PREVALENCE OF COLIFORMS AND MULTIPLE-ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* IN DAY-CARE CENTRES IN MINNA, NIGERIA .

BY
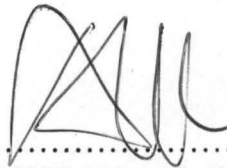
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APRIL, 2002

DECLARATION

I hereby declare that this work is original and has not been carried out elsewhere. All literatures cited have been listed in the references.

24-09-02

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Appendix 14: Statistical analysis of potency of test antibiotics in relation to their minimum inhibitory concentration (MIC) per Day-care Centre.

Day-care centre	Nalidixic acid	Gentamicin	Erythromycin	Tetracycline	Chloramphenicol	Sulphamethoxazol e t.m.p	Penicillin	Ampiclox	± sem
NDC	19.0 ± 2.55^{ab}	12.11 ± 4.31^{ab}	18.14 ± 2.59^{ab}	9.94 ± 4.67^{b}	10.89 ± 4.60^{a}	16.45 ± 2.69^{ab}	20.00 ± 2.09^{ab}	27.27 ± 3.27^{b}	±3.48
UDC	20.73 ± 3.53^{abcd}	10.45 ± 2.73^{ab}	27.27 ± 3.27^{cd}	6.36 ± 2.66^{ab}	4.99 ± 2.20^{a}	13.55 ± 3.06^{abc}	22.09 ± 4.62^{bcd}	33.09 ± 5.40^{d}	±3.58
GDC	29.40 ± 4.42^{c}	6.60 ± 2.02^{ab}	25.60 ± 3.11^{c}	1.48 ± 0.31^{a}	5.45 ± 2.18^{ab}	16.50 ± 3.17^{bc}	20.60 ± 2.27^{c}	21.93 ± 4.13^{c}	±2.97

Data on the same row carrying the same superscripts do not differ significantly from each other ($P> 0.05$).

ANOVA Table- Prevalence of coliforms from New Secretariat Day-care Centre (NDC).

Source Of Variation	*E. coli* Between groups	within groups	Total	*Ent.aerogenes* Between groups	within groups	Total	*Klebsiella* spp. Between groups	Within groups	Total	*Serratia* spp Between groups	Within groups	Total
Sum of Squares	143.8686	3.6200	147.4886	20.8286	8000	20.9086	12.4971	2.0000	14.4971	326.5973	312.9174	639.5147
Df	6	7	13	6	7	13	6	7	13	6	7	13
Ms	23.9781	0.5171		3.4714	0.0114		2.0829	.2857		54.4329	44.7025	
F-ratio	46.366			303.750			7.290			1.218		
Sign level	.0000			.0000			0.0096	0.3967				

Range test: Tukey
Confidence level: 95

APPENDIX 17:

ANOVA Table- Prevalence of coliforms from Unguwan-daji Day-care Centre (UDC)

Source Of Variation	*E. coli* Between groups	within groups	Total	*Ent.aerogenes* Between groups	within groups	Total	*Klebsiella* spp. Between groups	Within groups	Total	*Serratia* spp Between groups	Within groups
Sum of Squares	307.8186	486.3900	794.2086	1040.4286	4.3200	1044.7486	54.7542	5.4200	60.1743	283.3371 .2000	283.5371
Df	6	7	13	6	7	13	6	7	13	6 7	13
Ms	51.3031	69.4843		173.4048	61.71		9.1257	0.7742		47.2229 .0286	
F-ratio	.738	·		280.980			·11.786			999.999	·
Sign level	.738			.0000			.0023	.0000			

Range test: Tukey
Confidence level: 95

APPENDIX 18:

ANOVA Table- Potency of antibiotic determined from values of zones of inhibition

Source Of Variation	NDC			UDC			GDC		
	Between groups	Within groups	Total	Between groups	Within groups	Total	Between groups	Within groups	Total
Sum of Squares	2594.317	1068.048	13277.365	7871.867	1161.900	19133.767	7388.4844	6339.2625	13727.747
Df	7	80	87	7	80	87	7	72	79
Ms	370.61673	133.5381	·	1124.5524	140.7737	·	1055.4978	88.0453	
F-ratio	2.775			7.988			11.988		
Sign. level	0.0123			.0000			.0000		

