclusion may however be misleading since *S. lugdunensis*, *S. intermedius* and *S. schleferi* also show positive to coagulase test (Hinnebusch *et al.*, 1992). One clue that such species may not be *S. aureus* is penicillin susceptibility; more than 95% of *S. aureus* isolated from clinical specimens are penicillin resistant (Roberson *et al.*, 1992). Currently, phage typing and molecular methods are used to identify *S. aureus* (Betty *et al.*, 2007).



**2.3 Epidemiology of disease caused by *S. aureus***

*S. aureus* is the most frequently occurring bacterial pathogen among clinical isolates from hospital inpatients and is the second most prevalent bacterial pathogen among clinical isolates from outpatients (Styers *et al.*, 2006). According to Biedenbach *et al.*, (2004) *S. aureus* is the most common cause of nosocomial bactaeremia in North America, Latin America and Europe. Staphylococcal infections can be classified as nosocomial or community acquired. The incidence of staphylococcal nosocomial infections has increased greatly in recent years especially in Europe and the United States (Naber, 2009). Some of the risk factors associated with nosocomial staphylococcal infection include: hospitalization, skin ulcers and cellulitis at hospital admissions, indwelling catheters, immunosuppressive conditions, liver disease and type I diabetes mellitus (Mitchell and Howden, 2005). According to Mitchell and Howden, (2005) other risk factors associated with community acquired *S. aureus* infections are age and sex.

### 2.3.1 Prevalence of *S. aureus*

A study in the United States of America by Styers *et al*., (2006) between 1988 and 2005 on clinical isolates from inpatients revealed that 18.8% of all the isolates were *S. aureus*. They also reported a prevalence rate of *S. aureus* among outpatients to be 14.9%. A study in Europe by Biedenbach *et al.*, (2004) on the occurrence and antimicrobial resistance pattern of bloodstream infection isolates revealed that the prevalence of *S. aureus* was 26.0%.



Studies conducted by Manal *et al*., (2006) on trends in antibiotic susceptibility patterns and epidemiology of methicillin resistant *S. aureus* (MRSA) isolates from several hospitals in Riyadh, Saudi Arabia, revealed that prevalence of *S. aureus* among inpatients was 77.5%. Inpatient isolates were distributed in the following services: Intensive Care Unit: 96 (24.2%), Medicine: 59 (14.9%), Surgery: 54 (13.6%), Pediatric: 48 (12.1%), Burn and Plastic Surgery: 29 (7.3%), Renal: 18 (4.5%) and other unspecified wards: 66 (16.6%). Most isolates came from wounds (39.7%) followed by soft tissues (28.4%) while 22.5% prevalence was reported for outpatients.

A research conducted in Nigeria by Chigbu *et al*., (2003) on Antibiotic resistant *S. aureus* in Abia State of Nigeria, reported that out of 35 persons screened, 80% were positive for *S. aureus*. The report further showed that out of 16 persons from the hospital population screened, 14 (87.5%) were colonized either in the ear or nostril. Also, out of 19 persons from non-hospital population screened 14 (73.7%) were colonized either in the ear or nostril (Chigbu *et al*., 2003).

Another research on the resistance by bacteria to antimicrobial drugs in Ghana conducted by Newman *et al.,* (2006) showed that out of 4321 microorganisms isolated, 788 were *S. aureus* representing a prevalence rate of 18.24%. However, majority of the *S. aureus* were isolated from 48.4% of blood cultures and 17.5% of wound swabs.

### 2.3.2 Distribution of *S. aureus* in gender and different age groups

A study in Australia by Siranda *et al.,* (1999) on the continuing evolution of methicillin resistant *S. aureus*, revealed that the number of males and females colonized with *S. aureus* was approximately equal. However, they reported that 18% of the organism was recovered in children between the ages of 0 to 9 years and out of this age group, 58% of the isolates were recovered from children under the age of 2 years.

Manal *et al.*, (2006), reported that 64.4% of males and 34.6% of females carry *S. aureus* and among these two groups 41.5% of the isolates came from patients in the extreme age groups, (21.0% in patients greater than 60 years and 20.5% in patients less than 5 years of age). Similarly, other studies within the same country corroborated these findings. Mandani *et al.*, (2001) reported 26.1% of *S. aureus* prevalence from patients aged more than 60 years and 26.1% from patients less than a year old.

### 2.3.3 Transmission of *S. aureus*

Persons colonized with *S. aureus* strains are at increased risk of becoming infected with these strains. Most cases of nosocomial infection are acquired through exposure to the hands of health care workers after they have been transiently colonized with Staphylococci from

their own reservoir or from contact with an infected patient. Outbreaks may also result from exposure to a single long-term carrier or environmental sources, but these modes of transmission are less common (Sheretz *et al.*, 1996).

### 2.3.4 Temporal Trends in *S. aureus* infections

The numbers of both community-acquired and hospital-acquired staphylococcal infections have increased in the past 20 years (Naber, 2009). This trend parallels the increased use of intravascular devices (Tacconelli *et al.*, 2004). During the period from 1990 through 1992, *S. aureus* was the most common cause of nosocomial cases of pneumonia and surgical-wound infections and the second most common cause (after coagulase-negative staphylococci) of nosocomial bloodstream infections, according to data from the National Nosocomial Infections Surveillance system of the Centers for Disease Control and Prevention (CDC) (Emori and Gaynes, 1993).

A second trend, resulting in part from selective antibiotic pressure, has been the dramatic worldwide increase in the proportion of infections caused by methicillin-resistant *S. aureus* (Speller *et al.*, 1997). Initially noted in tertiary care hospitals, methicillin-resistant strains are increasingly found in the community (Naber, 2009).

Data from the National Nosocomial Infections Surveillance system (United States of America) for the period from 1987 to 1997 show that the number of methicillin-resistant *S. aureus* infections in intensive care units has continued to increase (Speller *et al.*, 1997). Methicillin-resistant strains have also become resistant to other antimicrobial agents (Chigbu *et al.*, 2003).

## 2.4 Diseases caused by *S. aureus*

*S. aureus* infection is a major cause of skin, soft tissue, respiratory, bone, joint, and endovascular disorders (Klodkowska-Farner, *et al*., 1995). The majority of these life threatening infections occur in persons with multiple risk factors for infection (Burnett *et al.*, 1996). Complications associated with *S. aureus* also increase disease burden (Naber, 2009). According to Nissenson *et al.*, (2005), among patients with end-stage renal failure, the cost of treatment increased significantly for patients with *S. aureus* infection who had more than one complication, compared with those who had no complications. Inappropriate initial antibiotic therapy also contributes to higher costs and mortality rates (Nissenson *et al.*, 2005).

### 2.4.1 Bacteraemia

*S. aureus* bacteraemia represents a significant burden on health care systems. According to an analysis of a large US database, *S. aureus* bacteraemia was associated with a longer median duration of hospital stay, higher median total treatment cost, and greater risk of mortality, compared with bacteraemia caused by any other pathogen (Shorr and Lodise, 2006).

According to Cosgrove *et al.*, (2006), there is substantial variation in the mortality rates (range, 0%–83%) reported for *S. aureus* bacteraemia. Mortality rates associated with *S. aureus* bacteraemia due to methicillin-resistant strains are particularly high among intensive care unit patients (Naber, 2009). In a Belgian study that consisted of a subset of critically ill patients, the methicillin resistant *S. aureus* bacteraemia–associated mortality rate was

23.4%, which was significantly higher than the corresponding methicillin susceptible *S. aureus* bacteraemia–associated mortality rate of 1.3% (Blot *et al.,* 2002).

An analysis of approximately 1000 US hospitals revealed that inpatients with *S. aureus* bacteraemia had a 3-fold longer mean duration of hospital stay than did inpatients without *S. aureus* bacteraemia and this resulted in a 3-fold increase in treatment costs (Noskin *et al.,* 2005).



### 2.4.2 Sepsis

A few cases of bactaeremia or local infections in patients progress into sepsis (Mylonakis and Calderwood, 2001). Risk factors for sepsis include advanced age, immunosuppression, chemotherapy and invasive procedures (Mitchell and Howden, 2005). *S. aureus* is one of the most common gram-positive pathogens in cases of sepsis. Severe cases progress to multi organ dysfunction, intravascular coagulation, lactic acidosis and death (Bone, 1994).

### 2.4.3 Toxic Shock Syndrome

Staphylococcal toxic shock syndrome came to prominence in 1980–1981, when numerous cases were associated with the introduction of super absorbent tampons for use during menstruation (Betty *et al.*, 2007). However, the toxin associated with this syndrome is also produced in men and in non-menstruating women by *S. aureus* present at sites other than the genital area such as in surgical wound infections (Prescott, 2002). According to Betty *et al.,* (2007) the toxic shock syndrome often develops from a site of colonization rather than infection. Although toxic shock syndrome toxin I accounts for more than 90 percent of cases

of the syndrome that are associated with menstruation, other enterotoxins account for 50 percent of cases unrelated to menstruation (Prescott, 2002). Non-menstrual cases have increased and now account for approximately one third of all cases. These non-menstrual cases have been associated with localized infections or surgery (Kenneth, 2004). Patients with non-menstrual toxic shock syndrome have a higher mortality rates than those with menstrual toxic shock syndrome (Park and Arthur, 2002).



### 2.5 Mechanism of pathogenicity

The ability to up-regulate virulence factors under stressful stimuli (e.g., host immune response or circulating antibiotics) is a key factor in the enabling of *S. aureus* to persist in the bloodstream, to seed deep tissues and to form secondary foci of infection (Naber, 2009). *S. aureus* strains have been effectively able to adhere to and colonize the skin and mucosa of nares, invade the bloodstream, evade host immunological responses, form protective biofilms and develop resistance to several antibiotics (Brenda and Lee, 2008). Consequently, despite the availability of many antibiotics with activity against wild-type strains, *S. aureus* is a highly successful and increasingly clinically important gram-positive pathogen.

#### 2.5.1 Adhesion and colonization

*S. aureus* can up-regulate a variety of virulence factors, enabling it to adhere to and colonize the nares and damaged skin or the surfaces of implanted devices or prostheses and to cause serious bloodstream infections (Park and Arthur, 2002). Teichoic acid, a polymer on the surface of *S. aureus*, is essential for this purpose (Weidenmaier *et al.*, 2004).

### 2.5.2 Invasion

*S. aureus* can disrupt the skin barrier by secreting exfoliative toxins, hemolysins (including α-hemolysin, which forms pores in skin cell membranes), and various enzymes that destroy tissue (Park and Arthur, 2002). Invasion may be triggered when the immune system is compromised, when there is a break in the physical integument, and/or when localized inflammation occurs (Prescott, 2002).



### 2.5.3 Evasion

*S. aureus* evades the host immune response by secreting anti-opsonizing proteins (e.g. chemotaxis inhibitory protein), which prevent phagocytosis by neutrophils (Park and Arthur, 2002). Protein A, located on the surface of *S. aureus* cells, also has antiphagocytic properties (Naber, 2009). Furthermore, *S. aureus* secretes leukotoxins (e.g. Panton-Valentine leukocidin), which lyse leukocytes and expresses superantigens (e.g. enterotoxins and toxic shock syndrome toxin I) (Prescott, 2002) which subvert the normal immune response by inducing strong, polyclonal stimulation and expansion of T cell receptor Vb–specific T cells (followed by the deletion or suppression of these T cells to ananergic state) (Naber, 2009).

### 2.5.4 Biofilm

*S. aureus* may form slimy biofilms on damaged skin, fitted medical devices, and healthy or damaged heart valves. The depletion of nutrients and oxygen causes bacteria to enter a non-growing state in which they are less susceptible to some antibiotics. In particular, small-colony variants of *S. aureus,* when adherent and in the stationary phase, demonstrate almost complete resistance to antimicrobial agents (Prescott, 2002). According to Prescott, (2002)

the biofilm matrix provides protection against immune cells and may restrict the penetration of some antibiotics.

### 2.6 Resistance of *S. aureus* to antibiotics

During the late 1950s and early 1960s, *S. aureus* caused considerable morbidity and mortality as a nosocomial or hospital-acquired pathogen. Since then, penicillinase-resistant, semi-synthetic penicillins have proved to be successful antimicrobial agents in the treatment of staphylococcal infections (Brenda and Lee, 2008). Unfortunately methicillin resistant *S. aureus* (MRSA) strains have recently emerged as a major nosocomial problem (Mandani *et al.,* 2001).

*S. aureus* strains carry a wide variety of multi-drug resistant genes on plasmids, which can be exchanged and spread among different species of Staphylococci (Neihart *et al*., 1988). In Ghana, a study by Newman *et al.,* (2006), in nine of the ten regional hospitals, showed that 42% of *S. aureus* isolates were multi-drug resistant.

One way in which *S. aureus* becomes resistant is through acquisition of a chromosomal gene (*mec A*) that encodes an alternate target protein which is not inactivated by methicillin (Prescott, 2002). The majority of the strains are resistant to several of the most commonly used antimicrobial agents, including macrolides, aminoglycosides and the beta-lactam antibiotics, including the latest generation of cephalosporins (Shakibaie *et al*., 2002). The multi-resistance determinants can be transferred to new bacterial hosts.

The situation is made more difficult in developing countries such as Ghana where antibiotics are readily available to consumers across the counter with or without prescription from a medical practitioner (Newman *et al.,* 2006). Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the Staphylococci (Donkor *et al.,* 2008). Serious infections by methicillin-resistant strains have been most often successfully treated with a potentially toxic antibiotic, vancomycin (Shakibaie *et al.,* 2002). However, some strains of *S. aureus* recently have become resistant to vancomycin (Aubry-Damon *et al*., 1998). Resistance to methicillin also may extend to the cephalosporins (Betty *et al.,* 2007).



## 2.7 General Characteristics of the Genus *Salmonella*

*Salmonella* is a Gram-negative, non-spore forming rod-shaped bacterium. It is named after Daniel Salmon (1850–1914), who, with Theobald Smith (1859–1934), isolated the bacterium from pigs in 1885 (Brenda and Lee, 2008). Since then, over 2,500 different serotypes of the bacterium have been found; the term serotype indicates the protein composition of the bacterial surface, which produces a distinct immune response by the host. The many different serotypes indicate that the surface of *Salmonella* is highly variable (Sebastien *et al.,* 2010).

They possess flagellae and are motile (Jawetz *et al.,* 2007). *Salmonella* characteristically ferments glucose and mannose without producing gas but does not ferment lactose and sucrose (Cowan and Steel, 1970).

These fermentation characteristics coupled with the ability of *Salmonella* to produce hydrogen sulphite when inoculated into triple sugar iron (TSI) agar are features that distinguish this genus from other enterobacteriaceae such as *Klebsiella* and *Proteus*. The production of hydrogen sulphite on selective media such as bismuth sulphite agar may cause the colonies of *Salmonella* to be confused with *Klebsiella* and *Proteus* (Murray *et al.*, 2002). *Salmonella* differs from other enterobacteriaceae by its inability to hydolyse urea to ammonia and carbon dioxide (Gloria *et al.*, 2003).



**2.8 Classification and identification of Salmonella**

The nomenclature and taxonomy of *Salmonella* are complex, controversial, have changed over the years and are still evolving. According to Kauffmann, (1963), the genus *Salmonella* can still be divided into three subgenera namely: *S. enteritidis, S. cholera-suis,* a zoonosis of swine and *S. arizonae*. Le Minor *et al.*, (1987) however held a different opinion; they thought that the genus *Salmonella* should be divided into two subgroups namely *Salmonella bongori* and *Salmonella enterica* which in their view should be further divided into six subspecies. The subspecies were classified into over 50 serogroups based on the O (somatic) antigen and $V_i$ (capsular) antigen and divided into more than 2400 serovars based on the H (flagellar) antigen.

Some serovars were ubiquitous and generalists, while others were specifically adapted to a particular host (Sebastien *et al.,* 2010). Each serotype had a species designation that reflected its source. For example, *S. typhimurium* (found in rats and mice) (Park and Arthur, 2002).

Cowan and Steel, (1970) observed that the serotype, *S. typhi*, was quite distinct both serologically and biochemically and warranted separate description; it differed from almost all the other serotypes of *Salmonella* in its inability to produce gas in the breakdown of carbohydrates and in being citrate negative.



Until the early 1960s, bacterial classification had been based primarily on Gram stain reaction, biochemical reaction and sugar fermentation test (Kauffmann and Edwards, 1952). These tests are still a major line of routine identification of bacteria in spite of newer techniques such as DNA-DNA hybridization and guanine-plus-cytosine (%G+C) determination (Britten and Kohne, 1965). Presently, biochemical and sugar fermentation tests and these newer techniques have enabled scientists to place new species into their correct genera (Britten and Kohne, 1965). DNA-DNA hybridization has shown all *Salmonella* to be genetically identical. For this reason the species name *S. enterica* proposed by Le Minor *et al.* (1987) did not gain widespread acceptance (Le Minor and Popoff, 1987). Microbiologists continue to refer to *Salmonella* by their serovar names (Betty *et al.*, 2007).

**2.9 Epidemiology of disease caused by *S. typhi***

*S. typhi* causes typhoid fever, a communicable disease found only in man (Singh, 2001). Typhoid fever is endemic throughout Africa and Asia and persists in the Middle East, a few southern and eastern European countries and central and South America. In the US and most of Europe, apart from occasional point source epidemics, typhoid fever is predominantly a disease of the returning traveller (Ackers *et al.*, 2000). According to Singh (2001), the

salient epidemiological risk features of typhoid fever are: age group, occupation, socio economic factors, environmental factors and reservoir infection.

### 2.9.1 Risk of typhoid fever among age groups

Typhoid fever may occur at any age but it is considered to be a disease mainly of children and young adults (Singh, 2001). Singh, (2001) also noted that in endemic areas such as Delhi, the highest attack rate occurs in children aged 8-13 years. Older people appear to be relatively immune, presumably because of frequently reinforced acquired immunity through numerous sub-clinical exposures to *S. typhi* (Parry *et al.*, 2005).

### 2.9.2 Occupational risk

Certain categories of persons handling infective materials and live cultures of *S. typhi* are at increased risk of acquiring infection. Food handlers, who are carriers, obviously pose a great threat of causing outbreaks. However, food handlers themselves are not at any increased risk to become carriers (Singh, 2001).

A study conducted by Feglo *et al.,* (2004) revealed that 1.16% of the subjects studied were carriers of *S. typhi*. Another study by Mensah *et al.,* (2002) in Accra reported a prevalence of 3.2% in a study involving 176 food vendors. A report from Spain also highlighted the risk posed by food handlers. In that report, one chronic carrier, a food handler was shown to have infected 70 others (Xercavins *et al.,* 1997).

### 2.9.3 Reservoir infection

Man is the only known reservoir of *S. typhi* infection, either in cases or carriers. A case is infectious as long as *S. typhi* appear in stool or urine (Hornick *et al.*, 1970). Carriers may be temporary or chronic. Temporary (convalescent or incubatory) carriers usually excrete *S. typhi* up to 6-8 weeks. By the end of one year, 3-4% of cases continue to excrete typhoid bacilli (Betty *et al.,* 2007). Persons who excrete the bacilli for more than a year after a clinical attack are called chronic carriers. A chronic carrier state can be expected to develop in about 3% of cases (Singh, 2001). Chronic carriers can excrete as many as $1x10^{10}$ cells of *S. typhi* per gram of faeces (Hornick, 1985). It was estimated in 1986, for instance, that there were 694 carriers per 100,000 habitants in Santiago, Chile (Edelman and Levine, 1986).

### 2.9.4 Environmental and Socio Economic factors

*S. typhi* can survive for prolonged periods in water, ice, dust and dried sewage and these may become sources of infection. Survival can be up to a month in ice and ice creams and up to 70 days in the soil irrigated with sewage (Velema *et al.*, 1997). Vegetables grown in sewage farms or washed in contaminated water are a health hazard (Singh, 2001). In endemic areas, peaks of transmission occur in dry weather or at the onset of rains when there may be a substantial increase in fly population. The flies act as vectors of *S. typhi* under such conditions (Black *et al.,* 1985).

