

**A STUDY OF ANTIBIOTIC RESISTANCE PROFILE OF DIARRHOEAGENIC
PATHOGENS ISOLATED FROM INFECTED CHILDREN IN ILE-IFE.**

BY

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IN

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This is to certify that the research in this thesis was carried out by Florence Olubunmi Akinwumi under the supervision of Professor A. Lamikanra.

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DEDICATION

This project is dedicated to God Almighty, maker of all that is seen and unseen

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ABSTRACT

This study identified the bacterial pathogens associated with diarrhoea in children aged 0-24 months, established the frequency of diarrhoea caused by each pathogen encountered in diarrhoea and determined the antibiotic resistance pattern of the children's faecal isolates obtained to some commonly used antibiotics. This was with a view to providing information on the aetiology and management of diarrhoea in Ile-Ife, South West, Nigeria.

A total of 107 children within the age range 0-24 months presenting with diarrhoea and 115 apparently healthy children (aged 0-24 months) receiving immunization at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Eleyele and State Hospital, Okeogbo both in Ile-Ife were recruited into this study. Stool samples or rectal swabs were collected using sterile swab sticks and sterile universal bottles. These samples were cultured for the isolation of enteric pathogens present in the stool using standard microbiological procedures. All recovered isolates were tested against 8 antibiotics using the disc diffusion method. Data were analysed by descriptive and inferential statistics.

The isolation of enteric pathogens was found to be significantly ($p < 0.0001$) higher among diarrhoeal children (388) than among the apparently healthy children who did not exhibit symptoms of diarrhoea (279). Children in the age group 13-18 months were mostly affected by diarrhoea diseases. *Campylobacter* spp. was the most frequently isolated enteric pathogen among diarrhoeal (17.8%) and apparently healthy (16.5%) children. There was no significant ($p > 0.05$) difference in the isolates recovered from both diarrhoeal and control subjects. The isolates were found to be multiply resistant to all the antibiotics tested. However, the incidence of resistance to ciprofloxacin, gentamicin, ceftriaxone and chloramphenicol was less than 50%.

The study concluded that more than one enteric pathogen may be responsible for diarrhoea diseases in a single patient and that the pathogens encountered were resistant to the antibiotics which were frequently administered to patients within the study

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Researchers have estimated that the human body contains 10^{14} cells, only 10% of which are not bacteria and belong to the human body proper (Savage, 1977). These bacteria are found all over the body but the mammalian intestinal tract represents a complex, dynamic and diverse ecosystem of interacting aerobic and anaerobic, non-pathogenic bacteria. According to Moore and Holdeman, 1974, this complex yet stable colony includes more than 400 separate species.

1.1.1 Diarrhoea

Reports from around the world have suggested that more than 4,000 children lose their lives to diarrhoea everyday which means that it kills more children worldwide than any other condition (Desmond *et al.*, 1996; World Health Organization (WHO), 2004a) with 3.3 million child deaths (Seung-Hak *et al.*, 2006) associated with more than one billion episodes each year (Bern *et al.*, 1992). It is characterized by increased frequency of bowel movements and increased liquidity of stool (Fine and Schiller, 1999); the second leading cause of death in children younger than 5 years globally (pneumonia, 17%; diarrhoea, 16%; malaria, 7%; measles, 4%; AIDS, 2%; neonatal causes, 37%; injuries, 4%; other causes, 13%), (WHO, 2004) with nearly one in every five child deaths, due to diarrhoea (WHO, 2009) and the fifth leading cause of death in low- and middle-income countries (WHO, 2012).

Infant diarrhoea and infant mortality are frequently associated with poor environmental health conditions, lack of access to public health services, malnutrition and poor hygiene, which

are conditions frequently found in underdeveloped nations. Infant diarrhoea is more prevalent and more serious in areas of precarious socio-economic conditions (Jones *et al.*, 2003; Thapar and Sanderson, 2004). According to Adegunloye (2005) acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria.

WHO (1995; 2009) defines diarrhoea as the passage of unusually loose or watery stools, usually at least three times in a 24-hour period. Although, the lay population may claim they have diarrhoea when they, in fact, have other problems, such as faecal impaction (Schiller, 2009). Therefore when evaluating a person with complaints of diarrhoea, it is imperative to closely question the patient about associated signs and symptoms. However, it is the consistency of the stools rather than the frequency that is important as frequent passing of formed stools is not diarrhoea. Babies fed only breast milk often pass loose, "pasty" stools; this also is not diarrhoea. A study by Adimora *et al.* (2011) suggests that mothers usually know when their children have diarrhoea and may provide useful working definitions in local situations. Blood in stool could indicate an acute diarrhoeal illness or dysentery, irrespective of frequency (Baqui *et al.*, 1991; WHO, 1994).