Range test: Tukey
Confidence level: 95

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APPENDIX 19:

ANOVA Table- Potency of antibiotic determined from values of zones of inhibition

Source Of Variation	NDC			UDC			GDC		
	Between groups	within groups	Total	Between groups	within groups	Total	Between groups	Within groups	Total
Sum of Squares	874.4432	1828.000	2702.4432	1436.7159	1193.2727	2629.9886	1507.3500	1053.4000	2562.7500
Df	7	82	89	7	80	87	7	72	79
Ms	124.9205	22.8500		205.2451	14.9159		215.3357	14.6583	
F-ratio	5.467			13.760			14.690		
Sign level	.0000			.0000			.0000		

Range test: Tukey
Confidence level: 95

APPENDIX 20 a:

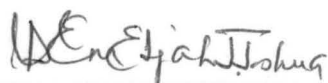ANOVA Table- Susceptibility of *E.coli* determined from values of zones of inhibition

Source Of Variation	Nalidixic acid			Gentamicin			Erythromycin			Tetracycline		
	Between groups	Within groups	Total	Between groups	Within groups	Total	Between groups	Within groups	Total	Between groups	Within groups	Total
Sum of	634.0091	3845.4909	4479.5000	165.7524	3237.2304	3402.9888	516.7399	2788.6273	3305.3672	376.1369	3181.7381	3557.8750
Df	2	29	31	2	29	31	2	29	2	29	31	
Ms	317.0046	132.6031		82.8762	111.6288		258.3699	96.1596	188.0685	109.7151		
F-ratio	2.391			.742			2.687			1.714		
Sign. level	.094			.484			0.0850			0.1979		

ZDI: Zone Diameter of Inhibition
Range test: Tukey
Confidence level: 95

ANOVA Table- Susceptibility of *E.coli* determined from values of zones of inhibition

Source Of Variation	Nalidixic acid			Gentamicin			Erythromycin			Tetracycline		
	Between groups	Within groups	Total	Between groups	Within groups	Total	Between groups	Within groups	Total	Between groups	Within groups	Total
Sum of	203.1627	3288.7572	3491.9198	62.0142	2725.9545	2787.9688	25.4097	3297.3091	3322.7188	654.8853	5919.0972	6573.9824
Df	2	29	31	2	29	31	2	29	31	2	29	31
Ms	101.5813	113.4054		31.0071	93.9984		12.7048	113.7003		327.4426	204.1068	
F-ratio	.896			.330			.112			1.604		
Sign. level	.4193			.7217			.8947			.2184		

Range test: Tukey
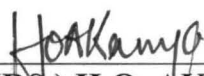**Confidence level:** 95

CERTIFICATION

This is to certify that this project work titled "Prevalence of coliforms and multiple-antibiotic resistant *Escherichia coli* in Day-Care Centres in Minna, Nigeria, was carried out under my supervision, and has been examined, read, and found to have met the regulations governing the award of Master of Technology (M.Tech.) degree in Environmental and Public Health Microbiology of Federal University of Technology, Minna, Nigeria, and is approved for its contribution to knowledge and literary presentation.

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Date

PROF. J. A. ABALAKA
(Dean, Post-Graduate School)

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Date

DR. K. B. TANYIGNA
(External Examiner)

24 – 09 – 02
Date

DEDICATION

This work is dedicated to the Almighty Allah from whom we expect forgiveness of all our sins.

ACKNOWLEDGEMENT

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ABSTRACT

Environmental surfaces (wall, funitures, door handle, toys, floor, utensils and toilet seats) in three day-care Centres (the New Secretariat Day-care Centre, NDC, Unguwan-daji Day-care Centre UDC and Government Day-care Centre, GDC), all located in Minna, Nigeria were sampled to determine the prevalence of coliforms and other bacteria on them. Coliforms were isolated from 95(13.7%) of 693 samples collected while non-coliform bacteria were isolated from 598 (86.3%) of the environmental samples examined. Walls, furnitures, toys and utensils had high prevalence of both coliform and non-coliforms suggesting that these items could act as reservoirs for the organisms. *Escherichia coli* was the most prevalent in UDC and GDC while *Enterobacter aerogenes* was most prevalent in NDC. The highest prevalence of non-coliform bacteria in the three centres was recorded for *Staphylococcus* species followed by *Bacillus* spp.. The *E.coli* isolates obtained were subjected to antibiotic sensitivity tests. The results revealed that *E.coli* isolates from the three centres exhibited varying levels of resistance to most of the antibiotics tested, particularly, penicillin and ampiclox. The organisms were however sensitive to chloramphenicol and tetracycline with 0.04ug/ml and 0.75ug/ml MIC values respectively. These results suggest that the two drugs can be effective in treating infections caused by *E.coli* and related bacteria.