The relatively long lifespan of *S. typhi* and social factors such as pollution of drinking water supplies, open air defecation, and urination, low standards of food and personal hygiene and health ignorance compound the problem of transmission (Morris *et al.*, 1984). A study

conducted in Uzbekistan by Padmini *et al.*, (2007) revealed that drinking untreated or unboiled water especially outside the home is a major risk factor in getting infected with *S. typhi.*

### 2.10 Transmission of *S. typhi* Infection

Typhoid is usually contracted by ingestion of food or water contaminated by faecal or urinary carriers excreting *S. typhi* (Black *et al.*, 1985). In developing countries, identified sources of transmission for the disease include eating food prepared outside the home, such as ice cream or flavored iced drinks from street vendors, drinking contaminated water, having a close contact or relative with recent typhoid fever, poor housing with inadequate facilities for personal hygiene and recent use of antimicrobial drugs (Parry *et al.*,2005)

### 2.11 Pathogenicity of *S. typhi*

Even though the infectious dose of *S. typhi* is as low as $10^3$ cells, the ability of the organism to cause infection depends on the gastric acid barrier (Hornick *et al.*, 1970). According to Parry *et al.*, (2005), achlorhydria due to ageing, previous gastrectomy, treatment with $H_2$ receptor antagonists, proton-pump inhibitors, large amounts of antacids and *Helicobacter pylori* infection increase susceptibility to *S. typhi* infection.

The virulence of *S. typhi* involves a number of biological features. These features include adhesion to epithelial surfaces, invasion and translocation to the intestinal lymphoid follicles (House *et al.*, 2001). *S. typhi* is able to survive and multiply within the mononuclear phagocytic cells of the lymphoid follicles, liver and spleen (Parry *et al.*, 2005).

## 2.12 Resistance of *S. typhi* to antibiotics

Antibiotic resistance by *S. typhi* is reported from all over the world and resistance levels are increasing yearly, making the resistant strains new pathogens, capable of causing severe outbreaks of typhoid fever (Crump *et al.*, 2004). A study on the emergence of multi-drug resistant *S. typhi* with reduced susceptibility to fluoroquinolones in Cambodia revealed that 56% of the isolates were multi-drug resistant. The study however went on to show that all the isolates were susceptible to ceftriaxone while 81% and 88% of the isolates demonstrated resistance to Nalidixic acid and ciprofloxacin respectively (Kasper *et al.*, 2010)

According to a research conducted by Mengo *et al.*, (2010) on the trends in *S. typhi* in Nairobi, Kenya from 2004 to 2008, 70% of all the *S. typhi* isolated were multi-drug resistant to ampicillin, chloramphenicol, tetracycline, co-trimoxazole, nalidixic acid, ciprofloxacin, ceftriaxone, gentamicin and cefuroxime. According to this research, the most effective drug was gentamicin with 99% of the isolates being susceptible. This was followed by ceftriaxone with susceptibility of 94%. Out of the 100 *S. typhi* strains isolated during the study, 82% were susceptible to cefuroxime and 65% were susceptible to nalidixic acid. The study also revealed that the least effective drugs were tetracycline and ampicillin where resistance to both drugs was 76%.

A study carried out in 11 hospitals in 7 regions in Ghana on the resistance to antimicrobial drugs by Newman *et al.*, (2006) reported that, 68 out of the 109 *S. typhi* isolates were multidrug resistant, representing 62% which was considered to be very high. The research

also revealed 95% resistance to ampicillin, 89% for chloramphenicol and co-trimoxazole and 84% for tetracycline. Lower resistances were recorded for gentamicin, ceftriaxone, cefuroxime and cefotaxime. cefotaxime recorded 3%, gentamicin 5%, cefuroxime 5% and ceftriaxone 0%.

In developing countries the antimicrobial agents most readily available for therapy against *S. typhi* are ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole (co-trimoxazole) (Kasper *et al.*, 2010). Unfortunately, strains of *S. typhi* resistant to all three agents have emerged (Islam *et al.*, 1993). According to Mills-Robertson *et al* (2003), ten out of fifty eight *S. typhi* strains isolated from patients in Accra, were resistant to Ampicillin, chloramphenicol and co-trimoxazole. There are also reports of resistance of *S. typhi* to cephalosporins (Newman *et al.*, 2006).

Due to the increasing resistance to antibiotics used traditionally for therapy, the use of fluoroquinolones, such as ciprofloxacin for the treatment of typhoid fever has become more common in the Asian countries. This in turn has led to an increase in fluoroquinolone resistance in *S. typhi* in those countries (Parry *et al.*, 2005). Sporadic cases of ciprofloxacin treatment failure in typhoid fever have been reported in Europe and more recently, in Asia (Parry *et al.*, 2005). Njinkeng *et al.*, (2005) reported that multidrug resistant (MDR) *S. typhi* and nalidixic acid resistant *S. typhi* (NARST) strains were found in Cameroon, Central Africa. Nalidixic acid resistant *S. typhi* have also been reported in East Africa (Karuiki *et al.*, 2000).

## 2.13 Antibiotic susceptibility testing

Antibiotic susceptibility testing is performed on bacteria isolated from clinical specimens to determine which antibiotic might be effective in treating infections caused by the bacteria. In clinical laboratories, susceptibility testing is usually performed by broth dilution, disc diffusion and the antibiotic gradient strip (E-test) methods.



For the broth dilution method, decreasing concentrations of the antibiotic(s) to be tested, usually prepared in serial twofold dilutions are placed in tubes of a broth medium that will support the growth of the test microorganism (CLSI, 2010). This technique measures the minimum inhibitory concentration (MIC) in microgram per milliliter. By comparing the MIC value with known concentrations of the drug obtained in serum or other body fluids, the likely clinical response can be assessed. A miniaturized version of this method called the broth microdilution test is currently being used.

The disc diffusion test, also known as the Kirby-Bauer test, has been widely used in clinical laboratories since 1966. A standardized suspension of bacteria is swabbed over the surface of Mueller-Hinton agar plate and paper discs containing single concentrations of each antibiotic are placed onto the inoculated surface. During incubation, the various drugs diffuse outward from the discs at a rate inversely proportional to their size, forming a concentration gradient of drug around each disc. A clear zone of inhibition around an antimicrobial disc reflects the degree of susceptibility of the organism to the drug (Mahon *et al*., 2007)

The E-test, a modification of the disc diffusion test, utilizes a strip impregnated with a gradient of concentrations of an antimicrobial drug. Multiple strips, each containing a different drug, are placed on the surface of an agar medium that has been uniformly inoculated with the test organism. During incubation the test organism will grow, and a zone of inhibition will form around the strip, but because of the gradient of concentrations, the zone of inhibition is shaped somewhat like a teardrop that will intersect the strip at some point. The MIC is determined by reading a number off the numerical scale printed on the strip at the point where the bacterial growth intersects it (Nester *et al*., 2004).

Currently, automated antibiotic susceptibility test methods have been developed. Some of these automated machines interpret growth endpoints of broth microdilution panels whereas others provide hands-off incubation and reading functions for microdilution trays. These machines use the principle of turbidimetric detection of bacterial growth in a broth by the use of a photometer. Another principle of growth detection is the detection of hydrolysis of a fluorogenic growth substrate incorporated in a special test medium. These automated methods of antibiotic susceptibility test may force the broth dilution, disc diffusion and the antibiotic gradient strip (E-test) methods out of laboratory diagnosis. However, the cost of these instruments and the reagents used may not allow laboratories in developing countries to discard broth dilution and disc diffusion methods which relatively cheaper.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 The Study Sites and Subjects

Three hospitals in Kumasi (Ashanti region, Ghana) namely, Kumasi South, North Suntreso and Tafo Hospitals were selected for the study. These hospitals were selected because they have well equipped laboratories which receive a large number of patients seeking medical attention.

The subjects for this study were patients who were suspected of bacterial infection and were requested to undergo medical diagnosis at the microbiology laboratories of these hospitals. From each hospital, a total of 150 subjects were selected randomly for the study. Out of this number, blood, urine and stool samples were collected from each of 100 subjects and a sample each of wound and nose swabs were collected from the remaining 50 subjects. A total of 1200 samples from the three hospitals were therefore collected and analyzed. Wound and nose swabs were collected at the wound dressing rooms of the hospitals. Records of the age and gender of the patients were also taken.

### 3.2 Isolation of bacteria

All the samples collected at the hospitals were inoculated into 10ml cooked meat broth (Oxoid CM 81) which is a transport medium (appendix 1) and placed in an incubator (an Gallenkamp Plus II) at 37$^{o}$C for 72 hours (Gloria *et al.*,2003).

A 20ml mannitol Salt agar (Oxoid CM0085) was melted and stabilized at 45$^{o}$C for 15 minutes. The agar was poured into sterile petri dishes whose bases were divided into four quadrants and allowed to set. Using a calibrated platinum loop, 2µl of each culture was transferred to the edge of each Petri dish and streaked back and forth over the first quadrant of the dish. The previous quadrant was cross streaked making sure that the loop extends into a second quadrant. This above procedure was repeated for each succeeding area until the 4 quadrants of the dish were inoculated. The inoculated petri dishes were then inverted and incubated at 37$^{o}$C for 24 hours (Betty *et al.*, 2007). The above method was repeated using blood agar (Oxoid CM 0331), bismuth sulphite agar (Oxoid CM0201) and MacConkey agar (Oxoid CM0109).

The colonies that were greyish, transparent to opaque on blood agar, colourless on MacConkey agar and black with zones of metallic sheen on bismuth sulphite agar were fished out and inoculated into nutrient broth and incubated at 37$^{o}$C for 24 hours. Colonies that were yellow with clear zones of haemolysis on blood agar and golden yellow on mannitol salt agar were also inoculated into nutrient broth and incubated at 37$^{o}$C for 24 hours. Nutrient agar (Oxoid CM0003) slants of the various isolates were prepared and stored at -8$^{o}$C for further studies.

### 3.3 Gram staining

A loopful of the culture was fixed on a slide. Ammonium oxalate crystal violet solution was applied for 20 seconds and washed with water. Lugol's iodine solution was applied for 30 seconds, then decolorized with 95% ethyl alcohol for 5 seconds and washed with water. It

was counterstained with 0.5% safranin for 30 seconds, washed with water and observed under the microscope (Betty *et al.,* 2007).

### 3.4 Identification of *S. typhi*

Various biochemical reactions including urease production, citrate utilization and triple sugar iron agar tests were used for the identification of *S. typhi* (Gloria *et al.,* 2003).



#### 3.4.1 Urease production test

Urease production test was carried out by the method described by Murray *et al.,* (2002) to distinguish between *Salmonella sp.* from *Proteus sp.* Both bacteria are non-lactose fermenters and therefore produce colourless colonies on MacConkey agar (Oxoid0109). A loopful of the test organism was inoculated onto urea agar (CM0053) slant. The tubes were then incubated at 37$^{o}$ C for 24 hours. *Proteus vulgaris* (ATCC13315) was used as the control organism. *P. vulgaris* is urease positive and changes the colour of the urea agar from red to pink (Gloria *et al.,* 2003).

#### 3.4.2 Citrate utilization

Citrate utilization test was carried out as described by Cowan and Steel (1970). A straightened inoculating loop was used to touch a pure colony of the test organism and inoculated into Koser's citrate medium. The tubes containing the Koser's citrate were covered with loose caps and incubated at 37$^{o}$C for 24 hours. An inoculated tube of *Klebsiella aerogenes* (ATCC9621) was used as a control.

### 3.4.3 Reaction in triple sugar iron agar

This was performed using the method described by Jawetz *et al.,* (2007). An inoculum of the test organism was picked using a straightened inoculating loop. The butt of prepared triple sugar iron agar (OxoidCM277) was stabbed and the slant streaked. The test tube was loosely capped and incubated at 37$^{o}$ C for 24 hours. An uninoculated triple sugar iron tube was used as a control (Gloria *et al.,* 2003).



### 3.5 Identification of *S. aureus*

*S. aureus* was identified and characterized using the coagulase and catalase biochemical tests as described by Betty *et al.,* (2007).

### 3.5.1 Coagulase test

Fresh horse blood (Oxoid SR0050C) was poured into a commercially prepared ethylenediaminetetraacetic acid tube (REF 367955 BD Vacutainer) and centrifuged with Hettich Centrifuge (D-78532 Tuttlingen) at 3622g for 30 minutes to obtain the plasma (Betty *et al.,* 2007).

Using an inoculating loop, 10 well isolated colonies of the test organism on the Nutrient agar (Oxoid CM0003) slant were completely emulsified into the plasma in a test tube. The test tube was capped and incubated at 37$^{o}$C for 4 hours (Reshma *et al.,* 2007). Coagulation of the plasma was interpreted as a positive result. A drop of distilled water in which a colony of the test organism was emulsified was used as a control (Betty *et al.,* 2007).

### 3.5.2 Catalase test

An inoculating loop was used to transfer a colony of the pure growth from the Nutrient agar slant onto the surface of a clean, dry glass slide. A drop of 3% hydrogen peroxide ($H_2O_2$) was placed on the colony on the slide. The evolution of bubbles of gas was recorded as positive (Park and Arthur, 2002)



### 3.6 Antibiotic Susceptibility Testing

The disk diffusion test (modified Kirby-Bauer method) was used to test the *in vitro* sensitivity of these isolates to antibiotics as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2010). Commonly used antibiotics for treating typhoid fever and *S. aureus* infections were tested against the *S. typhi* and *S. aureus* isolates. These antibiotics were 10µg of ampicillin, 30µg of chloramphenicol, 30µg of ceftriaxone, 25µg of co-trimoxazole 5µg of ciprofloxacin 10µg of gentamicin, 30µg of vancomycin and 15µg of erythromycin. The antibiotics were obtained from Oxoid, UK in the paper discs form.

### 3.7 Disc diffusion (Kirby-Bauer) method

A loopful of the isolated *S. aureus* was inoculated into 10ml peptone water and incubated at 37$^{o}$ C for 24 hours. It was then diluted to turbidity comparable to the 0.5 MacFarland turbidity standard (CLSI, 2010). The comparison between the inoculum and the 0.5 McFarland turbidity standard was done in adequate light against a white paper with contrasting black lines (Lalitha, 2004).

A sterile swab was dipped into the standardized inoculum and used to inoculate the surface of already prepared Mueller-Hinton agar (Oxoid CM0337). The swab was streaked over the

entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum. Finally, the entire edge of the agar was swabbed. The agar was left for 15 minutes for the surface moisture to dry (Gloria *et al.*, 2003).

Antibiotic discs were applied to the surface of the inoculated agar plate using a disc dispenser (Oxoid 6-place, 90mm) and incubated for 16 to 18 hours at 37$^{o}$C (Lalitha, 2004). The zones of inhibition for each antibiotic was measured in millimeters and compared with values provided by the Clinical and Laboratory Standards Institute (CLSI, 2010) as shown in appendix III. *Escherichia coli* (ATCC 25922) and *S. aureus* (ATCC25923) were used as controls in testing the sensitivity of *S. typhi* and *S. aureus* respectively (Gloria *et al*., 2003).

The laboratory results were analyzed using GraphPad prism 5 and Microsoft excel 2010 edition to address the objectives of the study.

# CHAPTER FOUR

## 4.0 RESULTS

A total of 128 *S. typhi* and 109 *S. aureus* strains were isolated from the 1200 clinical samples collected. The organisms were isolated based on their cultural, morphological and biochemical characteristics. The cultural, morphological and biochemical characteristics of the organisms isolated from the various clinical samples are presented in Appendix II.



**Table 4.1: Clinical samples screened and the number of *S. typhi* and *S. aureus* isolates obtained**

| Clinical samples | *S. typhi* | | | *S. aureus* | | |
|---|---|---|---|---|---|---|
| | Urine | Stool | Total | Wound | Nose | Total |
| Total clinical sample | 300 | 300 | 600 | 150 | 150 | 300 |
| Number of isolates | 49 | 79 | 128 | 53 | 56 | 109 |
| Percentage | 16.3 | 26.3 | 21.3 | 35.3 | 37.3 | 36.3 |

### 4.1 Distribution of *S. typhi* and *S. aureus* in relation to gender and age

The occurrence of isolates was higher in females (56.6%) than in males. A total of 117 males representing 49.2% from the three hospitals yielded both organisms (Table 4.2). The occurrence of *S. typhi* and *S. aureus* in the samples according to gender is as shown in Table 4.3. Samples which had *S. typhi* came from 72 (56.3%) females and 56 (43.7%) males (Table 4.3). *S. aureus* was detected in 48 females representing 44% and 61 males representing 56.0% (Table 4.3).

The incidence of isolates according to age is as shown in Figures 1 and 2. *S. typhi* occurred most frequently in the 30-39 age brackets while *S. aureus* occurred more frequently between the ages of 20-29.

**Table 4.2: Distribution of *S. typhi* and *S. aureus* isolates from the various hospitals according to gender**

| | FEMALE | | | MALE | | |
|---|---|---|---|---|---|---|
| **HOSPITAL** | Total no. | No. of isolates | % Occurrence | Total no. | No. of isolates | % Occurrence |
| **Kumasi South** | 63 | 43 | 68.3 | 87 | 49 | 56.3 |
| **North Suntreso** | 79 | 43 | 54.4 | 71 | 28 | 39.4 |
| **Tafo** | 70 | 34 | 48.6 | 80 | 40 | 50.0 |
| **Total** | 212 | 120 | 56.6 | 238 | 117 | 49.2 |

**Table 4.3: *S. typhi* and *S. aureus* isolated in relation to the gender of patients**

| **SPECIES** | **FEMALE** | | **MALE** | |
|---|---|---|---|---|
| | No: | % | No: | % |
| *S. typhi* | 72 | 60 | 56 | 47.8 |
| *S. aureus* | 48 | 40 | 61 | 52.2 |

**Table 4.4: Distribution of *S. typhi* and *S. aureus* among clinical samples**

| | *S. typhi* | | *S. aureus* | |
|---|---|---|---|---|
| | Urine | Stool | Wound | Nose |
| **Total** | 49 | 79 | 53 | 56 |
| **Percentage** | 38.3 | 61.7 | 48.6 | 51.4 |