Each year, an estimated 2.5 billion cases of diarrhoea occur among children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades (Boschi Pinto *et al.*, 2009). More than half of these cases are in Africa and South Asia (WHO, 2004), where bouts of diarrhoea are more likely to result in death or other severe outcomes.

The incidence of diarrhoeal diseases varies greatly with the seasons and a child's age. In temperate climates, bacterial diarrhoea occur more frequently during the warm season, whereas viral diarrhoea, particularly diarrhoea caused by rotavirus peaks during the winter. In tropical areas, rotavirus diarrhoea occurs throughout the year, increasing in frequency during the drier, cool months, whereas

bacterial diarrhoea peak during the warmer, rainy season. The incidence of persistent diarrhoea follows the same seasonal patterns as that of acute watery diarrhoea (WHO, 1992). The youngest children are most vulnerable with highest incidence in the first two years of life which declines as a child grows older (El-Gilany and Oni, 1996; Banerjee *et al.*, 2004; Hammad, 2005; Siraj *et al.*, 2008).

Africa and South Asia are home to more than 80 per cent of child deaths due to diarrhoea (United Nations International Children's Emergency Fund (UNICEF)/WHO, 2004) and just 15 countries account for almost three quarters of all deaths from diarrhoea among children less than five years of age annually with countries in Africa accounting for 696 million deaths due to childhood diarrhoea (WHO, 2007).

In the year 2000, Audu and his colleagues claimed that diarrhoea was the number one killer disease among children aged 1–5 years in Nigeria (Audu *et al.*, 2000). However, by 2010, UNICEF reported that diarrhoea is the second biggest killer of children in Nigeria, responsible for about 17 percent of child deaths every year, the majority (88% globally reported) of which is attributed to inadequate water, lack of sanitation and poor hygiene (UNICEF, 2010). Diarrhoeal disease is not just a health issue, but an economic one as well. In sub-Saharan Africa, for example, treating water-borne diseases like diarrhoea costs governments at least 12 percent of their total health budgets each year (United Nations Development Program (UNDP), 2006). The World Bank estimates that environmental health problems such as diarrhoea and associated malnutrition cost low-income governments up to 9 percent of their annual gross domestic products (GDP) (World Bank, 2008).

Several reports have shown that diarrhoeal diseases are causes of malnutrition, and malnutrition contributed to increase the risk for more diarrhoeal diseases (Black *et al.*, 1984; Checkley *et al.*, 2008; Guerrant *et al.*, 2008).

1.2 Aetiology of Diarrhoea

The aetiology of diarrhoea can be psychogenic, neurogenic, surgical, endocrine, irritant, osmotic, dietary, allergenic, malabsorptive, infectious and/or inflammatory (Banwell, 1990). Diarrhoeal infections may be caused by an array of bacterial (Zurawska-Olszewska *et al.*, 2002; von Seidlein *et al.*, 2006; Jafari *et al.*, 2009), viral (Van Man *et al.*, 2005; Ramani and Kang, 2009) or parasitic pathogens (Vargas *et al.*, 2004; Brianna *et al.*, 2011; Adimora *et al.*, 2011), both individually and together (O'ryan *et al.*, 2005; Nair *et al.*, 2010) most of which are spread by faeces-contaminated water. Together, coinfecting pathogens may cause more severe diarrhoea than infection with either pathogen alone (Gimprel *et al.*, 2008). The contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological, geographic, and socio-economic conditions (Reither *et al.*, 2007). Specific coinfecting pathogens may act synergistically, resulting in even greater pathogenesis and a larger contribution to the overall diarrhoeal disease burden (Bhavnani *et al.*, 2012). One of the most common causes of infectious diarrhoea is a lack of clean water (Brown *et al.*, 2013).

A number of microorganisms, including a variety of viral, bacterial, and protozoan agents are capable of causing diarrhoeal disease. The four most common viruses associated with acute gastroenteritis are rotavirus, norovirus, enteric adenovirus, and astrovirus (Clark and McKendrick, 2004). Norovirus is the most common cause of viral diarrhoea in adults (Patel *et al.*, 2009), but rotavirus is the most common cause of diarrhoea in children under five years old (Greenberg and Estes, 2009; Goodgame, 2001). Astrovirus infections occur predominantly in