CHAPTER ONE

1.0   INTRODUCTION

Coliforms are members of the family Enterobacteriaceae and make up approximately 10% of the intestinal microorganisms of human and other animals (Prescott *et al.*, 1990). The coliform group includes *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Citrobacter, Serratia* and *Hafnia*. They are facultatively anaerobic, gram negative, non-sporing, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at $35^{0}C$ (Prescott *et al*, 1990; Singleton, 1999).

Though enteric infections in urban areas populated mainly by humans may derive from man, the source of faecal Coliforms in the environment remains both human and animals. Movement of organisms through the air represents a major pathway for organisms to disperse. Thus, several bacteria diseases are spread through the atmosphere and outbreaks of some microganisms often follow prevailing winds (Ijah and Adekeye, 2000). Carmelita and Tauzon (1984) reported that some bacteria are dispersed by droplet transmission and by the organisms from the nose to the skin and then to the environment. Airborne bacteria may infect patients or people generally by inhalation or settling down directly in some susceptible places. For instance, transmission of coliforms among children and infants is known to be vigorous where hygiene is less than optimal (Feachem *et al.*, 1983). These investigators have also identified most members of the coliform group as the major cause of diarrhoeas especially among children in poor communities with undesirable waste disposal regimes.

Infant nurseries and day-care centres have been implicated as settings for the spread of communicable diseases, especially diarrhoea among young children (Antai and Ogbonna, 1988). Hadler *et al.* (1980) reported that specific agents such as *Shigella, Giardia* and Rotavirus have been associated with diarrheal illnesses in day- care centres.

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Although the cause of most diarrheal cases in these institutions is known, environmental surfaces such as furnitures, toys utensils and building surfaces may serve as the secondary and in some instances the primary routes of transmission of enteric pathogens. Thus, this is one of the focus of the present study. Gastroenteritis due to coliforms particularly the enterotoxigenic strain of faecal coliform have tended to be more endemic because of its habitation of the intestinal tract of man and many other animal species. The problem of coliform- induced diarrhoea has been compounded by the fact that virulence factors of faecal coilforms are plasmid- encoded and may be transmitted to many other members of the Enterobacteriaceae (Prescott *et al.,* 1990). Ijah and Mohammed (2001) reported the isolation of coilforms from environmental surfaces in the labour ward of the Minna General Hospital with a higher prevalence of *E.coli* compared to *Enterobacter aerogenes* found contaminating the toilet, the floor and babys' cot. The high prevalence of *E.coli* on toilet items, baby's cot and floor in the ward suggest that these items may act as vehicles of infection by this organism. *E.coli* has also been incriminated in food poisoning, urinary tract infection (UTI)and hemolytic uraemic syndrome (Ijar and Sar, 1996; Takeda, 1997, Koutkia *et al.,* 1997).

Escherichia coli is a common member of the normal flora of the large intestine. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals (Donnenberg *et al.,* 1993). Strains that acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasive factors become virulent and can cause either a plain, watery diarrhoea or inflammatory dysentery. These diseases are most familiar to Europeans as travellers diarrhoea, but they are also major health problems in endemic countries particularly among infants (Donnenberg *et al.,* 1993., Kawamura, 1997).three groups of *E.coli* are associated with diarrheal disease: they include enterotoxigenic *E.coli* (ETEC)which produces numerous types of enterotoxins, some which are cytotoxic, damaging the mucosal cells, whereas others are merely