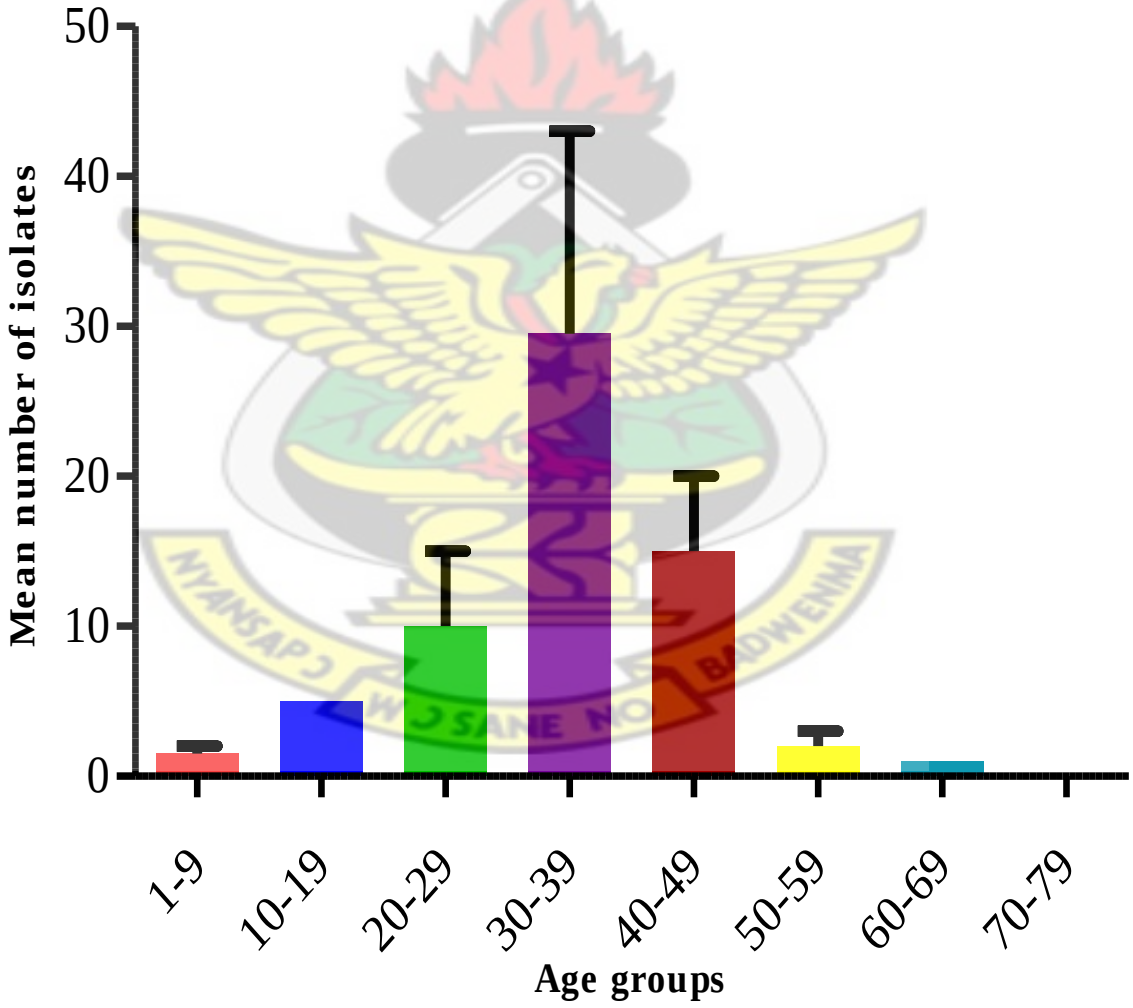



**Figure 4.1: *S. typhi* isolated in relation to age groups**

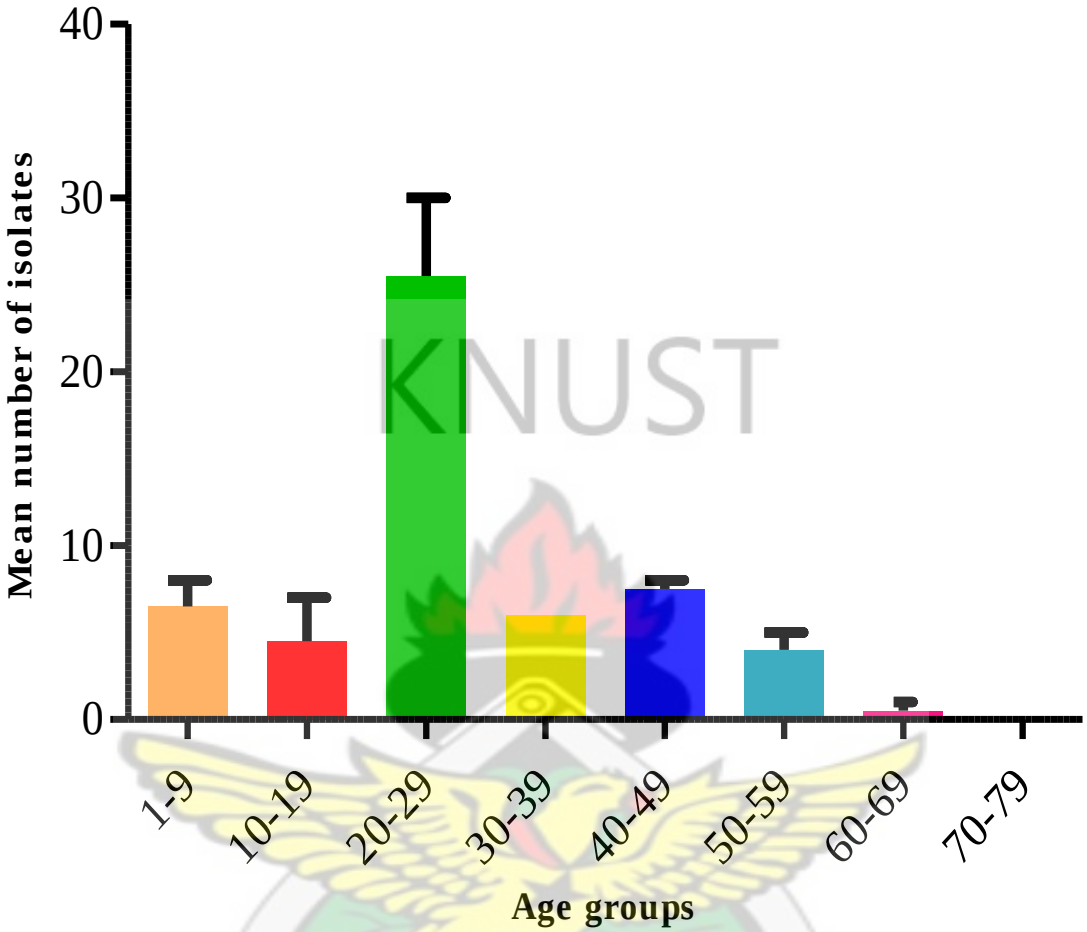



**Figure 4.2: *S. aureus* isolated in relation to age groups**

### 4.2 Distribution of *S. typhi* and *S. aureus* in clinical specimens

The occurrence of *S. typhi* and *S. aureus* in the various samples (urine, stool wound and nose) is shown in Table 4.1. A total of 128 *S. typhi* isolates was obtained from both urine and stool samples. Out of these isolates, 61.7% were from stool samples and 38.3% were from urine samples (Table 4.4). *S. aureus* isolates on the other hand, were obtained from wound and nose swabs. Out of the 109 *S. aureus* isolates, 48.6% came from wound swabs and 51.4% came from nose swabs (Table 4.4). Blood samples did not yield *S. typhi* and *S. aureus*.

### 4.3 Distribution of *S. typhi* and *S. aureus* in the various hospitals

As presented in Figure 4.3, Kumasi South hospital gave the highest number of *S. typhi* isolates of 50 representing 39.1%, followed by Tafo hospital with 44 isolates representing 34.4% and North Suntreso hospital recorded the lowest number with 34 isolates representing 26.6% of the total number of *S. typhi* isolates.



Kumasi South hospital also recorded the highest number of *S. aureus* isolates representing 38.5% while Tafo hospital recorded the lowest number of *S. aureus* isolates representing 27.5% (Figure 4.3).

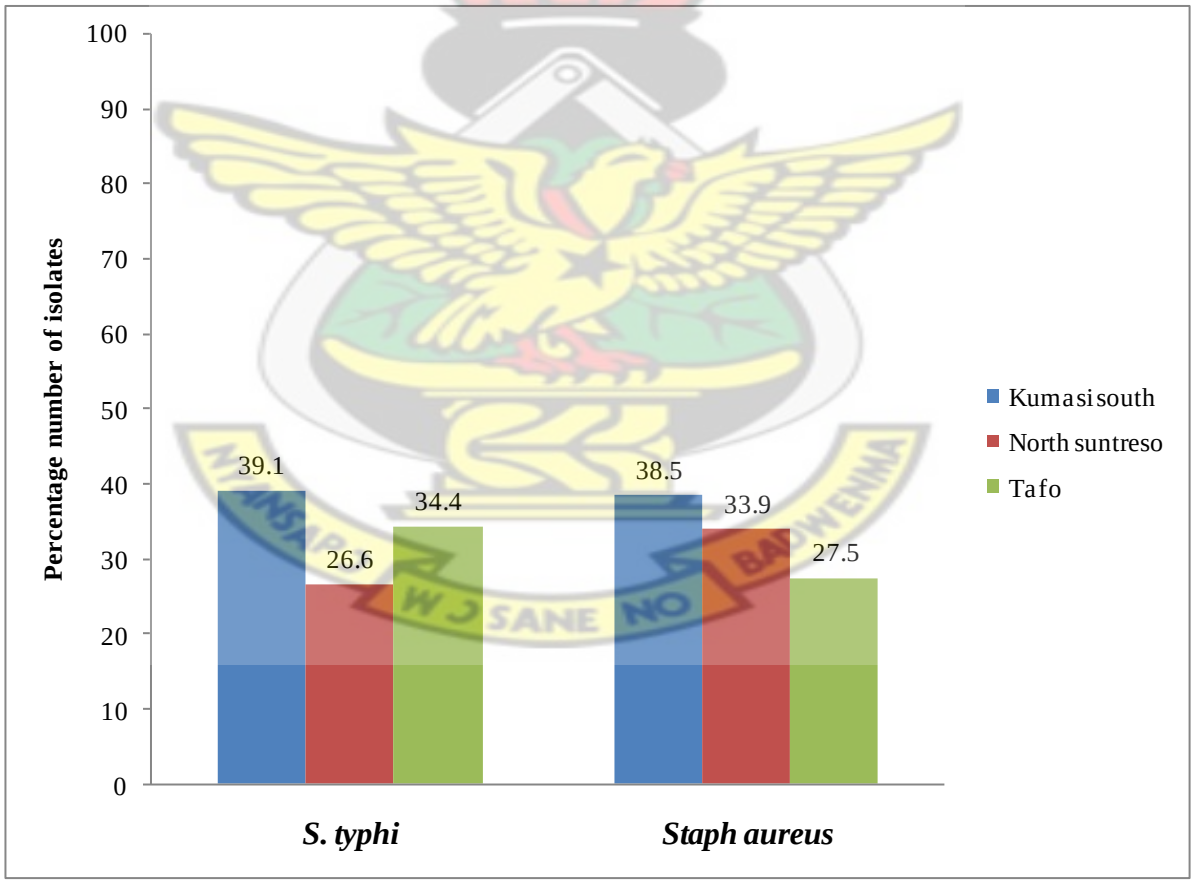



**Figure 4.3: Representation of *S. typhi* and *S. aureus* isolates in the various hospitals**

### 4.4 Antibiotic sensitivity of *S. typhi* and *S. aureus*

Microorganisms were classified as being susceptible, intermediate resistant and resistant to the antibiotics depending on the size of inhibition zone diameters when compared to the standard provided by the CLSI (Appendix III).

Antibiotic susceptibility patterns of *S. typhi* to individual antibiotics are presented in Figure 4.4. All the *S. typhi* isolates screened showed high susceptibility to ciprofloxacin (76.6%), co-trimoxazole (60.9%), ceftriaxone (70.3%) and chloramphenicol (58.6%).

Among the 128 *S. typhi* isolates, the overall rate of resistance was 52.3% to ampicillin, 32.8% to co-trimoxazole, 25% to chloramphenicol, 17.2% to ciprofloxacin and 14.8% to ceftriaxone (Figure 4.4). The isolates also showed intermediate resistance of 6.3% to ciprofloxacin and co-trimoxazole. Other intermediate resistance patterns were 12.5% to ampicillin, 14.8% to ceftriaxone and 16.4% to chloramphenicol (Figure 4.4).

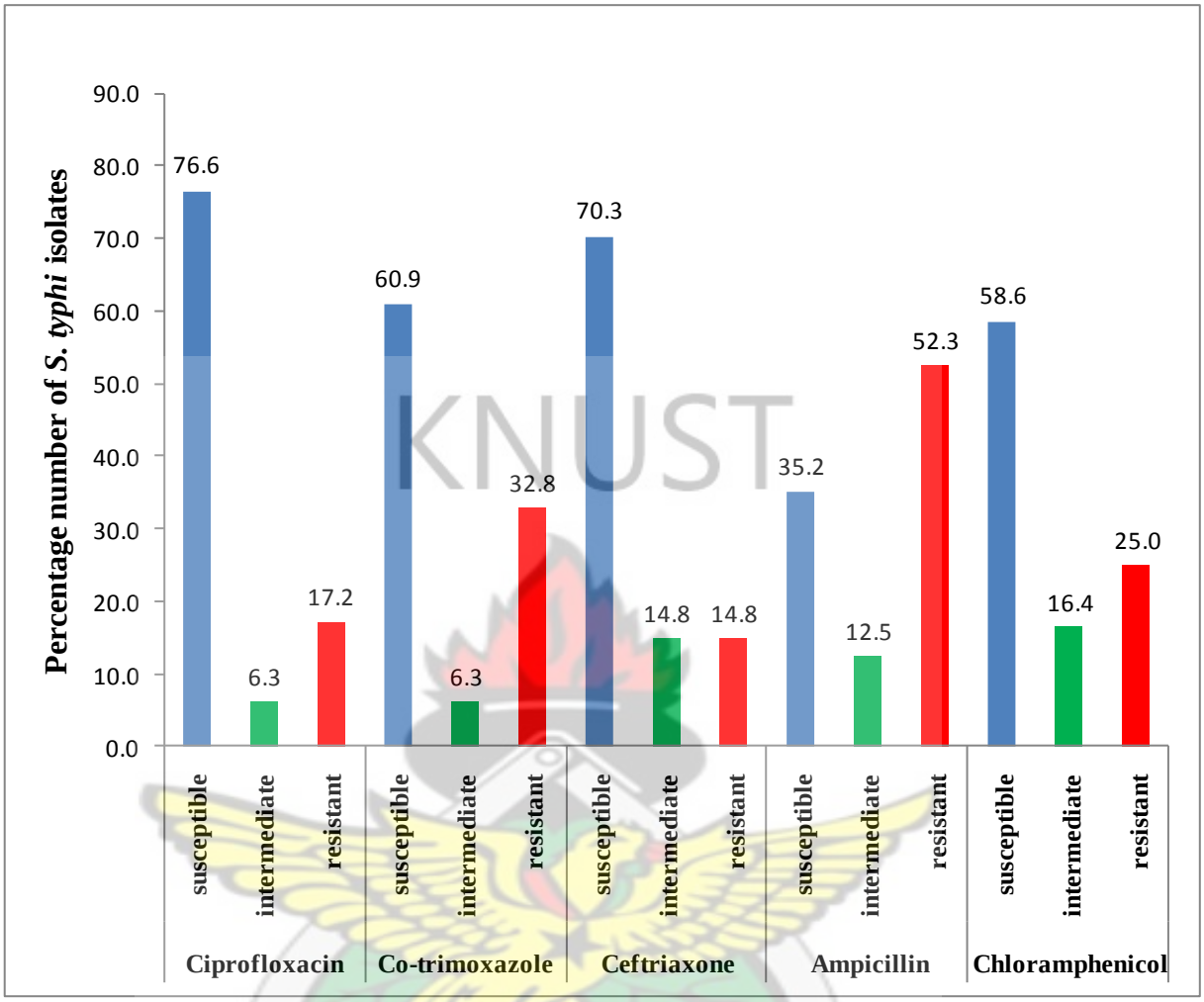



**Figure 4.4: Antibiotic susceptibility patterns of *S. typhi* to the antibiotics**

As shown in Figure 4.5, the *S. aureus* isolates showed variable susceptibility to the antibiotics tested. Vancomycin registered the highest susceptibility of 73.4% followed by ampicillin with 47.2%, gentamicin with 44.4%, ceftriaxone with 43.5% and erythromycin with 38.0%. Resistance to vancomycin, ampicillin, ceftriaxone, gentamicin and erythromycin were 20.4, 40.7, 43.5, 42.6 and 49.1% respectively (Figure 4.5). The *S. aureus* isolates showed intermediate resistance of 39.8% to ceftriaxone, 13.9% to ampicillin, erythromycin and gentamicin and 6.5% to vancomycin (Figure 4.5).

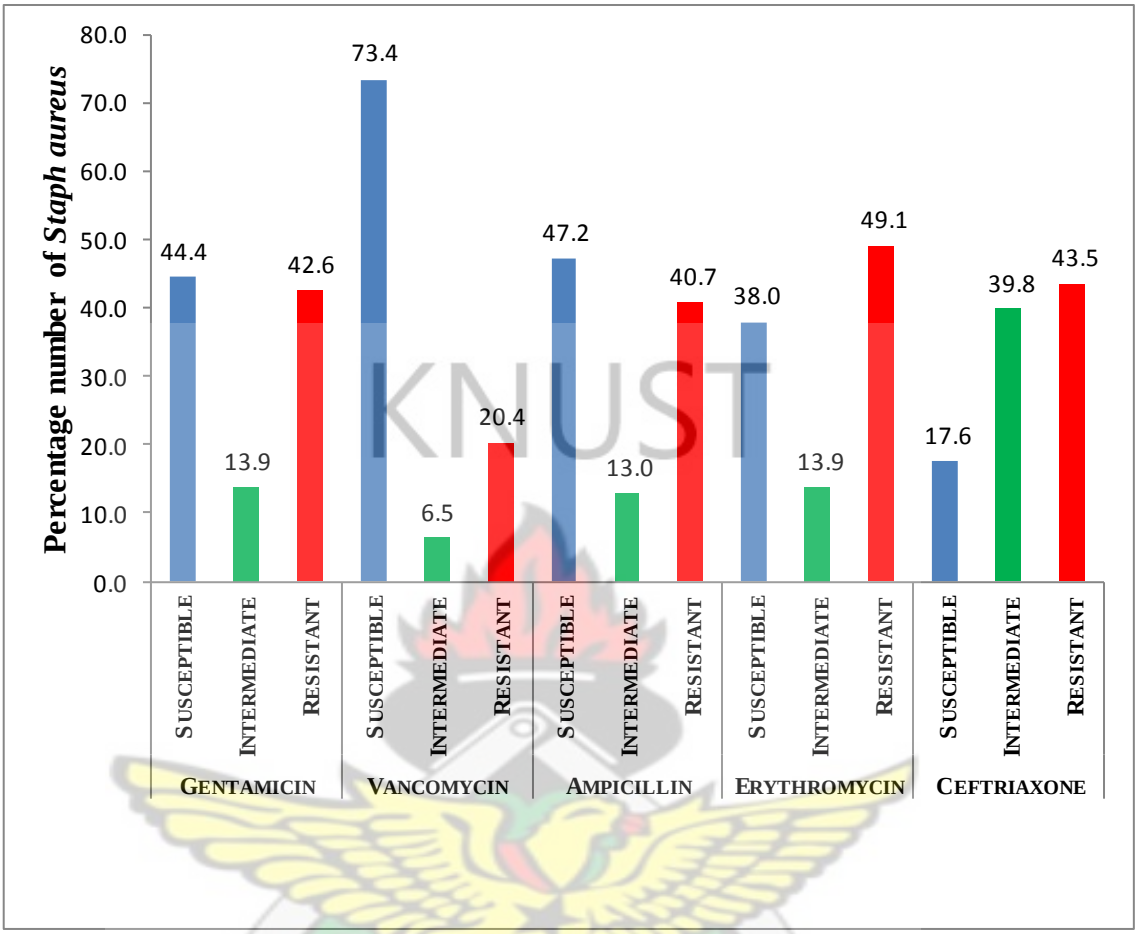



**Figure 4.5: Antibiotic susceptibility patterns of *S. aureus* to antibiotics**

### 4.5 Antibiotic susceptibility patterns of wound and nose isolates of *S. aureus*

The wound and nose isolates of *S. aureus* were sensitive to vancomycin with susceptibilities of 69.8% and 76.8% respectively. Generally, the number of the *S. aureus* isolates from both wound and nose that showed resistance to gentamicin, ampicillin, erythromycin and ceftriaxone were slightly high (Figure 4.6).