cytotonic, inducing only the secretion of water and electrolytes (Spangler, 1992, Tesh and O'Brien, 1992). A second group of *E.coli*, the enteroinvasive strains (EIEC)have invasion factors and cause tissue destruction and inflammation resembling the effects of *Shigella*. A third group of serotypes, called enteropathogenic *E.coli* (EPEC), are associated with outbreaks of diarrohea in newborn nurseries but produce no recognizable toxins or invasion factors (Blanco *et al.*, 1993). *Escherichia coli* 0157:Hz is an important human pathogen associated with haemorrhagic colitis, a syndrome characterized by severe abdominal pain, copious bloody diarrhoea, and little or no fever (Son *et al.*, 1997). There is also a report on person-to- person transmission (Cunin *et al.*, 1999). These investigators reported that the protracted course of the illness, poor sanitation and the length of time patients were infected with pathogens may have favoured transmission. In Japan, an epidemic involving more than 5000 cases was described in school children who ate contaminated food prepared in the school canteen in 1996 (Kawamura, 1997).In the Republic of Central Africa during the first isolation of *E.coli* 0157,food was the suspected vehicle *(Germanii et al.* 1996). The main reservoir of the germ is suspected to be cows and other ruminants (Allerberger *et al.*, 1997), but the association between *E.coli* 0157 and cows is not absolute (Chart, 1998). Transmission from person to person has been observed in a small number of cases, most often intrafamilially (Judwig *et al.*, 1997).

In nursery outbreaks, the main route of transmission is the hands of those nursing infected infants. Baron and Finegold (1999) reported that the hands of personnel, doctors, nurses, ward attendants play an important role in the distribution of microorganisms in hospital environments. These investigators also reported the occurrence of *Klebsiella aerogenes* on the hands of hospital staff and that transmission by hands may be an imporant mechanism by which the spread of this organism occurs in the hospital environment. It seems likely that faecal contamination of the environment,

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fomites, and hands constitute the primary means of transmission and infection among children and adults.

The presence of antibiotic-resistant members of the Enterobacteriaceae in the environment has been a cause for concern in recent years. The ability of these strains to transmit their resistance to other bacteria, mainly through R-plasmids, has been the subject of many studies (Breittmayer and Gauthier, 1990; Mezriou and Echab, 1995; Calva *et al*, 1996; Lamikanra and Okeke, 1997; Hoge *et al.*, 1998; Hart and Kariuki, 1998). Iruka *et al.* (2000) reported the prevalence of resistance to most drugs tested in *E.coli* isolates from apparently healthy Nigerian Students as being within a high range which had increased from 1986 to 1998. The findings by the investigators sound a warning because the indiscriminate use of antibiotics, along with poor hygiene and infection control (risk factors for antibiotic resistance in bacteria) are high in Nigeria and other developing countries. For instance in Mexico, a large water-borne outbreak involving R+plasmid bacteria led to a large number of dealths, due partly to the failure of the patients to respond to antibiotics of choice (Hassani, *et al*, 1999).

Prior to the antibiotics era (1940s) resistant bacteria were rare. With the use of antibiotics in clinical medicine and as animal feed additives, the situation has changed dramatically (Jacoby, 1985). The excellent properties of many antibacterial drugs notwithstanding, the continuing threat posed by the emergence of resistant bacteria emphasizes the need for studies on the antibiogram of bacteria in general and on *Escherichia coli* in particular. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control (O'Brien, 1997). Studies with *E.coli* are of particular relevance because this species can occupy multiple niches, including human and animal hosts (Levy, 1997). In addition, *E.coli* strains efficiently exchange genetic material with pathogens such as *Salmonella, Shigella, Yersinia,* and *Vibrio* species, as well as pathogenic *E.coli* (Tauxe *et al.*, 1989; Levy, 1997).

As the number of working mothers in Nigerian cities continues to increase with a corresponding increase in the numbers of nurseries and day-care centres the need for effective monitoring of the centres cannot be overemphasized.This will enhance the success of any epidemiologic investigation that may be undertaken to determine the extent of spread of antibiotic resistant strains of *Escherichia coli* in particular and coliforms in general and to define risk factors and possible remedial measures.

The objectives of this study were as follows:

1. To determine the prevalence of coliforms particularly *Escherichia coli* in day-care centres in Minna.

2. To isolate and characterize the coliform bacteria particularly *Escherichia coli* on environmental surfaces in day-care centres in Minna.