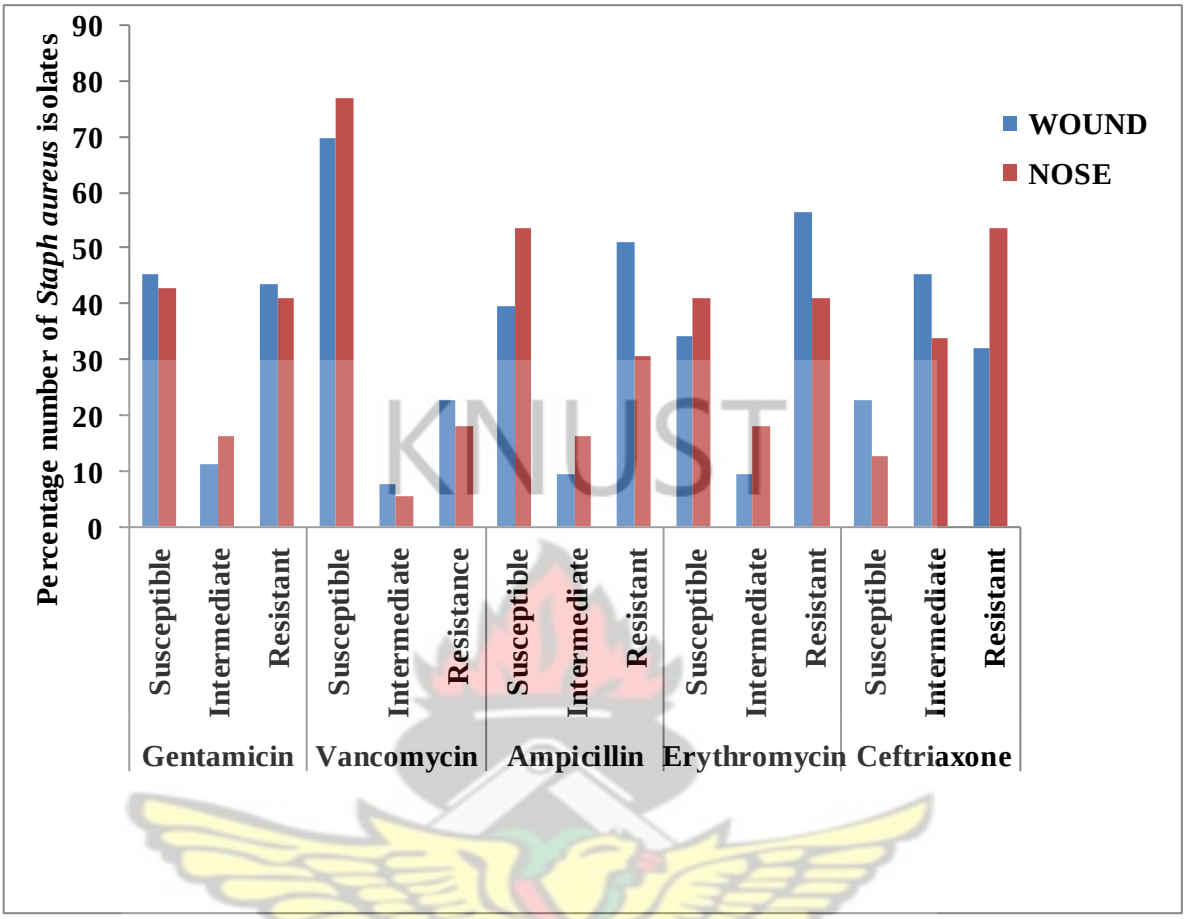



**Figure 4.6: Antibiotic susceptibility patterns of *S. aureus* from wound and nose samples**

### 4.6 Levels of antibiotic susceptibility of *S. aureus* in the various hospitals

Figure 4.7 shows the susceptibility trends of *S. aureus* isolates from the three hospitals. The isolates were more susceptible to vancomycin with susceptibilities of 76.7, 76.2 and 67.6% for Tafo, Kumasi South and North Suntreso hospitals respectively as compared to susceptibilities of 19.0, 18.9 and 13.3% to ceftriaxone by the isolates which were registered by Kumasi South, North Suntreso and Tafo hospitals respectively. In Tafo hospital, 53.3% of the *S. aureus* were resistant to erythromycin, 50% to both gentamicin and ceftriaxone and 46.7% to ampicillin whereas 47.6, 45.9, 37.8 and 33.3% of the *S. aureus* isolates from Kumasi South were resistant to erythromycin, ceftriaxone, ampicillin and gentamicin respectively.

As shown in Figure 4.7, 45.9% of the isolates from North Suntreso hospital exhibited resistance to erythromycin, gentamicin and ceftriaxone while 37.8% of these isolates showed resistance to ampicillin. The above shows similar resistance trends of the *S. aureus* isolates from Kumasi South, Tafo and North Suntreso hospitals to the antibiotics used in the study.

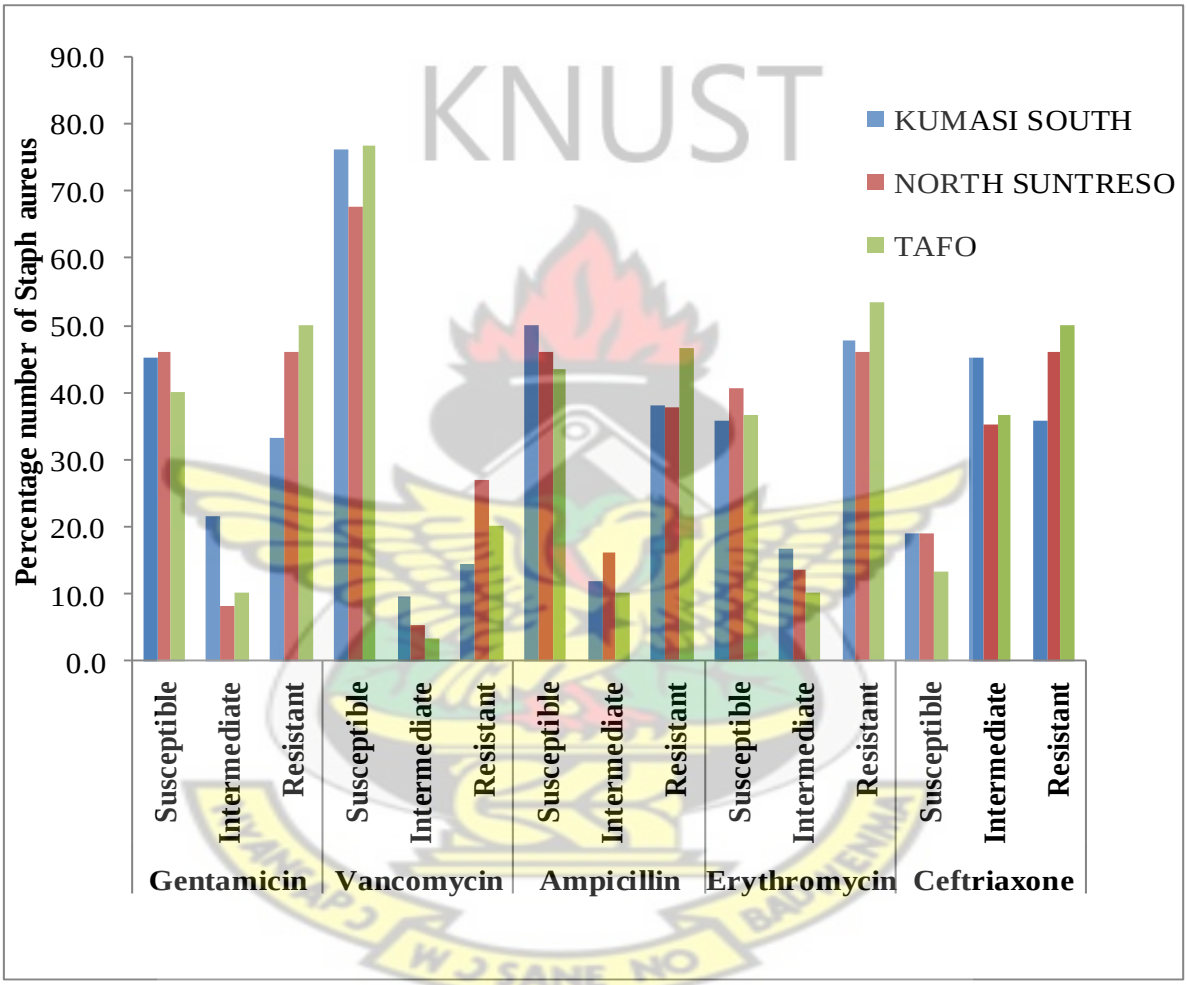



**Figure 4.7: Levels of antibiotic susceptibility of *S. aureus* in the various hospitals**

### 4.7 Antibiotic susceptibility patterns of stool and urine isolates of *S. typhi*

As shown in Figure 4.8, 84.8% of the *S. typhi* isolated from stool samples were susceptible to ciprofloxacin. Susceptibilities of 72.2, 69.6, 60.8 and 40.5% were shown to ceftriaxone, co-trimoxazole, chloramphenicol and ampicillin respectively.

The number of *S. typhi* isolated from urine samples that were susceptible to ceftriaxone were 67.3% while 63.3% were susceptible to ciprofloxacin, 55.1% were susceptible to chloramphenicol and 26.5% were susceptible to ampicillin. The *S. typhi* isolates from both stool and urine samples exhibited intermediate resistance to the five antibiotics tested (Figure 4.8).

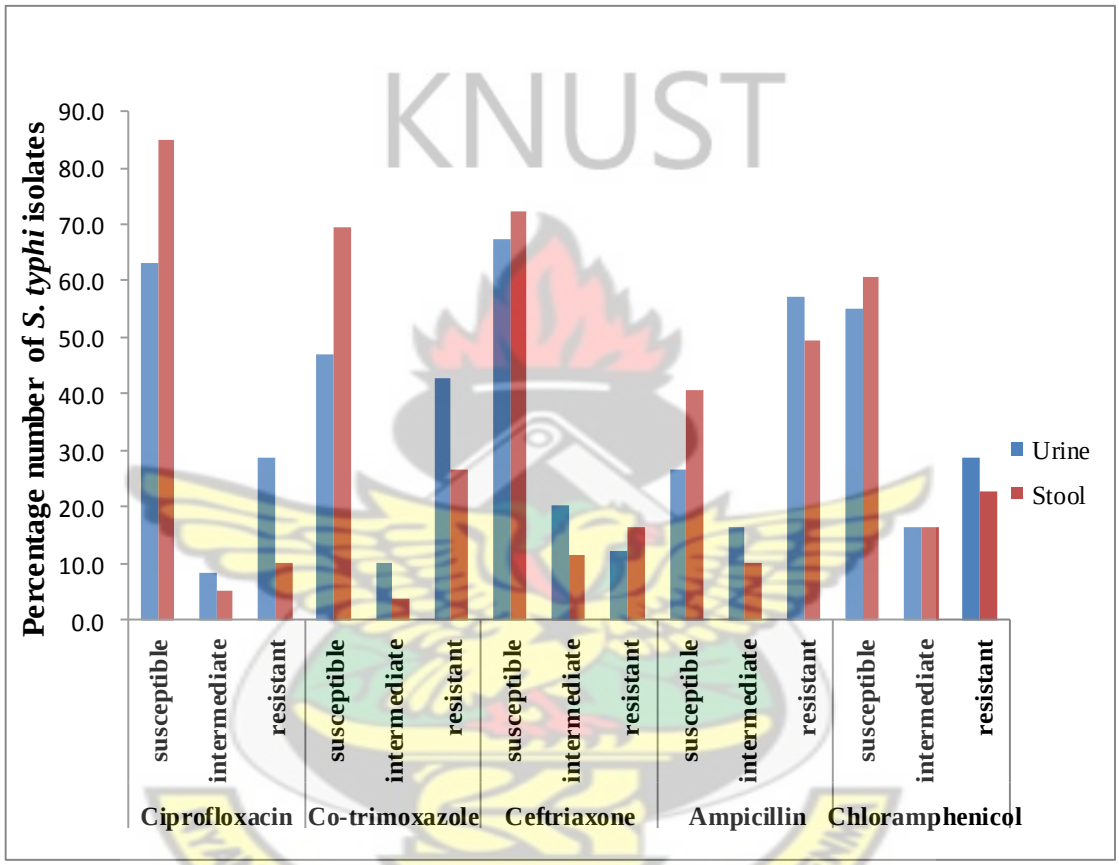



**Figure 4.8: Antibiotic susceptibility patterns of *S. typhi* isolates from stool and urine samples**

### 4.8 Antibiotic susceptibility patterns of *S. typhi* isolates from the various hospitals

The *S. typhi* isolates from the various hospitals were tested against the antibiotics and their antibiograms determined. The number of *S. typhi* isolates from Kumasi South hospital that showed susceptibility to chloramphenicol was 88.0%. Also, 80.0% and 78.0% of the isolates showed susceptibility to ciprofloxacin and ceftriaxone respectively. Furthermore,

susceptibilities of 48.0% and 32.0% were recorded for co-trimoxazole and ampicillin (Figure 4.9).

The number of resistant *S. typhi* isolates from Kumasi South hospital to ampicillin was 58.0%. The *S. typhi* isolates however showed a resistance of 6.0% to chloramphenicol. The resistances of these isolates to the other antibiotics were 8.0% against ceftriaxone, 14.0% against ciprofloxacin and 46.0% against co-trimoxazole. The *S. typhi* isolates showed intermediate resistance level of 6.0% to chloramphenicol, ciprofloxacin and co-trimoxazole. Ampicillin and ceftriaxone also recorded 10.1% and 14.0% respectively (Figure 4.9).

The numbers of susceptible *S. typhi* from North Suntreso hospital to ceftriaxone, ciprofloxacin and co-trimoxazole were 63.6, 61.8 and 58.8% respectively. However, only 38.6% and 17.6% of these isolates were susceptible to chloramphenicol and ampicillin respectively. A large number of the *S. typhi* isolates (70.6%) recorded resistance to ampicillin. The number of resistant *S. typhi* to co-trimoxazole, chloramphenicol, ciprofloxacin and ceftriaxone were 38.2, 35.3, 26.5 and 23.5% respectively.

Among the same *S. typhi* isolates from North Suntreso hospital, 23.5% registered intermediate resistance to chloramphenicol, 11.8% to both ciprofloxacin and ampicillin, 8.8% to ceftriaxone and 2.9% to co-trimoxazole (figure 4.9).

Also, 84.1% of the *S. typhi* from Tafo hospital were susceptible to ciprofloxacin, 77.3% to co-trimoxazole, 63.6% to ceftriaxone and 52.3% to ampicillin. The number of *S. typhi*

isolates sensitive to chloramphenicol was 38.6%. A total of 38.6, 31.8 and 15.9% of the *S. typhi* displayed resistance to chloramphenicol, ampicillin and ceftriaxone. The number of resistant isolates to ciprofloxacin and co-trimoxazole was 13.6%. In Tafo hospital, the number of *S. typhi* that showed intermediate resistance to chloramphenicol was 22.7%.

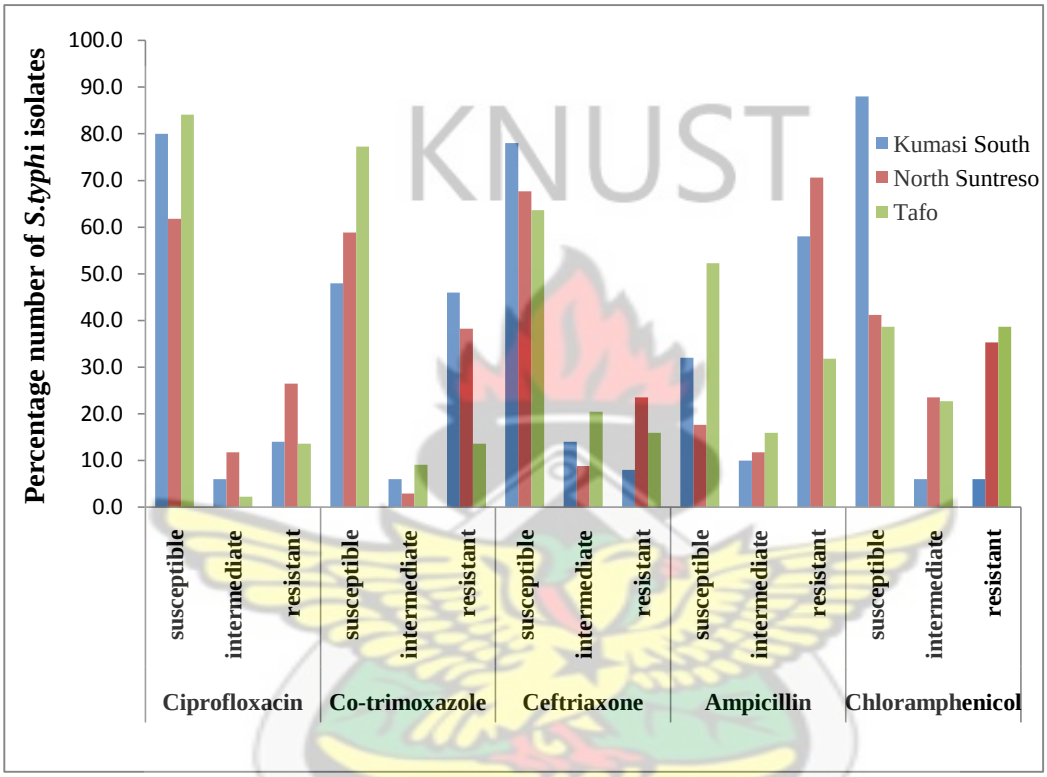



**Figure 4.9: Antibiotic susceptibility patterns of S. typhi from the various hospitals**

### 4.9 Multiple-drug resistance among *S. aureus* and *S. typhi* isolates

A total of 32.1% of the *S. aureus* isolates were multiple-drug resistant whereas 19.5% of *S. typhi* were found to be multiple-drug resistant. Multiple-drug resistance was defined as resistance of bacteria to at least three different antibiotics (Feglo *et al.*, 2010).

**Table 4.5: Multiple-drug resistant (MDR) *S. aureus***

| HOSPITAL | No. of MDR *S. aureus* | | Total No. of MDR *S. aureus* | Total No. of *S.aureus* isolates | Percentage MDR |
|---|---|---|---|---|---|
| | WOUND | NOSE | | | |
| Kumasi south | 5 | 3 | 8 | 42 | 19.0 |
| North Suntreso | 11 | 3 | 14 | 37 | 37.8 |
| Tafo | 8 | 5 | 13 | 30 | 43.3 |
| Total | 24 | 11 | 35 | 109 | 32.1 |



**Table 4.6: Multiple-drug resistant (MDR) *S. typhi***

| HOSPITAL | No. of MDR *S. typhi* | | Total No. of MDR *S. typhi* | Total No. of *S. typhi* | Percentage MDR |
|---|---|---|---|---|---|
| | STOOL | URINE | | | |
| Kumasi | 3 | 7 | 10 | 50 | 20.0 |
| North Suntreso | 3 | 6 | 9 | 34 | 26.5 |
| Tafo | 5 | 1 | 6 | 44 | 13.6 |
| Total | 11 | 14 | 25 | 128 | 19.5 |

# CHAPTER FIVE

## 5.0 DISCUSSION

*S. typhi* isolates were obtained from 49 (16.3%) of the 300 urine samples and 79 (26.3%) of the 300 stool samples (Table 4.1). The presence of *S. typhi* in urine and stool samples depicted a carrier state among the patients (Jones and Falkow, 1996). According to Singh, (2001) carriers may shed *S. typhi* either through their stool or urine continuously or intermittently and the carrier state is usually the source of contamination. Carriers could therefore serve as reservoirs for the dissemination of *S. typhi* causing serious public health problems. The high stool yield of *S. typhi* observed in this study confirms an earlier report by Singh (2001) that faecal carriers were more frequent than urinary carriers. However, statistical comparison of the stool and urine samples did not show any significant difference.

All the blood samples investigated in this study did not yield any growth of *S. typhi*. This observation is similar to a report by Alfred and Comfort, (2004). According to Parry *et al*, (2005), counts of *S. typhi* in blood of patients with typhoid fever indicate a median concentration of one bacterium per milliliter. In order to isolate *S. typhi* from blood cultures, Parry et al, (2005) recommended that at 20ml of blood from an individual patient should be subcultured. This could be the reason why this study did not record growth of *S. typhi* on any of the media subcultured with the blood samples since one milliliter of blood was taken from each patient and used.

No growth of *S. aureus* was recorded in the blood samples analyzed and the implication could be that no patient recruited for the study had bacteraemia. Blood is sterile in healthy

persons and the presence of *S. aureus* is normally an indication of bacteraemia and possible sepsis (Mylonakis and Calderwood, 2001). Some of the risk factors for bacteraemia include old age, immunosuppression, chemotherapy and invasive procedures (Mitchell and Howden, 2005).

The high yield of *S. aureus* from nose swabs (37.3%) is an indication that the nostrils serve as a habitat for the organism (Prescott, 2002) and could therefore be one of the major sources of *S. aureus* infection.

Out of the 150 wound swabs collected, a total of 53 representing 35.3% yielded *S. aureus. S. aureus* is a common cause of surgical wound or ulcer infections (Emori and Gaynes, 1993). Most cases of wound infection are acquired either through the hands of a healthcare worker who is colonized with *S. aureus* or from the patient's own reservoir (Sheretz *et al.*, 1996). There was no significant difference between the number of nose swabs and wound swabs that yielded *S. aureus*.

This study showed that contrary to findings by Okome-Nkoumou, (2000); Akinyemi *et al*., (2005) and Mandeep *et al*., (2006), the isolation of *S. typhi* was not correlated with gender implying that both sexes are predisposed to *S. typhi* infection. However, statistical analysis of variance showed that the age distribution of *S. typhi* varied significantly between some age groups. For instance, *S. typhi* isolates were obtained more in patients between the ages of 30 to 39 than the other age groups (Figure 4.1). People within the 30 to 39 years bracket

fall within the active work group in Kumasi (KMA report, 2006) and usually work outside their homes where they may eat food sold by hawkers that are liable to contamination.