3. To determine the extent of resistance of the *Escherichia coli* to some antibiotics

4. To determine the Minimum Inhibitory Concentration (MIC) of a antibiotics to *Escherichia coli* isolates.

2.0   **LITERATURE REVIEW**

2.1   MICROBIAL CONTAMINATION OF ENVIRONMENTAL SURFACES

Environmental surfaces such as the floor, building surfaces, furnitures, eating utensils, bathrooms, toilets, skin surfaces, the air, and other objects, have often acted as reservoirs of many different microorganisms most of which are pathogenic (Antai and Ogbonna, 1988; Breittmayer and Gauthier, 1990; Mezrioui and Echab, 1995; Tuttle *et al.*, 1995; Shears, 1996; Kawanura, 1997). It is therefore not unexpected that these surfaces may be involved in the transmission of diseases. A major pathway for microbial dissemination is through aerial suspension. Thus, the transmission of several bacterial diseases may be through the atmosphere as determined by the prevailing weather conditions (Ijah and Adekeye, 2000). It has been reported that microbial transmission may also occur from person to person, often through the hands and most often intra familiarly before finally settling on environmental surfaces (Tuttle *et al.*, 1995; Shears, 1996; Judwig *et al.*, 1997). Oral absorption of pathogenic microorganisms from contaminated surfaces or direct inhalation of suspended bacteria may infect people generally, particularly in environments where several different unhygienic forms of behaviour are associated with the incidence of transmissible pathogens in the environment (Martens *et al.*, 1990; Mitchel, 1991).

Baron and Finegold (1990) have reported that the distribution of microorganisms in the environment maybe through the intimate environment and infected patients. Ijah and Mohammed (2001) reported that the major pathway of environmental contamination is the indirect transmission of the organisms from the nose of the carrier to the various portions of his clothings and beddings which form the final vehicle for the infection of

susceptible persons.The hospital environment offers a good example of the convergence of many potential sources of infection. Cross infections and endemic nosocomial infections are therefore, continual threats in such environments.Talaro and Talaro (1993) reported that infections acquired in hospital environments are common and resist strenous efforts to eliminate them. In most Nigerian hospitals, the labour wards are frequently unhygienic and may habour pathogenic microorganisms that can contaminate the birth canals and facilities used during labour (Ijah and Mohammed, 2001). Medical personnel directly involved in deliveries also offer potential means of transmission of microorganisms, some of which may be pathogenic to susceptible patients that may already be immunocompromised. Ijah and Mohammed (2001) have also reported the role played by the hands of hospital personnel, doctors, nurses and ward attendants in the transmission of microorganisms in the hospital. The investigators are of the opinion that the high nasal carrier state often found among hospital personnel is an important factor in the transmission of infections due to *Staphylococcus aureus* as well as the perpetuation of virulent strains in the hospital environment.

Mitchell (1991) reported the passage of *Klebsiella* spp. from the hands of patients to nurses in an intensive care nursery during simple, routine, "clean" nursing procedures. The investigator further reported the contamination of the hands of nurses by *Klebsiella pneumoniae* and *Escherichia coli* which accounted for 55% of the total isolates. Arduina and Murray (1995) reported that one of the most serious threats of infections due to microbial contamination of environmental surfaces is from our own microbiota as exemplified by serious infections due to *Escherichia coli, Klebsiella preumoniae, Staphylococcus aureaus, Candida albicans, Enterococci, Proteus mirabilis* and some *Actinomycetes*. Antai and Ogbonna (1988) reported the involvement of environmental surfaces such as floors, furniture, building surfaces and children toys in the transmission or transfer of pathogens in day-care centres in Portharcourt, Nigeria. The investigators

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isolated viable faecal coliforms and *Staphylococcus aureus* from the environmental surfaces in day-care centres and infant nurseries. Mitchell (1991) reported the occurrence of *Shigella sonnei* on the hands of about 49% of children following a visit to the toilet for urination. Enteropathogenic strains of *Escherichi coli* have been associated with outbreaks of diarrhoea in the environment of newborn nurseries (Blanco *et al.*, 1993). At the Obafemi Awolowo University Nigeria, Iruka *et al.* (2000) identified possible source of microbial contamination of environmental surfaces as food, water, and person- to-person transfer. The investigators believed that sub- optimal sanitary conditions and overcrowding in student hostels may facilitate the spread of the organisms. Similarly, the investigators observed rapid increases in the prevalence of resistance to drugs among the commensals (such as *Bacillus* spp; *Aeromonas, Streptococcus* spp, *Alcaligenes* e.t.c) found in the sources identified. Not only were these strains potential causes of infection, they are also potential reservoirs of resistant genes that could be transferred to pathogens (Iruka *et al.*, 2000).

Reichler *et al.* (1992) reported the isolation of multiple antibiotic resistant preumococcal organisms among children at a day-care centre, their family members and members of their surrounding community in Cleveland, Ohio. The investigators believed this discovery to be of substantial importance because of the potential of these organisms to cause invasive disease. Resistant strains recovered from similar environments in South Africa have been identified as significant causes of invasive disease (Collet *et al.*, 1991). Studies in other developing countries have shown that the trend in environmental pathogens is torward increasing antibiotic resistance (Hoge *et al.*, 1998). There is therefore a pressing need to monitor commensal organisms as well as pathogens in the environment and pay greater attention to higher sanitary standards in human and animal related environments.

2.2 GENERAL CHARACTERISTICS OF COLIFORMS

The coliforms belong to one of the two major subdivisions of the family Enterobacteriaceae, and like other members of the family are gram- negative, non sporing bacilli, typically 0.3-1x1-6 (m and grow steadily in ordinary laboratory madia under both aerobic and anaerobic conditions (Singleton, 1999). It is also reported that members of the family Enterobacteriacea are chemoorganoheterotrophic, able to grow well in/on basal media, produce gas from dextrose, reduce nitrate to nitrite and are oxidase negative (Adelberg *et al.*, 1980; Singleton 1999). Adelberg *et al.* (1980) reported that all except a few strains are catalase positive, existing as parasites, pathogens or commensals in man and other animals, and as saprotrophs in soil and water. The genera and species of the coliforms are differentiated by biochemical tests such as IMViC (--++) urease and decarboxlase tests. Most Enterobacteriaceae but not *Shigella* produce gas as well as acid from glucose although strains among the family which do not ferment lactose may do so when they acquire a lac plasmid that encodes the uptake and metabolism of lactose (Elliot and Nataro, 1995).

Muhldorfer and Hacker (1994) have identified six genera in the coliform group. These are *Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia* and *Hafnia*. The investigators stated that the bacilli grow at 37^0C and normally possess the enzyme β-galactosidase. Adelberg *et al.* (1980) and Miihldorfer and Hacker (1994) distinguish four distinct types of antigens in the coliform group; these include a thermostable somatic antigen chemically resembling those of the *Salmonella* group. H or flagella antigens, K or surface antigens which when present masks the agglutinability of the organism by O antiserum.

Individual characteristics of members of the coliform group are further examined.

2.2.1 *ESCHERICHIA COLI*

Escherichia coli is a typical coliform organism of the intestinal tract and is present in large numbers in faeces of man and other animals. It is non-capsulated, occurs as a short rod ($0.5 \times 2\text{-}5\mu m$) in size, short coccobacillary and long filamentous forms and occasionally observed on solid media (Taylor, 1995) they occur singly or in pairs and produce relatively large colonies which are usually smooth and glistening. On blood agar, *Escherichia* is usually non-haemolytic, typically motile (peritrichously flagellate) and fimbriate, aerobic and facultatively anaerobic with an optimum cultivation temperature of 37^0C (but with a range of $20 - 440C$), ferments nitrate anaerobically, and glucose via the mixed acid fermentation pathway (Buxton and Fraser, 1977; Adelberg *et al.*, 1980; Singleton 1999).

Escherichia coli is subdivided into numerous stereotypes some of which seem to cause infection in man particularly groups 26, 55, 111, 119, 127 and 128, which are associated with gastroenteritis of infants (Cowan and Steel, 1983.) The investigators have identified *Escherichia coli* as the typical type species of the group that gives negative reactions to citrate utilization, KCN and gelatin hydrolysis. They do not ferment adonitol and inositol acids and give negative voges-proskauer, H2S and urease reactions in addition to gluconate malonate and phenyl alanine.