*S. aureus* on the other hand recorded higher male infections than female even though there was no significant difference. This observation is corroborated by Manal *et al.*, (2006) who also reported a higher percentage of *S. aureus* isolates (64.4%) in male patients from a Saudi Arabian hospital. There was a significant difference of *S. aureus* isolates among the various age groups with 20-29 years age group recording the highest mean value of 51 (Figure 4.2). The high mean isolates of *S. aureus* in this age bracket could be as a result of body contact such as hugging and handshakes which young adults usually indulge in. Body contact is one of the means through which *S. aureus* is transmitted in the community. This observation may also be attributed to the level of contamination arising from the habitat of the patients, low personal hygiene and poor health education which still persists in many African countries (Agwu *et al.*, 2006).

The *S. typhi* isolated showed a susceptibility of 76.6% to ciprofloxacin and 70.3% to ceftriaxone (Figure 4.4). These findings agree with a research by Kasper *et al.*, (2010) in Cambodia where *S. typhi* susceptibility to ciprofloxacin and ceftriaxone were 79% and 81% respectively. Similarly, a study on ciprofloxacin resistant *S. typhi* and treatment failure in the United Kingdom by Threlfall *et al.*, (2001) also revealed susceptibility of 63.3% and 67.3% for ciprofloxacin and ceftriaxone respectively which were considered to be high. The high number of susceptible *S. typhi* to ciprofloxacin and ceftriaxone recorded at the three hospitals may be due to the fact that these antibiotics may not have been on the Ghanaian

market for long and may not have been subjected to abuse as compared to ampicillin (Newman *et al.,* 2006). Also, ceftriaxone is very expensive and usually administered parenterally making it difficult for patients to administer it themselves.

The *S. typhi* on the other hand showed susceptibilities of 60.9% and 58.6% to co-trimoxazole and chloramphenicol respectively. These susceptibilities however, differ from reports by Newman *et al.,* (2006) and Donkor *et al.,* (2008) who recorded lower susceptibilities of *S. typhi* to these antibiotics.

Chloramphenicol is a broad spectrum but toxic antibiotic and only used in life-threatening situations when no other antibiotic is effective (Prescott, 2002). The reduced susceptibility (58.6%) of the *S. typhi* isolates to chloramphenicol recorded in this study could be as a result of noncompliance with prescription regulations. In Ghana many people get access to this antibiotic over the counter without any prescription leading to improper use. This practice could result in an increase in the resistance level of the *S. typhi* to chloramphenicol. The *S. typhi* isolates exhibited high resistance to ampicillin which may be due to the production of β-lactamase which destroys the β-lactam ring of the ampicillin rendering it ineffective (Julian and Vera, 2004).

As shown in Figure 4.4, intermediate resistant strains of *S. typhi* were found to have emerged against all the five antibiotics namely, ciprofloxacin (6.3%), co-trimoxazole (6.3%), ceftriaxone (14.8%), ampicillin (12.5%) and chloramphenicol (16.4%). This trend shows an increasing minimum inhibitory concentration (MIC) of these antibiotics against

these strains (Kasper *et al.*, 2010) and higher doses of the antibiotics may therefore be needed in the treatment of typhoid fever caused by these intermediate resistant strains. Intermediate resistance occurs when patients fail to complete the recommended dose of prescription and so the bacteria are exposed to concentrations of the antibiotic that are lower than the minimum inhibitory concentration (Kunin, 1993). The exposure of the bacteria to the antibiotic leads to gradual development of resistance.



Susceptibility patterns of stool and urine isolates of *S. typhi* showed that 57.1% of the urine isolates and 49.4% of the stool isolates were resistant to ampicillin. This makes ampicillin the least active antibiotic against the *S. typhi* isolates. Also, a total of 42.9% of *S. typhi* from urine were resistant to co-trimoxazole whereas 26.6% of the *S. typhi* from stool samples were resistant to this antibiotic. Even though 29.5% of the *S. typhi* from urine were resistant to both ciprofloxacin and chloramphenicol, the number of susceptible *S. typhi* to these antibiotics was high with ciprofloxacin recording 84.8% and chloramphenicol registering 60.8%. There was however no significant difference in sensitivity, intermediate resistance and resistance between the *S. typhi* isolates from urine and stool.

*S. typhi* isolated from Kumasi South hospital produced high susceptibilities to chloramphenicol (88.0%), ciprofloxacin (80.0%) and ceftriaxone (78.0%). Unlike Kumasi South hospital, the *S. typhi* isolates displayed a susceptibility of 38.6% to chloramphenicol in both North Suntreso and Tafo hospitals indicating that a significant number of the isolates were resistant to chloramphenicol (Figure 4.8). The *S. typhi* isolates from Tafo hospital were susceptible to co-trimoxazole by 77.3% as compared to 58.8% in North Suntreso hospital

and 48.0% in Kumasi South hospital. North Suntreso hospital recorded the highest number of ampicillin resistant *S. typhi* (70.6%) when compared with 58.0% and 31.8% of the *S. typhi* isolates being resistant to ampicillin in Kumasi south and Tafo hospitals respectively.

The susceptibility patterns of *S. typhi* observed in the three hospitals could be attributed to the line of treatment adopted by the three hospitals. Probably, the antibiotic of choice in the treatment of typhoid fever in North Suntreso and Tafo hospitals is chloramphenicol whereas authorities in Kumasi south and North Suntreso hospitals prescribe Co-trimoxazole. The high number of *S. typhi* isolates resistant to ampicillin could be due to the critical role this antibiotic plays when it comes to empirical treatment over the years.

*S. aureus* on the other hand, exhibits remarkable versatility in its behavior towards antibiotics and the capability of this bacterium to cause human diseases has not diminished even with the introduction of antibiotics (Obiazi *et al.*, 2007). In this study, the susceptibility of the *S. aureus* isolates to gentamicin, vancomycin, ampicillin, erythromycin and ceftriaxone were 44.4%, 73.4%, 47.2%, 38.0% and 17.6% respectively. This trend of susceptibility is similar to a report by Reshma *et al.*, (2007) but contrary to another report by Obiazi *et al.*, (2007) who observed higher susceptibility of *S. aureus* to the antibiotics mentioned above. All the antibiotics used in this study except vancomycin were considered to be ineffective against *S. aureus*. Vancomycin has not been included as one the antibiotics to be used in the management of *S. aureus* infections in Ghanaian hospitals by the Ghana National Drugs Programme (GNDP) of the Ministry of Health (GNDP, 2004). The use and

subsequent abuse of this antibiotic by patients is therefore limited and could be the reason why vancomycin was very effective against the *S. aureus* isolates.

The *S. aureus* isolates from both wound and nose exhibited similar intermediate resistance to gentamicin, ampicillin and erythromycin. However, many *S. aureus* isolates registered intermediate resistance to ceftriaxone from both sites (Figure 4.6).



The number of resistant *S. aureus* isolates from wounds were 56.6% for erythromycin, 50.9% for ampicillin, 43.4% for gentamicin, and 32.1% for ceftriaxone whereas 53.6% of nose isolates were resistant to ceftriaxone, 41.1% resistant to both gentamicin and erythromycin and 30.4% registering resistance to ampicillin (Figure 4.6). Considering the results above, *S. aureus* is developing resistance to most of the older, less expensive antibiotics used in the treatment of infections. These inexpensive antibiotics are widely available to patients in pharmacies without prescription from authorized health personnel (Newman, *et al* 2006) and this leads to indiscriminate use which promotes selective pressure favouring the emergence of resistant bacteria (Levy, 1998). Not only are these resistant bacterial strains potential causes of recurrent infections but they are also reservoirs of resistance genes that could be transferred to other pathogens. For this reason, the antibiotic susceptibility trends seen with the *S. aureus* from both wound and nose swabs may also occur with other bacterial pathogens. The susceptibility patterns of *S. aureus* revealed no significant difference in susceptibility, intermediate resistance and resistance between wound and nose isolates of *S. aureus* implying that *S. aureus* from these sites have equal

chances of developing resistance to gentamicin, vancomycin, ampicillin, erythromycin and ceftriaxone.

More *S. aureus* isolates from the three hospitals exhibited intermediate resistance to ceftriaxone as compared to the other antibiotics tested. The number of *S. aureus* from Kumasi South hospital that recorded intermediate resistance to ceftriaxone was 45.2% while 36.7% and 35.1% of the *S. aureus* from Tafo and North Suntreso hospitals respectively displayed intermediate resistance to ceftriaxone. Also, an analysis of variance of susceptibility, intermediate resistance and resistance between *S. aureus* from Tafo, Kumasi South and North Suntreso hospitals did not show any significant difference.

Multiple-drug resistance among *S. aureus* isolates did not vary much from one hospital to another (table 4.5). Kumasi South hospital recorded a multiple-drug resistance of 19.0% among the *S. aureus* isolates. North Suntreso hospital had 37.8% of the *S. aureus* being multiple-drug resistant while Tafo hospital had 43.3% multiple-drug resistant *S. aureus* isolates. The overall multiple-drug resistance among the 109 *S. aureus* isolates was 32.1%.

Unlike trends observed for multiple-drug resistant *S. aureus*, the number of multiple-drug resistant *S. typhi* observed in Tafo hospital was 13.6%. Kumasi South and North Suntreso hospitals registered 20.0% and 26.5% of multiple-drug resistant *S. typhi* respectively. A total of 19.5% (25/128) of all the *S. typhi* isolates were multiple-drug resistant (Table 4.6). The multiple-drug resistance values in this study are lower than the findings of Newman *et al.*,

(2006), who reported the multiple-drug resistance of *S. typhi* and *S. aureus* isolated in nine regions of Ghana to be 62.4% and 42.3% respectively.

The presence of multiple-drug resistant strains of *S. typhi* and *S. aureus* among the isolates may be attributed to two main reasons. Firstly, antibiotic misuse arising from self-medication in suspected bacterial infections (Newman *et al.*, 2006). Self-medication prevents early reporting of patients to hospitals at the onset of disease symptoms, except where complications had occurred (Akinyemi *et al.*, 2005).

Secondly, either clonal and/or extrachromosomal resistant genes may be potential mechanisms for the level of multiple-drug resistance as noticed in this study. The implication of the multiple drug resistance recorded in this study is that efficacy of the relatively cheap empirical therapy for *S. typhi* and *S. aureus* infections could be jeopardized. Thus, rational use of antibiotics should be practiced (Donkor and Nartey, 2008).

Some other factors identified in literature as the drivers of bacterial resistance to antibiotics such as unnecessary prescriptions and substandard antibiotics could also be the cause of the *S. typhi* and *S. aureus* resistance observed against the antibiotics in this study. The usefulness of these antibiotics will therefore depend on the effective interventions put in place by health authorities to curb the spread of resistance among bacterial strains to antibiotics (Levy, 1998).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION

### 6.1 Conclusion

*S. typhi* isolates were obtained from stool and urine while *S. aureus* were found in wound and nose swabs. Both organisms were found in males and females, with no significant difference between the frequencies of occurrence. *S. typhi* isolates were found among all age groups with no significant difference. There was significant variation in the occurrence of *S. aureus* infections among the various age groups with the 20-29 years age group registering the highest number of isolates.

The study revealed that, the *S. typhi* was most sensitive to ciprofloxacin and ceftriaxone and most resistant to ampicillin. The study also revealed that *S. aureus* isolates were most sensitive to vancomycin and least sensitive to gentamicin, erythromycin, ampicillin and ceftriaxone. Both *S. typhi* and *S. aureus* also exhibited varied degrees of intermediate resistance to all the antibiotics tested.

Less than half of the *S. typhi* (25%) and *S. aureus* (32.1%) were found to be multiple-drug resistant. This could be attributed to widespread indiscriminate use of antibiotics in treating infections caused these pathogens.

## 6.2 Recommendations

The findings of the study have important implications for practicing physicians with regard to empirical antibiotic selection. Therefore, authorities involved in hospital formulary decisions, development of policies regarding antibiotic utilization, infection control and public healthcare should be made aware of the rising antibiotic resistance levels.



Since standard antibiotic discs were used in this study, it is also recommended that antibiotics available in the Ghanaian market should be used in subsequent studies to determine the susceptibility patterns of bacteria to them.

## REFERENCES


Ackers, M.L., Puhr N.D., Tauxe, R.V., Mintz, E.D. (2000). Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. *JAMA*; 283:2668-73.

Agwu, E., Ohihion, A.A., Agba, M.I., Okogun, G.R.A., Okodua, M., Tatfeng, Y.M., Nwobu, GO., (2006). Incidence of Streptococcus pneumoniae Infections among Patients Attending Tuberculosis Clinics in Ekpoma, Nigeria. *Shiraz Electronic Medical Journal*, 7(1)

Akinyemi, K.O., Smith, S.I., Bola, O.A., Oyefolu, A., Coker, O., (2005) Multidrug resistance in *Salmonella enterica* serovar *typhi* isolated from patients with typhoid fever complications in Lagos, Nigeria. *Public Health*. 119, 321-327.

Alfred, Y.I and Comfort, J.A., (2004) Correlation studies on Widal agglutination reaction and diagnosis of typhoid fever. Diagnosis of typhoid fever, Nigeria. *Southeast Asian J. Trop. Med. Public Health*, 35(1) 88-91.

Aubry-Damon H., Soussy, C.J., Courvalin P., (1998). Characterization of mutation in the rpob gene that confer rifampicin resistance in *Staphylococcus aureus*. *Antimicrob. Agent Chemother*.42 (1) 2950 – 2954.

Baldwin, J.N., Rheins, M.S., Sylvester, R.F., (1957). Staphylococcal infections in newborn infants. *American Journ. of Dis. in Children* 9(4)107 – 116.

Betty, F., Daniel, S., Alice, W., (2007). Bailey & Scott's Diagnostic Microbiology. 12th edition. Elsevier Inc; New York; 322-325.

Bhutta, Z.A., (1996) Impact of age and drug resistance on mortality in typhoid fever. *Arch. Dis. Child.* 75(33) 214-7



Biedenbach, D.J., Moet, G.J., Jones, R.N., (2004). Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002). *Diagn. Microbiol. Infect. Dis*; 50:59–69.

Black, R.E., Cisneros, L., Levine, M.M., Banfi, A., Lobos, H., Rodriguez, H (1985). Case-control study to identify risk factors for paediatric endemic typhoid fever in Santiago, Chile. *Bulletin of WHO*; 63:899-904.

Blot, S.I., Vandewoude, K.H., Hoste, E.A., Colardyn, F.A., (2002) Outcome and attributable mortality in critically ill patients with bactaeremia involving methicillin-susceptible and methicillin-resistant *S. aureus*. *Arch. Intern. Med*, 162 (19) 2229-35

Bone, R.C., (1994). Gram-positive organisms and sepsis. *Arch. Intern. Med* ; 154:26-34.

Brenda, W.L., Lee, KL., (2008). Infectious diseases in context, Farmington Hills, Mich: Thomson Gale. 1 (10) 173-210

Britten, R.J., Kohne, D.E., (1965). Nucleotide sequence repetition in DNA. *Carnegie Inst. Wash. Year Book*.65:78–91.

Bryant, R.G., Jarvis, J., Gilbert, G., (1998). Selective enterotoxin production by a *Staphylococcus aureus* strain implicated in a food borne outbreak. *J. Food Prot*. 51(1)130-131.



Burnett, G.W., Henry, W.S., Schuster, S.G., (1996). *Staphylococcus* and Staphylococcal infections. In Oral microbiology and infectious disease. 1st edition, Williams and Wilkins, 405 – 416.

Chigbu, C., Chinwe, O., Ezeronye, O.U., (2003). Antibiotic resistant *Staphylococcus aureus* in Abia state of Nigeria. *Afr. Journ. of Biotechnol*, 2(10): 374-378.

Chikere, C.B., Chikere, B.O., Omoni, V.T., (2008). Antibiogram of clinical isolates from a hospital in Nigeria. *Afr. J. Biotechnol*. 7 (24): 4359-4363

Clinical and Laboratory Standards Institute (2010). Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement. M100-S17. CLSI,Wayne, PA.

Cowan, S.T., Steel, J.K., (1970). Manual for the identification of medical bacteria. Cambridge university press. London. 5: 50-53.

Crump, J.A., Luby, S.P., Mintz, E.D., (2004). The global burden of typhoid fever. *Bull. World Health Org.* 82:346–353.

Doig, C.M., (1981). Nasal carriage of *Staphylococcus* in a general surgical unit. *Brit. J. Surg.* 58(3): 113.



Donkor, E.S., Nartey, E., (2008). Nasal colonization of drug resistant bacteria in Ghanaian children less than five years . *The Internet Journ. of Microbiol*. 5 (2).

Donkor, E. S., Nortey T., Opintan, J. A., Dayie N. and Akyeh M. L. (2008) Antimicrobial susceptibility of *Salmonella typhi* and *Staphylococcus aureus* isolates and the effect of some media on susceptibility testing results . *The Internet Journ. of Microbiol.* 4(2): 1937-8289.

Edelman, R., Levine., M.M., (1986). Summary of an international workshop on typhoid fever. *Rev.Infect. Dis*. 8(4) 329-349.

Emori, T.G., Gaynes R.P., (1993). An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin. Microbiol. Rev*; 6: 428-42.

Evans, J.B., Bradford, W.L. Jr., Niven, C.F.Jr., (1955). Comments concerning the taxonomy of the genera micrococcus and staphylococcus. *Int. Bull. Bact. Nom. Tax*. 7(37) 1-123.

Feglo, P.K., Frimpong, E.H., Essel-Ahun, M., (2004). Salmonellae carrier status of food vendors in Kumasi, Ghana. *East African Med. Journ.* 81(7): 2293-3204

Feglo, P.K., Gbedema, S.Y., Quaye, S.A., Adu-Sarkodie, Y, and Opoku-Okra, C. (2010). Occurrence, Species distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. *Intern. Journ. of Pharma Sci. and Research.* 1(9), 347-352.

Fowler, V.G Jr., Miro, J.M., Hoen, B., (2005).*Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* ; 293:3012–21.

Gasem, M.H., Dolmans, W.M., Keuter, M.M., Djokomoeljanto, R.R., (2001). Poor food hygiene and housing as risk factors for typhoid fever in Semarang, Indonesia. *Trop Med Int Health.* 6(5):484-90.

Ghana National Drugs Programme (Ministry of Health), (2004) Standard treatment guidelines, fifth edition, Yemens press Ltd, Accra., 6:172-181.

Gloria, A., Cheryl, B., John E., Richard, F., Joan, S.K., Tanja, P., Joy, W., Scott, F.D., (2003). Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Centers for

Disease Control and Prevention, Atlanta, Georgia, USA and World Health Organization: Department of Communicable Disease Surveillance and Response. 12:103-118.

Harley, J.P., Prescott, L.M., (1990). Laboratory exercises in microbiology Wm.C. Brown publishers; 5:190-192.

Hinnebusch, C., Glenn, D., Bruckner, D.A., (1992). Potential misidentification of *Staphylococcus* species when using rapid identification tests that detect clumping factor. Abstr.General meeting, Amer. Soc. Microbial. American society for microbiology, Washington, D.C. C-485,498.

Hornick, R.B., Greisman, S.E., Woodward, T.E., DuPont, H.L., Dawkins, A.T., Snyder, M.J., (1970). Typhoid fever: pathogenesis and immunologic control. *N Engl J Med*; 283:686-91, 739-46.

Hornick, R.B., (1985). Selective primary health care: strategies for control of disease in the developing world. XX**.** Typhoid fever. *Rev. Infect. Dis*. 7:536-546.

House, D., Bishop, A., Parry, C.M., Dougan, G., Wain, J., (2001). Typhoid fever: pathogenesis and disease. *Curr. Opin Infect. Dis*;14(5):73-8.

Islam, A., Butler, T., Kabir, I. and Alam, N.H., (1993). Treatment of typhoid fever with Ceftriaxone for 5 days of Chloramphenicol for 14 days: a randomized clinical trial. *Ant. Ag. Chem.* 37(10):572-1575.

Jawetz, M., Melnick, I., Adelberg S., (2007). Medical microbiology, 24th edition. Prentice Hall International Inc. 7:214-217.



Jones, B.D., and S. Falkow. S (1996). Salmonellosis: host immune responses and bacterial virulence determinants. *Annu. Rev. Immunol*. 14(23):533–561

Julian, D., Vera, W., (2004). The Desk Encyclopedia of Microbiology: Antibiotic resistance in bacteria. Elsevier Ltd. UK. 3:25-47.