E. coli gives a negative oxidase reaction and does not form pigment in culture (Elliot and Nataro,1995). The authors reported further that the organism gives a positive catalase reaction, ferments arabinose, lactose, glucose, mannitol and gives a positive O-nitrophlenyl-β-D-galactopyranoside in phosphate solution (O.N.P.G) reaction and has a guanine-cytosine percentage (GC%) of 48-52. IMViC reaction as given by the organism are indole positve,V-P positive, Methyl red negative and citrate negative. Taylor (1995) described the biochemical varieties of *E.coli* (commune and communior) formed on the basis of different sugar reactions be disregarded and upheld the modern view that

lactose-negative strains be acceptable in the group. The investigator reported that water bacteriologists who find *Escherichia* of considerable value in bacteriological examination of water use a classification based on indole and gas production from lactose at 44^0C, and that many strains of *E.coli* are actually non-motile, or only feebly motile on first isolation, and permanently non-motile on the anaerogenic strains.

2.2.2 CITROBACTER

Singleton (1999) described these members of the *Citrobacter* group as showing considerable resemblance to *Escherichia coli*. They reported the genus as including all the lactose-fermenting Bethesda ballerup strains formally classified as paracolon baccilli and occur as intestinal commensals. Cowan and steel (1983) reported that the Common water and soil forms are rapid lactose fermenters and from *Citrobacter freundii* with the less common variety producing indole. The authors reported further that the Ballerup and Bethesda groups are non-lactose or late – lactose fermenters of the *Citrobacter* group by the characteristically foul odor they produce.

Citrobacter are typically gram-negative rods, motile, positive to catalase reaction, H_2S reaction, citrate reaction, KCN reaction and positive O.N.P.G. reaction. Sugars attacked fermentatively by the group include lactose, dulcitol, sucrose and glucose. The organisms do not ferment adonitol, inositol, and give negative indole reaction and gluconate fermentation as well as negative lysine decarboxylate and phenyl alanine reactions. They however produce organic dihydrolase but do not produce pigment in cultures (Swerdlow and Griffin, 1997).

2.2.3 KLEBSIELLA

In contrast to *E. coli*, *Klebsiella* is capsulated non-motile, facultatively anaerobic, attacks sugars fermentatively (with gas production) and is usually shorter and plumper in appearance than *Escherichia* (0.5 to1.5 x 1-2cm). The cells which are characterized by high mucoid colonies on McConkey agar may appear singly or in pairs (usually in short

chains). They occur in soil, water and as parasites or pathogens in man and other animals (Elliot and Nataro, 1995). The investigator described six species in the group; these include: *Klebsiella aerogenes, Klebsiella pneummoniae, Klebsiella edwardsii. Var. edwardsii, Klebsiella edwardsii.var atlantae, Klebsiella ozaenae* and *Klebsiella rhinoscleromatis,* all with a GC% of 53-58. With few variations, *Klebsiella* is catalase, KCN and citrate positive, ferment lactose, glucose, maltose, mannitol, saccharose, inositol and in a few cases dulcitol. The exceptions as given by Cowan and Steel (1983) are *Klebsiella rhinoscleromatis* which is negative to citrate utilization, *Klebsiella pneumoniae,* negative to KCN, *Klebsiella edwardsii.v.edwardsii* and *Klebsiella rhinoscleromatis* negative to glucose fermentation, *K. rhinoscleromatis* negative to lactose fermentation, and the four species of *K.edwardsii.v.edwardsii, K.edwardsii.v.atlantae, K. ozaenae and K. rhinoscleromatis* negative to dulcitol. *Klebsiella* species do not hydrolse gelatin and they give a negative indole reaction, a positive voeges-proskaver reaction except *Klebsiella pneumoniae, K.ozoenae,*and *K. rhinoscleromatis* negative. H_2S reaction, positive urease and lysine decarboxylase reactions except *Klebsiella rhiroscleromatis,* negative arginine dihydrolase, ornithine decarboxylase reactions but a positive gluconate fermentation except *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis.* The others are positve to malonate utilzation except *Klebsiella edwardsii.v.atlantae* and *K. ozaenae* and a positive O.N.P.G. reaction with the exception of *Klebsiella rhinoscleromatis* (Cowan and Steel, 1983).

2.2.4 *ENTEROBACTER*

Adelberg *et al.* (1980) and Cowan and Steel (1983) described the *Enterobacter* as a group of motile organisms with IMViC (--++) reactions respectively. Compared to *E.coli, Enterobacter* has less mucoid growth, is often motile, has small capsules and exists as a commensal in the intestinal tract Muhldorfer and Hacker, (1994). There are three species in the group, some of which produce a non-differentiable yellow pigment.