Kariuki, S., Gilks, C., Revathi, G., Hart, C.A., (2000). Genotypic analysis of multidrug-resistant *Salmonella enterica* Serovar *typhi* in Kenya. *Emerg. Infect. Dis*. 6(1):64-65.

Kasper, M.R., Sokhal, B., Blair, J.P., Wierzba, F.T., Putnam, D.S., (2010). Emergence of multidrug-resistant *Salmonella enterica* serovar *typhi* with reduced susceptibility to fluoroquinolones in Cambodia. *Diagn. Microbiol. and Infect. Dis*. 66(4):207-209

Kauffmann, F., (1963). On the species definitions. *Int. Bull. bact. Nom. Tax*.13(181)

Kauffmann, F., Edwards, PR., (1952). Classification and nomenclature of enterobacteriaceae. *Int. Bull. bact. Nom. Tax*. 2(2).

Kenneth, J.R., (2004). Medical microbiology. An introduction to infectious diseases, 4th edition. McGraw Hill companies: 3:261-271.

Kenneth, T., (2008) Todar's Online Textbook of Bacteriology, (http://www.textbookofbacteriology.net/ken_todar.htm), (accessed 2010 November 10)

Klodkowaska-Farner, E., Zwolska-Kwiet, Z., Wojciechowka, M., Bestry, I., Pacocha, W., Podsiadlo, B., Otto, T., (1995). Pneumonia in Patients after extra corporal circulation. *Pneumonol. Alergol. Pol*. 63(8): 371 – 377.

Kloos, W.E., Bannerman, T., (1995). *Staphylococcus* and *Micrococcus*: Manual of Clinical Microbiology. 6th ed. ASM, 12:282-298.

Kocur, M., Martinec, T., (1962).Taxonomikastudierodu micrococcus. *Folia Fac.Sci. nat., brne.* 3(3).

Kumasi Metropolitan Assembly report, (2006). (http://www.ghanadistricts.com/districts1on1/kma/?arrow=atd&_=6&sa=5477), (accessed 2011 March 10)

Kunin, C.M., (1993). Resistance to antimicrobial drugs – a worldwide calamity. *Ann. Int. Med*. 1(18): 557 – 561.

Lalitha, M.K., (2004). Manual on antimicrobial susceptibility testing. 7th edition. Indian Association of Medical Microbiologists. Delhi. 10-47.

Le Minor, L., Popoff, M., Laurent, B., Hermant, D., (1987). Characterization of a 7th subspecies of *Salmonella*: *S. choleraesuis* subsp. *indica* subsp. *nov*. *Ann. Inst. Pasteur Mic*. 137B: 211–217.

Le Minor, L., Popoff, M.Y., (1987). Designation of *Salmonella enterica* sp. *nov*., nom. Rev., as the type and only species of the genus *Salmonella*. *Int. J. Syst. Bacterial*. 3(7):465.

Lesse, AJ., Mylotte JM., (2006). Clinical and molecular epidemiology of nursing home-associated *Staphylococcus aureus* bacteremia. *Am. J. Infect. Control*. 3(4):642–50.

Levy, S., (1998). The antibiotic paradox: How miracle drugs are destroying the miracle. *Plenum publishers*,10(10):1 – 11.

Lin, F.Y., Ho, V.A., Bay, P.V., (2000). The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *Am. J. Trop. Med. Hyg*. 62(6):44-8.

Luby, S.P., Faizan, M.K., Fisher-Hoch, S.P., (1998). Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. *Epidemiol. Infect*. 1(20):129-38.

Luxemburger, C., Chau, M.C., Mai, N.L., (2001). Risk factors for typhoid fever in the Mekong Delta, southern Viet Nam: a case-control study. *Trans. R Soc. Trop. Med. Hyg*. 9(5):19-23.



Mahon, C.R., Lehman, D.C., Manuselis, G., (2007) A textbook of diagnostic microbiology, 3rd edition, Saunders Elsevier, St. Louis, Missouri, USA, 13:319-351.

Mandani, T.A., Al-Abdullah, N.A., Al-Sanousi, A.A., Ghabrah, T.M., Afandi, S.Z., Bajunid HA., (2001). Methicillin resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia**.** *Infect Control Hosp. Epidemiol*, 2(12):211-216.

Mandeep, B.P., Koirala, J., Dahal, R.K., Mishra, S.K., Khadga, P.K., Tuladhar, N.R., (2006). Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes *Typhi* and *Paratyphi* A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *Int J Infect Dis*; 16(9):788-98

Manal, M.B., Manal, M.A., Amal, J.F., (2006). Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. *Annals of Clin. Microbiol .and Antimicrob.* 5(30)

Martinez – Martinez, L., Paschal, A., Jacoby, G.A., (1998). Quinolone resistance from a transferable plasmid. *Lancet* 35(1):797–799

Mathieu, J.J., Henning, K.J., Bell, E., Frieden, T.R., (1994). Typhoid fever in New York City, 1980 through 1990. *Arch. Int. Med***.** 15(4): 1713-1718.

Mengo, D.M., Karuiki, S., Ann, W.T., Gunturu, N.R., (2010). Trends in *Salmonella enterica* serovar *typhi* in Nairobi, Kenya from 2004 to 2006. *J. Infect. Dev. C'tries*. 4(6) 393-396.

Mensah, P., Owusu-Darko, K., Yeboah-Manu, D., (2002). The role of street food vendors in the transmission of enteric pathogens in Accra. *Ghana Med. J.* 33(2)**:**19-29.

Mermin, J.H., Villar, R., Carpenter J., (1999). A massive epidemic of multidrug- resistant typhoid fever in Tajikistan associated with consumption of municipal water. *J Infect Dis*. 17(9):1416-22.

Mills-Robertson, F.C., Newman, M.J., Mensah, P., Addy, M.E. (2003) Multiple resistant *Salmonella* in Accra, Ghana. *Ghana Med. J*. 37(5):165 - 169.

Mirza, S.H., Beeching, N.J, Hart, C.A., (1996). Multidrug resistant *Salmonella typhi*: A global problem. *J. of Med. Mic*. 44(3):17-319.

Mitchell, D.H., Howden, B.P., (2005). Diagnosis and management of *Staphylococcus aureus* bacteraemia. *Intern. Med. J.* 35(2):S17–24.

Morris, D.O., Kathryn, A.R., Frances, S.S., Shelley, C.R., (2006). Screening of *Staphylococcus aureus, Staphylococcus intermedius,* and *Staphylococcus schleiferi* isolates obtained from small companion animals for antimicrobial resistance: a retrospective review of 749 isolates (2003-04*). Euro. Soc. Veter. Dermatol*. 17(3):32-337

Morris, J.G., Ferreccio, C., Garcia, J., (1984). Typhoid fever in Santiago, Chile: a study of household contacts of pediatric patients. *Am J Trop Med Hyg*, 3(3): 1198-202.

Mossel, D.A.A., (1962). Attempt in classification of catalase-positive Staphylococci and micrococci. *J. bact*. 84(1140)

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., (2002). Manual of Clinical Microbiology. Washington D.C.: ASM Press. 20:23-88

Mylonakis, E and Calderwood, S.B (2001). Infective Endocarditis in adults. *New Eng. Journ. of Med*. 345(18): 1318-1330

Naber, C.K., (2009). *Staphylococcus aureus* bacteremia: epidemiology, pathophysiology and management strategies. *Clin. Infect. Dis*: 48( 4) S231-7.

Neihart, R.E., Fried, J.S., Hodges, G.R., (1988). Coagulase – positive Staphylococci. *South Med. J*. 81(2): 491 – 500.

Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N and Martha, T.N., (2004) Microbiology: A human perspective, 4th edition, McGraw Hill Companies 21:507-521.

Newman, M.J., Frimpong, E., Asamoah-Adu A. and Sampane-Donkor E. (2006). Resistance to Antimicrbial Drugs in Ghana. The Ghanaian – Dutch collaboration for Health Research and Development 1-6.

Njinkeng, J., Nkemngu, E., Asonganyi, D.N., Anna, L.N., (2005). Treatment failure in a typhoid patient infected with nalidixic acid resistant *S. enterica* serovar *Typhi* with reduced susceptibility to Ciprofloxacin: a case report from Cameroon. Biomedicine central infectious diseases (http://www.biomedcentral.com/content/pdf/1471-2334-5-49.pdf), (accessed 2010 June 12)

Noskin, G.A., Rubin, R.J., Schentag, J.J., (2005) The burden of Staphylococcus aureus infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. *Arch. Intern. Med*, 165 (15) 1756-61

Obiazi, H.A.K., Nmorsi, O.P.G., Ekundayo, A.O., Ukwandu, N.C.D., (2007). Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37 and 44OC from Irrua, Nigeria. *Afr. J. Microbiol. Res*. 1 (5): 57-60

Ohene, A. (1997). Bacterial pathogens and their antimicrobial susceptibility in Kumasi, Ghana, *East Afri Med. J*. 74(45): 450 – 455.



Okome-Nkoumou, M., Ayo, E., Nkana, J., Békalé, R., Kombila, M., (2000). Typhoid and paratyphoid fever in adults in the Internal Medicine Department at Libreville (Gabon) Sante. 10 (3):205-9

Padmini, S., Sagdullo, V., Stephen, P.L., Tharwat, I., Kenneth, E., Ne'mat, K., Gregory, J., Crump, J.A., Frank, J.M., (2007). Epidemiology and risk factors for endemic typhoid fever in Uzbekistan. *Trop Med and Int. Health.* 12(7) :838–847.

Park, T., Arthur, K.T., (2002). Foundations in microbiology.4th edition. McGraw Hill companies. 13: 345-546.

Parry C.M., Tran, T.H., Gordon, D., Nicholas, J.W., Jeremy, J.F., (2005). Medical progress: Typhoid fever. *New Eng. Journ. of Med*. 347(22): 32-59

Paterson, D.L., (2000). Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin Microbiol Infect*; 6(9): 460-3.

Petersberg, W., (2001). Endogenous Antimicrobial Molecules: An Ecological Perspective, *Clin Microbiol Infect* 25(15): 215-228



Prescott, L.M., (2002). Introduction to microbiology, 5th edition, McGraw Hill companies. 20: 919-920.

Reshma, F., Asma, B., Talat, Y., Mujahid, R., Nayar, J., (2007). Antibiotic resistance profile: Isolation and characterization of clinical isolates of *Staphylococcus aureus* from patients with community acquired infections. *Pak J. Pharm. Sci*. 20(4): 295-299.

Roberson, J.R., Fox, L.K., Hancock, D.D., and Besser, T.E. (1992). Evaluation of methods for differentiation of coagulase positive staphylococci. *J.Clin. microbial*. 30(3):2-17

Sebastien, C., Sabbagh, C., Forest, G., Christine, L., Jean-Mathieu, L., France D., (2010). So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars Typhimurium and Typhi. *FEMS Microbiol Lett*. 305 (2010) 1–13

Shakibaie, M.R., Mansouri, S., Hakak, S., (2002). Plasmid pattern of antibiotic resistance in beta lactamase producing *Staphylococcus aureus* isolated from hospital in Karman. Iran. (http://www.sums.ac.Ir./AIM/9922/shakibaie 9922.html.). Accessed, 10th June, 2010

Shaw, C., Stitt, J.M., and Cowan, S.T., (1951). Staphylococci and their identification. *J. gen. microbial*. 5(10)10.



Sheretz, R.J., Reagan, D.R., Hampton, K.D., (1996). A cloud adult: the *Staphylococcus aureus*-virus interaction revisited. *Ann Intern Med*. 1(24): 539-47.

Shittu, A.O., Johnson, L., (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus .BMC. Infect. Dis*. 12(5): 2334-2336.

Smith, S.M., Palumbo, P.E., Edelson, P.J., (1984). Salmonella strains resistant to multiple antibiotics: therapeutic implications. *Pediatr. Infect. Dis*. 3(4):55-60.

Speller, D.C.E., Johnson, A.P., James, D., Marples, R.R., Charlett, A., George, R.C., (1997). Resistance to methicillin and other antibiotics in isolates of *Staphylococcus aureus* from blood and cerebrospinal fluid, England and Wales, 1989-95. *Lancet*. 35(10):323-325.

Singh, B., (2001) Symposium: Typhoid fever, epidemiology. *J. Indian Aca. of Cl. Med*. 2(1 and 2).

Stephen, H.G., Kathleen, B.B., (2000). Medical microbiology and infection at a glance, Blackwell sciences. 1:26-27.

Styers, D., Sheehan, D.J., Hogan, P., Sahm, D.F., (2006). Laboratory-based surveillance of current antimicrobial resistance patterns and trends among *Staphylococcus aureus:* 2005 status in the United States. *Ann Clin. Microbiol. Antimicrob*. 5(2).



Siranda, T., Christine, R., Thomas, V.R., (1999). The Continuing Evolution of Methicillin-Resistant Staphylococcus aureus in Western Australia. *Infect. Control. Hosp. Epidemiol.* 20(11):33-35.

Tacconelli, E., Venkataraman, L., De Girolami, PC., Dagata, EM., (2004). Methicillin-resistant *Staphylococcus aureus* bacteraemia diagnosed at hospital admission: distinguishing between community-acquired versus healthcare- associated strains. *J. Antimicrob. Chemother*. 53(47):4–9.

Threlfall, E.J., Ward, L.R., Skinner, J.A., Smith, H.R., Lacey, S., (1999). Ciprofloxacin resistant *Salmonella typhi* and treatment failure. *Lancet*. 353:1590-1.

Tuo, P., Montobbio, G., Callarino, R., Tumolo, M., Calero, M.G., Massone, M.A., (1995). Nosocomial infection caused by multi–resistant staphylococci in a neonatal and pediatric intensive care unit. *Pediatric-Med. Clin*. 1(7): 117 – 122.

Velema, J.P., Van Wijnen, G., Bult, P., Van Naerssen, T., Jota, S., (1997). Typhoid fever in Ujung Pandang, Indonesia: high risk groups and high risk behaviors. Tro*p. Med. Int. Health*. 2(10):88-94.

Weidenmaier, C., Kokai-Kun, J.F., Kristian, S.A., (2004). Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nat Med.* 10(2):43–55

Xercavins, M., Llovet, T., Navarro, F., (1997). Epidemiology of an unusual outbreak of typhoid fever in Terrasa, *Spain. Clin. Inf. Dis.* 3(24):506-510.

## APPENDICES

## APPENDIX I: PREPARATION OF SOLID MEDIA

**A. MacConkey Agar No. 2 (Oxoid CM0109)**

| Composition | g/L |
|---|---|
| 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 |

This was prepared by suspending 51.5 grams of powder in 1 litre of distilled water. It was then boiled shortly to dissolve completely. Sterilization was done by autoclaving at 121oC for 15 minutes.

**Mode of differentiation/identification**

This medium is used as a differentiated medium, differentiating organisms into lactose and non-lactose fermenting organisms. The lactose-fermenting organisms produce acids which act upon the bile salt and absorb the neutral red, giving red colonies. The non-lactose fermenting colonies give an alkaline reaction, do not absorb the neutral red and produce colorless colonies.

**B. Mueller-Hinton Agar (Oxoid CM0337)**

| Formula | g/L |
|---|---|
| Beef infusion | 300.0 |
| Casein hydrolysate | 17.5 |
| Starch | 1.5 |
| Agar | 17.0 |
| pH | 7.2-7.4 |

To prepare the medium 35 grams of the powder was dissolved in 1 litre of distilled water. It was then boiled shortly to dissolve the medium completely. It was sterilized by autoclaving at 121 $^{o}$C for 15 minutes.

**Mode of differentiation/identification**

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

**C. Mannitol salt agar (Oxoid CM0085)**

| Formula | g/L |
|---|---|
| Lab-lemco powder | 1.0 |
| Peptone | 10 |
| Mannitol | 10 |

| | |
|---|---|
| Sodium Chloride | 75 |
| Phenol red | 0.025 |
| Agar | 15 |

111 grams was suspended in 1 litre of distilled water and brought to the boil to dissolve completely. It was distributed into test tubes, corked firmly and autoclaved at 121$^{o}$C for 15 minutes. It was stabilized at 45$^{o}$C for 15 minutes and poured into sterile Petri dishes.

**D. Triple Sugar Iron Agar (Oxoid CM277)**

| Formula | g/L |
|---|---|
| Lab-Lemco Powder (Oxoid L29) | 3 |
| Yeast Extract (Oxoid L20) | 3 |
| Peptone (Oxoid L37) | 20 |
| Sodium Chloride | 5 |
| Lactose | 1 |
| Sucrose | 10 |
| Dextrose | 0.3 |
| Ferric citrate | 0.3 |
| Sodium thiosulphate | 0.3 |
| Phenol red | 0.025 |
| Agar No. 3 (Oxoid L13) | 12.0 |

65 grams of the powder was suspended in 1 litre of distilled water and brought to the boil to dissolve completely. It was mixed well into the appropriate containers, sterilized by autoclaving at 121$^{o}$C for 15 minutes and then allowed to set in sloped forms.

**E. Columbia Blood Agar Base (Oxoid0331)**

| Approximate Formula | g/ L |
|---|---|
| Pancreatic Digest of Casein | 10.0 |
| Peptone | 5.0 |
| Yeast Extract | 5.0 |
| Beef Heart Digest | 3.0 |
| Starch | 1.0 |
| Sodium Chloride | 5.0 |
| Agar | 15.0 |

To prepare this medium 39g of the dehydrated powder was added to 1 litre of distilled water. It was then boiled to dissolve the medium completely and distributed in 20ml quantities in test tubes. They were then sterilised by autoclaving at 121°C for 15 minutes, cooled to 50°C and 5% sterile defibrinated Horse blood added.

**Mode of differentiation/identification**

Traditionally, blood agar bases have been either casein hydrolysate or meat infusion media. The advantage of the first lies in the rapid production of large colonies, and

of the second in clearly defined zones of haemolysis and good colonial differentiation.

**F. Bismuth sulphite agar (CM0201)**

| Typical Formula | g/L |
|---|---|
| Peptone | 5.0 |
| Lab-Lemco' powder | 5.0 |
| Glucose | 5.0 |
| Disodium phosphate | 4.0 |
| Ferrous sulphate | 3.0 |
| Bismuth sulphite indicator | 8.0 |
| Brilliant green | 0.016 |
| Agar | 12.7 |
| pH | 7.6 ± 0.2 at 25°C |

20g of the powder was suspended in 500ml of distilled water in a 1 litre flask. It was heated gently with frequent agitation until the medium just began to boil and was allowed to simmer for 30 seconds to dissolve the agar. The medium was cooled to 50-55 $^{o}$C and poured into petri dishes (25 ml medium per plate). It was then allowed to solidify with the dish uncovered. As a precaution in the preparation of Bismuth Sulphite agar, it was not autoclaved.

**Mode of differentiation/identification**

Bismuth Sulphite Agar is a modification of the original Wilson and Blair selective medium for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological material, sewage, water supplies, food and other products suspected of containing these pathogens. In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of salmonellae. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide. *S. typhi* produce Black `rabbit-eye' colonies with a black zone and metallic sheen surrounding the colony after 18 hours but uniformly black after 48 hours incubation.

**G. Cooked Meat Medium (CM81)**

An excellent medium for the primary growth and maintenance of aerobic and anaerobic organism

| Typical Formula | g/L |
|---|---|
| Heart muscle | 454.0 |
| Peptone | 10.0 |
| `Lab-Lemco' powder | 10.0 |
| Sodium chloride | 5.0 |
| Glucose | 2.0 |
| pH | 7.2 ± 0.2 at 25°C |

To prepare this medium, 10g of the powder was suspended in 100ml of distilled water (or 1g amounts in 10ml volumes of water in tubes). The broth was allowed to stand for 15 minutes until the meat particles were thoroughly wetted. The medium was then sterilised by autoclaving at 121°C for 15 minutes.

**Mode of differentiation/identification**

Cooked Meat Medium prepared from heart tissue is a well-established medium for the cultivation of anaerobic and aerobic organisms. It has the ability to initiate bacterial growth from very small inocula and to maintain the viability of cultures over long periods of time. Mixed cultures of bacteria survive in Cooked Meat Medium without displacing the slower growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic bacteria. The addition of glucose to the formulation allows rapid, heavy growth of anaerobic bacteria in a short time and leads to a more rapid identification of important anaerobes. The improved growth also enhances identification of anaerobic bacteria. The improved clarity of the supernatant broth permits earlier detection of growth especially when combined with the increased growth of most organisms. Slower growing isolates will yield detectable growth within 45 hours incubation.

**H. Nutrient Broth (CM0001)**

A nutritious medium suitable for the cultivation of fastidious pathogens and other micro-organisms.

| Typical Formula | g/L |
|---|---|
| `Lab-Lemco' powder | 10.0 |
| Peptone | 10.0 |
| Sodium chloride | 5.0 |
| pH | 7.5 ± 0.2 |

25g was added to 1 litre of distilled water. It was the mixed well, distributed into final containers and sterilised by autoclaving at 121°C for 15 minutes.

**Mode of differentiation/identification**

It gives good growth from small inocula and is recommended for sterility testing for aerobic organisms. Nutrient Broth complies with the recommendations in the `British Pharmacopoeia' for the composition of a sterility testing medium for aerobes. The medium is ideally suited for sub-culture, particularly as a secondary growth medium for staphylococci which are to be tested for

coagulase production. Nutrient Broth made up at double strength corresponds to the medium recommended by the British Standards Institution for use in the determination of the Rideal-Walker Coefficient of Disinfectants.

**I. Nutrient Agar (CM0003)**

| Typical Formula | g/L |
|---|---|
| `Lab-Lemco' powder | 1.0 |
| Yeast extract | 2.0 |
| Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |
| pH | 7.4 ± 0.2 at 25°C |

To prepare nutrient agar, 28g was suspended in 1 litre of distilled water. It was then boiled to dissolve completely and 20ml each was distributed into test tubes. Thee tubes were sterilised by autoclaving at 121°C for 15 minutes.

**Mode of differentiation/identification**

Nutrient Agar is a basic culture medium used to subculture organisms for maintenance purposes or to check the purity of subcultures from isolation plates prior to biochemical or serological tests. In semi-solid form, agar slopes or agar butts, the medium is used to maintain control organisms. Nutrient Agar is suitable for teaching and demonstration purposes. It contains a concentration of 1.5% agar to permit the addition of up to 10% blood or other biological fluid, as required. The medium, without additions, may be used for the cultivation of organisms which are not exacting in their nutritional requirements.

**APPENDIX II: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES**

**Table A: STOOL ISOLATES FROM TAFO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacConkey | Mannitol salt | Urease | Citrate | $H_2S$ | Slant | Butt | |
| tfs10M6 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfs1F3 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfs2F18 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs30F12 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs4M16 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs40F40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs13M41 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs24M36 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs11M32 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs27M39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs50M34 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs55M40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs43M37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs32F35 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs42F26 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs56F36 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs53M39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs26M32 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs7F35 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs59M21 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs61F27 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs33M30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs18M37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs80M39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs72F26 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs16F37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs74F30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs66F21 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs38F39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| tfs89F22 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |

tfs-Tafo stool sample(no:) gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table B: STOOL ISOLATES FROM KUMASI SOUTH HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Urease | Citrate | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacConkey | Mannitol salt | | | $H_2S$ | Slant | Butt | |
| chs 50F47 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| chs 30F36 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| chs 42F39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 56M38 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 39M30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs46M34 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 43M37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 19M38 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 92M32 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs75F54 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 10F35 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs8F10 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs14F21 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs11F27 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs6F40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs1M50 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs7F30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs16M22 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs5M36 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs4M31 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs2M39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs25M27 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs21M24 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs22M48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs28M30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs24M33 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs17F37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs36F39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs 15F33 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chs88M40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |

chs-Kumasi south stool sample(no:) gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table C: URINE ISOLATES FROM TAFO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Urease | Citrate | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacCon key | Mannitol salt | | | $H_2S$ | Slant | Butt | |
| tfu2M11 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu6F50 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu14F38 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu46M48 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu11M45 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu31M44 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu20F22 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu72M33 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu69M48 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu38F41 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu13F43 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu26F20 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu53F35 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| tfu17F32 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |

tfu-Tafo urine sample(no:) gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table D: STOOL ISOLATES FROM SUNTRESO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacCon key | Mannitol salt | Urease | Citrate | $H_2S$ | Slant | Butt | |
| ss1F15 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss2M20 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss7F51 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss10M39 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss9M34 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss4F44 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| ss14M30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss54M30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss21M23 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss30F35 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss43F39 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss60F28 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss61M36 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss76F48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss89F21 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss72F23 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss86F31 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss97F65 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| ss50F42 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |

ss-Suntreso stool sample(no:)gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table E: URINE ISOLATES FROM KUMASI SOUTH HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacCon key | Mannitol salt | Urease | Citrate | $H_2S$ | Slant | Butt | |
| chu6M22 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| chu2M26 | | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| chu4M62 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu9F46 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu7M40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu5F48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu16M41 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu23F45 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu14F42 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu12M45 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu26M33 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu35F48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu24F37 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu61M40 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 30F48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 10F38 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 54F43 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 44F30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 21F41 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| chu 73F5 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |

chu-Kumasi south urine sample(no:) gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table F: URINE ISOLATES FROM SUNTRESO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Triple Sugar Iron Agar | | | |
| | Blood | Bismuth sulphite | MacCon key | Mannitol salt | Urease | Citrate | $H_2S$ | Slant | Butt | |
| su4M10 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| su6F38 | G/T | B/M | colourless | N/G | – | – | + | Red | Yellow | *S. typhi* |
| su9F30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su1F30 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su8F34 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su2F48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su10F44 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su5F42 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su3M42 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su7F15 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su18F46 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su21M48 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su23F15 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su39F21 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |
| su47F18 | G/T | B/M | colourless | N/G | – | – | + | Red | yellow | *S. typhi* |

su-Suntreso urine sample(no:) gender(age) G/T-grey and transparent B/M-black with metallic sheen N/G-no growth

**Table G: WOUND ISOLATES FROM TAFO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey agar | Mannitol salt | Coagulase | Catalase | |
| tfw1M29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw2M21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw5M24 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw10M23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw8M9 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw12M23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw13F42 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw14M45 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw18M29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw20M40 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw23M25 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw22F51 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw25F30 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw31F39 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw40M13 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tfw35F7 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

tfw-tafo wound swab(no:) gender(age) Y/H-yellow with haemolysis N/G-no growth

**Table H: WOUND ISOLATES FROM SUNTRESO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey | Mannitol salt | Coagulase | Catalase | |
| sw10M5 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw13M31 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw15M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw50M8 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw11F22 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw7F26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw9M28 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw4F33 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw5F21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw12F29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw1F42 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw19F38 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw23M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw25M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw30M28 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw32M27 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw40M20 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw34M24 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sw37M54 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

sw-suntreso wound swab(no:) gender(age) Y/H-yellow with haemolysis N/G-no growth

**Table I: NOSE ISOLATES FROM KUMASI SOUTH HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey | Mannitol salt | Coagulase | Catalase | |
| cn2M19 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn3M7 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn4F22 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn5M27 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn7M23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn10F28 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn13F1 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn14F20 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn17F14 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn18F29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn21F31 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn22F61 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn23M24 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn24F16 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn25F33 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn26F23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn27M18 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn29M59 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn30M21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn33M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn34M27 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn37F47 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn39M44 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| cn40F21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

cn-Kumasi south nose swab(no:)gender(age) Y/H-yellow with haemolysis N/G-no growth

**Table J: NOSE ISOLATES FROM TAFO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey | Mannitol salt | Coagulase | Catalase | |
| tn5M2 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn9M32 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn10F29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn14F45 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn15F52 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn17M23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn18M8 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn25M35 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn31F21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn34M48 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn35F21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn37F40 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn39M12 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| tn40M10 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

tn-Tafo nose swab (no:) gender (age) Y/H-yellow with haemolysis N/G-no growth

**Table K: WOUND ISOLATES FROM KUMASI SOUTH HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey | Mannitol salt | Coagulase | Catalase | |
| chw6F7 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw7F16 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw8M49 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw3M55 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw10F22 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw13M | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw17M47 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw19F33 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw20M25 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw21M27 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw25M29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw29M20 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw30F23 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw1M25 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw32M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw33M21 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw35M27 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| chw39M40 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

chw- Kumasi south wound swab (no:)gender(age) Y/H-yellow with haemolysis N/G-no growth

**Table L: NOSE ISOLATES FROM SUNTRESO HOSPITAL**

| Specimen No./Gender/Age | Colony Characteristics on Agar media | | | | Biochemical tests | | Inference |
|---|---|---|---|---|---|---|---|
| | Blood | Bismuth Sulphite | MacConkey | Mannitol salt | Coagulase | Catalase | |
| sn1F55 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn3F12 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn4F29 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn7F25 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn6F28 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn11F58 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn13M9 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn18M26 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn17F4 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn25F47 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn24F49 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn29M20 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn21M9 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn30F50 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn32M40 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn33F38 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn36M37 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |
| sn38F5 | Y/H | N/G | N/G | Golden yellow | + | + | *Staph aureus* |

sn- Suntreso nose swab (no:) gender (age)  Y/H- yellow with haemolysis  N/G-no growth

**APPENDIX III: ZONES OF INHIBITION AND THEIR INTERPRTATION FOR THE ISOLATES FROM THE VARIOUS HOSPITALS**

**Table A: Zones of inhibition and their interpretation for selected antibiotics appropriate for *S. typhi* and *S. aureus* as recommended by CLSI (2010).**

| Antibiotics | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|
| | Susceptible | Intermediate resistant | Resistant |
| Ampicillin | ≥ 17 | 14-16 | ≤13 |
| Ceftriaxone | ≥23 | 15-22 | ≤14 |
| Chloramphenicol | ≥18 | 13-17 | ≤12 |
| Ciprofloxacin | ≥ 21 | 16 – 20 | ≤ 15 |
| Co-trimoxazole | ≥16 | 11-15 | ≤10 |
| Erythromycin | ≥18 | 14-17 | ≤13 |
| Gentamicin | ≥15 | 13-14 | ≤12 |
| Vancomycin | ≥12 | 10-11 | ≤9 |

**Table B: NOSE ISOLATES OF *S. AUREUS* FROM KUMASI SOUTH HOSPITAL**

| Sample | Gentamicin (10µ) | | Vancomycin (30µ) | | Ampicillin (10µ) | | Erythromycin (15µ) | | Ceftriaxone (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| cn2M19 | 13 | I | 13 | S | 16 | I | 6 | R | 6 | R |
| cn3M7 | 10 | R | 22 | S | 15 | I | 13 | R | 23 | S |
| cn4F22 | 6 | R | 12 | S | 6 | R | 6 | R | 6 | R |
| cn5M27 | 17 | S | 6 | R | 17 | S | 19 | S | 26 | S |
| cn7M23 | 21 | S | 18 | S | 25 | S | 6 | R | 6 | R |
| cn10F28 | 18 | S | 16 | S | 13 | R | 23 | S | 13 | R |
| cn13F1 | 21 | S | 23 | S | 6 | R | 16 | I | 20 | I |
| cn14F20 | 21 | S | 19 | S | 6 | R | 6 | R | 6 | R |
| cn17F14 | 11 | R | 13 | S | 18 | S | 14 | I | 10 | R |
| cn18F29 | 16 | S | 22 | S | 22 | S | 6 | R | 6 | R |
| cn21F31 | 6 | R | 25 | S | 22 | S | 20 | S | 26 | S |
| cn22F61 | 13 | I | 13 | S | 19 | S | 14 | I | 18 | I |
| cn23M24 | 22 | S | 15 | S | 22 | S | 20 | S | 24 | S |
| cn24F16 | 18 | S | 18 | S | 8 | R | 6 | R | 23 | S |
| cn25F33 | 13 | I | 16 | S | 22 | S | 19 | S | 6 | R |
| cn26F23 | 10 | R | 15 | S | 13 | R | 11 | R | 13 | R |
| cn27M18 | 13 | I | 22 | S | 18 | S | 13 | R | 10 | R |
| cn29M59 | 13 | I | 10 | I | 6 | R | 17 | I | 21 | I |
| cn30M21 | 16 | S | 18 | S | 6 | R | 12 | R | 22 | I |
| cn33M26 | 18 | S | 10 | I | 22 | S | 15 | I | 16 | I |
| cn34M27 | 16 | S | 22 | S | 21 | S | 10 | R | 13 | R |
| cn37F47 | 14 | I | 6 | R | 16 | I | 23 | S | 6 | R |
| cn39M44 | 6 | R | 6 | R | 22 | S | 21 | S | 16 | I |
| cn40F21 | 6 | R | 13 | S | 22 | S | 19 | S | 13 | R |

**S- Susceptible I- Intermediate resistance R- Resistant**

**Table C: WOUND ISOLATES OF *S. AUREUS* FROM KUMASI SOUTH HOSPITAL**

| Sample | Gentamicin (10µ) | | Vancomycin (30µ) | | Ampicillin (10µ) | | Erythromycin (15µ) | | Ceftriaxone (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| chw6F7 | 6 | R | 6 | R | 8 | R | 7 | R | 20 | I |
| chw7F16 | 6 | R | 20 | S | 16 | I | 30 | S | 22 | I |
| chw8M49 | 15 | I | 18 | S | 16 | I | 30 | S | 15 | I |
| chw3M55 | 17 | S | 7 | R | 7 | R | 7 | R | 22 | I |
| chw10F22 | 16 | S | 8 | R | 11 | R | 13 | R | 20 | I |
| chw13M | 8 | R | 20 | S | 17 | S | 9 | R | 18 | I |
| chw17M47 | 11 | R | 16 | S | 18 | S | 25 | S | 24 | S |
| chw19F33 | 16 | S | 19 | S | 17 | S | 31 | S | 16 | I |
| chw20M25 | 22 | S | 18 | S | 18 | S | 27 | S | 30 | S |
| chw21M27 | 13 | I | 10 | I | 10 | R | 12 | R | 16 | I |
| chw25M29 | 6 | R | 23 | S | 25 | S | 9 | R | 19 | I |
| chw29M20 | 13 | I | 16 | S | 13 | R | 12 | R | 12 | R |
| chw30F23 | 17 | S | 15 | S | 13 | R | 18 | S | 25 | S |
| chw1M25 | 12 | R | 20 | S | 10 | R | 11 | R | 12 | R |
| chw32M26 | 30 | S | 11 | I | 29 | S | 12 | R | 19 | I |
| chw33M21 | 18 | S | 15 | S | 23 | S | 14 | I | 20 | I |
| chw35M27 | 17 | S | 21 | S | 24 | S | 20 | S | 20 | I |
| chw39M40 | 6 | R | 14 | S | 10 | R | 15 | I | 17 | I |

**S- Susceptible I- Intermediate resistance R- Resistant**

**Table D: STOOL ISOLATES OF *S. TYPHI* FROM KUMASI SOUTH HOSPITAL**

| Sample | Ciprofloxacin (5μ) | | Co-trimoxazole (25μ) | | Ceftriaxone (30μ) | | Ampicillin (10μ) | | Chloramphenicol (30μ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| chs 50F47 | 21 | S | 26 | S | 28 | S | 11 | R | 23 | S |
| chs 30F36 | 35 | S | 26 | S | 27 | S | 12 | R | 28 | S |
| chs 42F39 | 27 | S | 24 | S | 27 | S | 30 | S | 30 | S |
| chs 56M38 | 36 | S | 25 | S | 32 | S | 19 | S | 31 | S |
| chs 39M30 | 39 | S | 29 | S | 34 | S | 26 | S | 32 | S |
| chs46M34 | 26 | S | 26 | S | 30 | S | 16 | I | 24 | S |
| chs 43M37 | 30 | S | 11 | I | 29 | S | 12 | R | 26 | S |
| chs 19M38 | 30 | S | 6 | R | 28 | S | 11 | R | 28 | S |
| chs 92M32 | 28 | S | 6 | R | 27 | S | 6 | R | 31 | S |
| chs75F54 | 31 | S | 6 | R | 29 | S | 11 | R | 27 | S |
| chs 10F35 | 28 | S | 6 | R | 20 | I | 7 | R | 29 | S |
| chs8F10 | 6 | R | 6 | R | 26 | S | 8 | R | 12 | R |
| chs14F21 | 32 | S | 21 | S | 30 | S | 13 | R | 29 | S |
| chs11F27 | 31 | S | 10 | R | 30 | S | 13 | R | 25 | S |
| chs6F40 | 27 | S | 8 | R | 15 | I | 12 | R | 26 | S |
| chs1M50 | 31 | S | 8 | R | 28 | S | 8 | R | 34 | S |
| chs7F30 | 6 | R | 8 | R | 9 | R | 8 | R | 15 | I |
| chs16M22 | 6 | R | 6 | R | 32 | S | 6 | R | 18 | S |
| chs5M36 | 20 | I | 20 | S | 22 | I | 20 | S | 28 | S |
| chs4M31 | 30 | S | 26 | S | 30 | S | 30 | S | 24 | S |
| chs2M39 | 25 | S | 29 | S | 27 | S | 6 | R | 21 | S |
| chs25M27 | 10 | R | 15 | I | 20 | I | 23 | S | 28 | S |
| chs21M24 | 17 | I | 25 | S | 29 | S | 11 | R | 30 | S |
| chs22M48 | 22 | S | 28 | S | 24 | S | 24 | S | 25 | S |
| chs28M30 | 33 | S | 27 | S | 30 | S | 21 | S | 30 | S |
| chs24M33 | 26 | S | 20 | S | 33 | S | 14 | I | 22 | S |
| chs17F37 | 28 | S | 26 | S | 26 | S | 26 | S | 32 | S |
| chs36F39 | 32 | S | 30 | S | 31 | S | 29 | S | 27 | S |
| chs 15F33 | 31 | S | 10 | R | 22 | I | 19 | S | 24 | S |
| chs88M40 | 18 | I | 20 | S | 19 | I | 12 | R | 28 | S |

**S- Susceptible I- Intermediate resistance R- Resistant**

**Table E: URINE ISOLATES OF *S. TYPHI* FROM KUMASI SOUTH HOSPITAL**

| Sample | Ciprofloxacin (5µ) | | Co-trimoxazole (25µ) | | Ceftrixone (30µ) | | Ampicillin (10µ) | | Chloramphenicol (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| chu6M22 | 30 | S | 6 | R | 27 | S | 13 | R | 11 | R |
| chu2M26 | 33 | S | 26 | S | 30 | S | 11 | R | 16 | I |
| chu4M62 | 6 | R | 7 | R | 21 | I | 9 | R | 14 | I |
| chu9F46 | 27 | S | 18 | S | 27 | S | 9 | R | 21 | S |
| chu7M40 | 6 | R | 6 | R | 14 | R | 8 | R | 10 | R |
| chu5F48 | 12 | R | 6 | R | 35 | S | 16 | I | 30 | S |
| chu16M41 | 29 | S | 8 | R | 26 | S | 10 | R | 35 | S |
| chu23F45 | 28 | S | 8 | R | 12 | R | 19 | S | 33 | S |
| chu14F42 | 6 | R | 6 | R | 29 | S | 6 | R | 22 | S |
| chu12M45 | 6 | R | 8 | R | 27 | S | 7 | R | 19 | S |
| chu26M33 | 26 | S | 7 | R | 32 | S | 8 | R | 21 | S |
| chu35F48 | 6 | R | 8 | R | 12 | R | 6 | R | 22 | S |
| chu24F37 | 6 | R | 8 | R | 26 | S | 6 | R | 18 | S |
| chu61M40 | 30 | S | 22 | S | 28 | S | 14 | I | 27 | S |
| chu 30F48 | 24 | S | 27 | S | 26 | S | 29 | S | 30 | S |
| chu 10F38 | 33 | S | 30 | S | 31 | S | 28 | S | 25 | S |
| chu 54F43 | 28 | S | 25 | S | 29 | S | 27 | S | 25 | S |
| chu 44F30 | 24 | S | 26 | S | 30 | S | 16 | I | 35 | S |
| chu 21F41 | 23 | S | 14 | I | 26 | S | 12 | R | 27 | S |
| chu 73F5 | 30 | S | 7 | R | 32 | S | 24 | S | 32 | S |

**S- Susceptible I- Intermediate resistance R- Resistant**

**Table F: NOSE ISOLATES OF *S. AUREUS* FROM NORTH SUNTRESO HOSPITAL**

| Sample | Gentamicin (10µ) | | Vancomycin (30µ) | | Ampicillin (10µ) | | Erythromycin (15µ) | | Ceftriaxone (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| sn1F55 | 19 | S | 6 | R | 19 | S | 21 | S | 6 | R |
| sn3F12 | 6 | R | 6 | R | 18 | S | 23 | S | 27 | S |
| sn4F29 | 10 | R | 13 | S | 18 | S | 14 | I | 20 | I |
| sn7F25 | 15 | S | 23 | S | 16 | I | 12 | R | 10 | R |
| sn6F28 | 20 | S | 20 | S | 16 | I | 19 | S | 6 | R |
| sn11F58 | 25 | S | 19 | S | 21 | S | 6 | R | 6 | R |
| sn13M9 | 10 | R | 11 | I | 14 | I | 12 | R | 18 | I |
| sn18M26 | 18 | S | 21 | S | 20 | S | 22 | S | 7 | R |
| sn17F4 | 7 | R | 12 | S | 10 | R | 16 | I | 11 | R |
| sn25F47 | 16 | S | 18 | S | 19 | S | 22 | S | 8 | R |
| sn24F49 | 18 | S | 16 | S | 18 | S | 20 | S | 16 | I |
| sn29M20 | 6 | R | 20 | S | 16 | I | 6 | R | 22 | I |
| sn21M9 | 14 | I | 19 | S | 16 | I | 25 | S | 15 | I |
| sn30F50 | 13 | I | 16 | S | 12 | R | 6 | R | 9 | R |
| sn32M40 | 11 | R | 28 | S | 22 | S | 19 | S | 21 | I |
| sn33F38 | 20 | S | 6 | R | 19 | S | 21 | S | 6 | R |
| sn36M37 | 6 | R | 22 | S | 24 | S | 8 | R | 20 | I |
| sn38F5 | 17 | S | 6 | R | 6 | R | 6 | R | 22 | I |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table G: WOUND ISOLATES OF *S. AUREUS* FROM NORTH SUNTRESO HOSPITAL**

| Sample | Gentamicin (10µ) | | Vancomycin (30µ) | | Ampicillin (10µ) | | Erythromycin (15µ) | | Ceftriaxone (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| sw10M5 | 13 | I | 16 | S | 13 | R | 9 | R | 6 | R |
| sw13M31 | 11 | R | 22 | S | 13 | R | 33 | S | 6 | R |
| sw15M26 | 30 | S | 22 | S | 19 | S | 24 | S | 13 | R |
| sw50M8 | 6 | R | 9 | R | 6 | R | 6 | R | 6 | R |
| sw11F22 | 19 | S | 9 | R | 8 | R | 7 | R | 30 | S |
| sw7F26 | 12 | R | 6 | R | 6 | R | 6 | R | 16 | I |
| sw9M28 | 23 | S | 19 | S | 19 | S | 28 | S | 31 | S |
| sw4F33 | 8 | R | 6 | R | 12 | R | 7 | R | 6 | R |
| sw5F21 | 8 | R | 22 | S | 24 | S | 8 | R | 20 | I |
| sw12F29 | 21 | S | 18 | S | 25 | S | 9 | R | 20 | I |
| sw1F42 | 10 | R | 14 | S | 12 | R | 11 | R | 31 | S |
| sw19F38 | 17 | S | 20 | S | 18 | S | 16 | I | 32 | S |
| sw23M26 | 9 | R | 6 | R | 6 | R | 19 | S | 15 | I |
| sw25M26 | 24 | S | 19 | S | 17 | S | 26 | S | 29 | S |
| sw30M28 | 15 | S | 15 | S | 13 | R | 8 | R | 9 | R |
| sw32M27 | 12 | R | 20 | S | 12 | R | 30 | S | 10 | R |
| sw40M20 | 7 | R | 11 | I | 14 | I | 8 | R | 16 | I |
| sw34M24 | 20 | S | 16 | S | 18 | S | 19 | S | 28 | S |
| sw37M54 | 6 | R | 8 | R | 10 | R | 12 | R | 12 | R |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table H: STOOL ISOLATES OF *S. TYPHI* FROM NORTH SUNTRESO HOSPITAL**

| Sample | Ciprofloxacin (5μ) | | Co-trimoxazole (25μ) | | Ceftriaxone (30μ) | | Ampicillin (10μ) | | Chloramphenicol (30μ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| ss1F15 | 25 | S | 25 | S | 28 | S | 6 | R | 14 | I |
| ss2M20 | 25 | S | 7 | R | 25 | S | 6 | R | 12 | R |
| ss7F51 | 19 | I | 7 | R | 12 | R | 6 | R | 21 | S |
| ss10M39 | 39 | S | 25 | S | 33 | S | 13 | R | 11 | R |
| ss9M34 | 26 | S | 26 | S | 28 | S | 12 | R | 25 | S |
| ss4F44 | 26 | S | 25 | S | 27 | S | 9 | R | 6 | R |
| ss14M30 | 12 | R | 28 | S | 31 | S | 16 | I | 17 | I |
| ss54M30 | 25 | S | 25 | S | 27 | S | 8 | R | 24 | S |
| ss21M23 | 30 | S | 23 | S | 29 | S | 8 | R | 26 | S |
| ss30F35 | 28 | S | 26 | S | 28 | S | 17 | S | 13 | I |
| ss43F39 | 27 | S | 21 | S | 27 | S | 19 | S | 18 | S |
| ss60F28 | 29 | S | 28 | S | 30 | S | 23 | S | 20 | S |
| ss61M36 | 26 | S | 23 | S | 23 | S | 10 | R | 25 | S |
| ss76F48 | 28 | S | 27 | S | 29 | S | 17 | S | 22 | S |
| ss89F21 | 23 | S | 25 | S | 14 | R | 27 | S | 8 | R |
| ss72F23 | 32 | S | 22 | S | 13 | R | 24 | S | 12 | R |
| ss86F31 | 12 | R | 27 | S | 25 | S | 8 | R | 16 | I |
| ss97F65 | 10 | R | 8 | R | 11 | R | 12 | R | 23 | S |
| ss50F42 | 15 | R | 10 | R | 10 | R | 6 | R | 19 | S |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table I: STOOL ISOLATES OF *S. TYPHI* FROM NORTH SUNTRESO HOSPITAL**

| Sample | Ciprofloxacin (5µ) | | Co-trimoxazole (25µ) | | Ceftriaxone (30µ) | | Ampicillin (10µ) | | Chloramphenicol (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| su4M10 | 12 | R | 27 | S | 26 | S | 13 | R | 15 | I |
| su6F38 | 34 | S | 28 | S | 31 | S | 11 | R | 12 | R |
| su9F30 | 19 | I | 8 | R | 11 | R | 7 | R | 11 | R |
| su1F30 | 6 | R | 6 | R | 16 | I | 10 | R | 10 | R |
| su8F34 | 19 | I | 6 | R | 9 | R | 9 | R | 13 | I |
| su2F48 | 26 | S | 7 | R | 25 | S | 6 | R | 17 | I |
| su10F44 | 25 | S | 10 | R | 28 | S | 10 | R | 25 | S |
| su5F42 | 31 | S | 22 | S | 13 | R | 13 | R | 29 | S |
| su3M42 | 13 | R | 8 | R | 24 | S | 7 | R | 17 | I |
| su7F15 | 21 | S | 7 | R | 25 | S | 13 | R | 26 | S |
| su18F46 | 9 | R | 11 | R | 16 | I | 16 | I | 8 | R |
| su21M48 | 27 | S | 13 | I | 20 | I | 14 | I | 25 | S |
| su23F15 | 20 | I | 8 | R | 25 | S | 15 | I | 6 | R |
| su39F21 | 12 | R | 30 | S | 23 | S | 12 | R | 7 | R |
| su47F18 | 30 | S | 26 | S | 30 | S | 10 | R | 10 | R |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table J: URINE ISOLATES OF *S. TYPHI* FROM TAFO HOSPITAL**

| Sample | Ciprofloxacin (5µ) | | Co-trimoxazole (25µ) | | Ceftriaxone (30µ) | | Ampicillin (10µ) | | Chloramphenicol (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| tfu2M11 | 15 | R | 20 | S | 26 | S | 14 | I | 22 | S |
| tfu6F50 | 35 | S | 13 | I | 20 | I | 22 | S | 12 | R |
| tfu14F38 | 35 | S | 14 | I | 19 | I | 21 | S | 20 | S |
| tfu46M48 | 33 | S | 21 | S | 20 | I | 6 | R | 18 | S |
| tfu11M45 | 30 | S | 12 | I | 17 | I | 10 | R | 21 | S |
| tfu31M44 | 32 | S | 23 | S | 32 | S | 21 | S | 16 | I |
| tfu20F22 | 13 | R | 28 | S | 25 | S | 14 | I | 10 | R |
| tfu72M33 | 25 | S | 21 | S | 23 | S | 19 | S | 26 | S |
| tfu69M48 | 16 | I | 18 | S | 19 | I | 9 | R | 21 | S |
| tfu38F41 | 11 | R | 17 | S | 20 | I | 6 | R | 6 | R |
| tfu13F43 | 25 | S | 22 | S | 26 | S | 23 | S | 18 | S |
| tfu26F20 | 14 | R | 29 | S | 25 | S | 15 | S | 13 | I |
| tfu53F35 | 15 | R | 32 | S | 24 | S | 26 | S | 12 | R |
| tfu17F32 | 10 | R | 19 | S | 23 | S | 21 | S | 7 | R |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table K: NOSE ISOLATES OF *S. AUREUS* FROM TAFO HOSPITAL**

| Sample | Gentamicin (10μ) | | Vancomycin (30μ) | | Ampicillin (10μ) | | Erythromycin (15μ) | | Ceftriaxone (30μ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| tn5M2 | 15 | S | 18 | S | 21 | S | 12 | R | 23 | S |
| tn9M32 | 6 | R | 6 | R | 12 | R | 22 | S | 16 | I |
| tn10F29 | 6 | R | 20 | S | 23 | S | 21 | S | 8 | R |
| tn14F45 | 19 | S | 24 | S | 13 | R | 22 | S | 17 | I |
| tn15F52 | 21 | S | 16 | S | 7 | R | 7 | R | 13 | R |
| tn17M23 | 10 | R | 17 | S | 20 | S | 28 | S | 12 | R |
| tn18M8 | 9 | R | 21 | S | 23 | S | 15 | I | 6 | R |
| tn25M35 | 13 | I | 15 | S | 11 | R | 17 | I | 9 | R |
| tn31F21 | 22 | S | 8 | R | 17 | S | 9 | R | 13 | R |
| tn34M48 | 12 | R | 17 | S | 17 | S | 12 | R | 6 | R |
| tn35F21 | 8 | R | 6 | R | 6 | R | 12 | R | 19 | I |
| tn37F40 | 7 | R | 14 | S | 21 | S | 22 | S | 12 | R |
| tn39M12 | 10 | R | 15 | S | 14 | I | 17 | I | 22 | I |
| tn40M10 | 6 | R | 20 | S | 12 | R | 18 | S | 21 | I |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**Table L: WOUND ISOLATES OF *S. AUREUS* FROM TAFO HOSPITAL**

| Sample | Gentamicin (10μ) | | Vancomycin (30μ) | | Ampicillin (10μ) | | Erythromycin (15μ) | | Ceftriaxone (30μ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| tfw1M29 | 12 | R | 17 | S | 23 | S | 11 | R | 22 | I |
| tfw2M21 | 6 | R | 18 | S | 12 | R | 12 | R | 20 | I |
| tfw5M24 | 21 | S | 15 | S | 19 | S | 22 | S | 26 | S |
| tfw10M23 | 13 | I | 24 | S | 7 | R | 21 | S | 16 | I |
| tfw8M9 | 19 | S | 16 | S | 6 | R | 8 | R | 12 | R |
| tfw12M23 | 8 | R | 11 | I | 12 | R | 6 | R | 23 | S |
| tfw13F42 | 13 | I | 22 | S | 25 | S | 15 | I | 12 | R |
| tfw14M45 | 10 | R | 7 | R | 12 | R | 9 | R | 16 | I |
| tfw18M29 | 18 | S | 20 | S | 19 | S | 23 | S | 21 | I |
| tfw20M40 | 20 | S | 16 | S | 16 | I | 12 | R | 10 | R |
| tfw23M25 | 25 | S | 30 | S | 6 | R | 9 | R | 13 | R |
| tfw22F51 | 7 | R | 6 | R | 6 | R | 7 | R | 12 | R |
| tfw25F30 | 21 | S | 23 | S | 18 | S | 22 | S | 16 | I |
| tfw31F39 | 15 | S | 19 | S | 10 | R | 6 | R | 6 | R |
| tfw40M13 | 12 | R | 7 | R | 6 | R | 6 | R | 13 | R |
| tfw35F7 | 15 | S | 18 | S | 30 | S | 21 | S | 26 | S |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

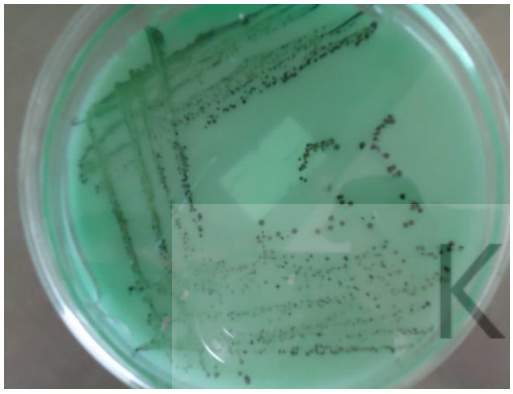
**Table M: STOOL ISOLATES OF *S. TYPHI* FROM TAFO HOSPITAL**

| Sample | Ciprofloxacin (5µ) | | Co-trimoxazole (25µ) | | Ceftriaxone (30µ) | | Ampicillin (10µ) | | Chloramphenicol (30µ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| tfs10M6 | 35 | S | 27 | S | 31 | S | 15 | I | 10 | R |
| tfs1F3 | 40 | S | 6 | R | 30 | S | 6 | R | 13 | I |
| tfs2F18 | 35 | S | 30 | S | 30 | S | 6 | R | 18 | S |
| tfs30F12 | 27 | S | 28 | S | 13 | R | 29 | S | 9 | R |
| tfs4M16 | 39 | S | 26 | S | 27 | S | 15 | I | 14 | I |
| tfs40F40 | 34 | S | 30 | S | 30 | S | 10 | R | 6 | R |
| tfs13M41 | 31 | S | 6 | R | 26 | S | 15 | I | 25 | S |
| tfs24M36 | 26 | S | 11 | R | 10 | R | 11 | R | 16 | I |
| tfs11M32 | 26 | S | 6 | R | 6 | R | 6 | R | 9 | R |
| tfs27M39 | 23 | S | 31 | S | 14 | R | 17 | S | 11 | R |
| tfs50M34 | 30 | S | 10 | R | 22 | I | 12 | R | 12 | R |
| tfs55M40 | 25 | S | 19 | S | 28 | S | 29 | S | 19 | S |
| tfs43M37 | 33 | S | 29 | S | 29 | S | 19 | S | 14 | I |
| tfs32F35 | 28 | S | 25 | S | 10 | R | 6 | R | 12 | R |
| tfs42F26 | 32 | S | 28 | S | 25 | S | 30 | S | 17 | I |
| tfs56F36 | 24 | S | 25 | S | 33 | S | 20 | S | 20 | S |
| tfs53M39 | 33 | S | 28 | S | 28 | S | 27 | S | 23 | S |
| tfs26M32 | 29 | S | 27 | S | 30 | S | 26 | S | 28 | S |
| tfs7F35 | 26 | S | 26 | S | 13 | R | 19 | S | 19 | S |
| tfs59M21 | 27 | S | 39 | S | 25 | S | 27 | S | 21 | S |
| tfs61F27 | 26 | S | 19 | S | 34 | S | 12 | R | 25 | S |
| tfs33M30 | 30 | S | 20 | S | 15 | I | 26 | S | 6 | R |
| tfs18M37 | 34 | S | 26 | S | 30 | S | 16 | I | 15 | I |
| tfs80M39 | 40 | S | 30 | S | 30 | S | 20 | S | 13 | I |
| tfs72F26 | 24 | S | 32 | S | 24 | S | 18 | S | 11 | R |
| tfs16F37 | 39 | S | 34 | S | 23 | S | 13 | R | 16 | I |
| tfs74F30 | 26 | S | 10 | R | 11 | R | 6 | R | 22 | S |
| tfs66F21 | 32 | S | 13 | I | 15 | I | 18 | S | 12 | R |
| tfs38F39 | 37 | S | 31 | S | 28 | S | 15 | I | 10 | R |
| tfs89F22 | 30 | S | 21 | S | 24 | S | 27 | S | 8 | R |

**S- Susceptible** **I- Intermediate resistance** **R- Resistant**

**APPENDIX IV**
**SOME RESULTS OF THE ISOLATES OBTAINED FROM SELECTIVE AND DIFFERENTIAL MEDIA**

Black colonies of *S. typhi* on bismuth sulphite agar



*Staph aureus* growing on Mannitol salt agar

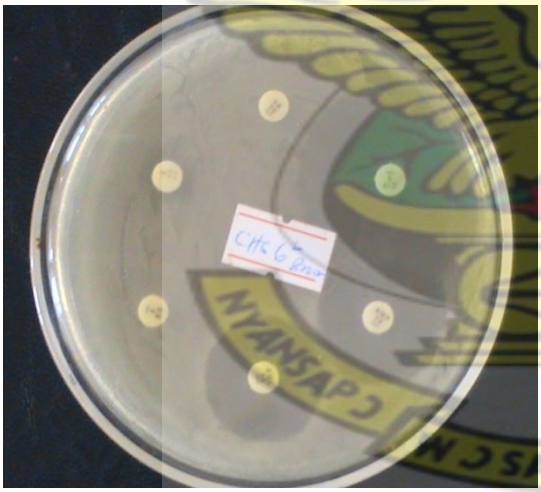
Pink colonies of a lactose fermenter and colourless colonies of a non-lactose fermenter growing on MacConkey agar

Greyish colonies of isolates on Blood agar

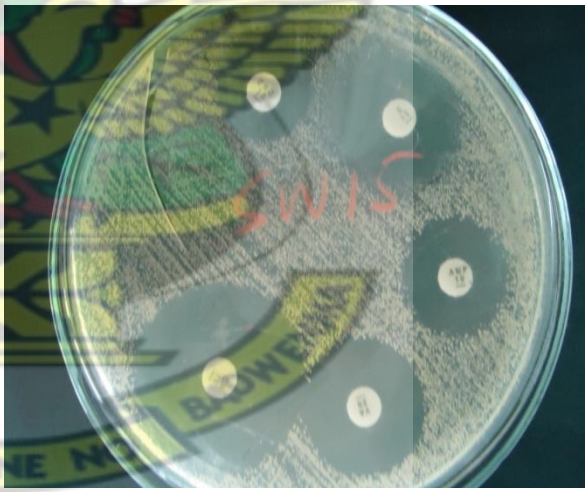
**APPENDIX V**
**SOME RESULTS OF THE BIOCHEMICAL REACTIONS AND SENSITIVITY TESTS**

Pink colour of Urease positive reaction and and yellow colour of Urease negative reaction

Yellow butt and pink slant of *S. typhi* on TSI agar

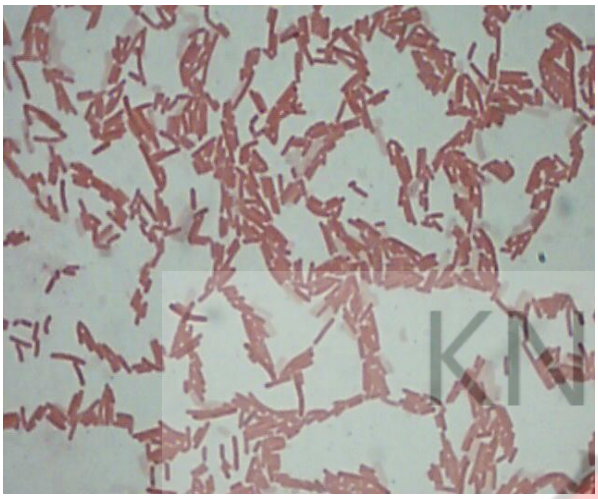


Sensitivity of *S. typhi* to selected antibiotics



Sensitivity of *S. aureus* to selected antibiotics

**APPENDIX VI**
**SOME RESULTS OF GRAM STAIN**



Gram negative bacilli of *S. typhi*

Gram positive cocci of *S. aureus*