These are *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Enterobacter liquifaciens* with the biochemical reactions of the latter species varying according to the temperature of growth. For example, about $2/3^{rds}$ of strains of *E.Liquifaciens* are positive to Voges-proskauer in cultures grown at 25^0C and only about 1/3rds are positive in 37^0C culture. The biochemical reactions generally elaborated by the group are negative gram reaction, slow gelatin liquefaction, catalase and oxidase positive reaction and facultative anaerobiosis (Taylor,1995). The *Enterobacter* are typically motile and attack sugar fermentatively (usually with gas production and give positive gluconate, O.N.P.G. and voges-proskauer reactions. They in addition produce ornithine decarboxylase, are all indole, H_2S phenyl alanine negative (Lennette *et al.*, 1985; Singleton, 1999).the organisms do not produce arginine dihydrolase with the exception of *Enterobacter cloacae*. However, they show positive urease reaction except *Enterobacter aerogenes* which gives a negative reaction.

2.2.5 *SERRATIA*

Serratia are free-living, small, gram-negative motile rods that produce a delayed but intense red pigment on culture due to the production of prodigiosin (Taylor, 1995). Singleton (1999) reported the occurrence of the orgnism in soil, water, on plants, in man and other animals. Other characteristics of the organisms are a GC% of 52-60, a negative methyl red reaction,a positive voges-proskauer reaction at 30^0C but a negative reaction at 37^0C, a citrate positive reaction and a variable lactose fermentation reaction according to species. The organism ferments glucose via the Entner-Doudoroff pathway (Singleton, 1999).

2.2.6 *HAFNIA*

Cowan and Steel (1983) described the *Hafnia* group as the non-lactose fermenting counterpart of the *Enterobacter* group. Lennette *et al.* (1985) described the genus *Hafnia*

containing a single species, *Hafnia alvei* and as the most prominent type species in the group. The investigators described organisms classified as *H. alvei* as being previously considered as *Enterobacter hafniae*. Brenner (1984) and Lennette *et al.* (1985) reported that *Hafnia alvei* is characterized by positive reactions for lysine decarboxylase, motility, ornithine decarboxylase, growth in K C N, O.N.P.G, and fermentation of arabinose, mannitol, rhamnose trehalose and xylose *H.alvei* isolates are negative for adonitol, arginine, indole, and urease (Brenner, 1984). Although infrequently encontered compared with other Enterobacteriaceae, isolates of *H. alvei* reportedly may cause a variety of extraintestinal infections (Lennette *et al.*, 1985; Singleton, 1999).

2.3 PATHOGENESIS OF COLIFORM INFECTIONS

Coliforms within the intestines generally do not cause disease and may even contribute to the normal function and body nutrition (Adelberg *et al.*, 1980). These organisms remain benign commensals and become pathogenic only when they reach tissues outside the intestinal tract or when they acquire genetic elements encoding for virulence factors (Donnenberg *et al.*, 1993). Strains that acquire bacteriophage or plasmid-DNA encoding enterotoxins or invasion factors become virulent and cause diseases that range in severity from a plain, watery diarrhoea or an inflammatory dysentery to complicated cases of haemolytic uraemic syndrome (HUS), toxic shock syndrome (TSS) or otitis media (Evans and Evans, 1990).

Coliforms produce numerous types of enterotoxins; Some of these toxins are cytotoxic, damaging the mucosal cells, whereas others are merely cytotonic, inducing only the secretion water and electrolytes (Spangler, 1992). It has also been reported that other groups have invasion factors and cause tissue destruction and inflammation resembling the effects of *Shigella* while some are associated with disease outbreaks in newborn nurseries with sub optimal environmental hygiene standards (Spangler, 1992; Tesh and O'Brien, 1992). Diseases may also be contracted orally by ingestion of food or

water contaminated with pathogenic strains shed by an infected person or by direct inhalation of the aerially suspended organisms. (Tesh and O'Brien, 1992). Coliform-related diseases occur in all age groups, but mortality is most common in infants, particularly in the most undernourished or malnourished infants in developing nations (Tesh and O'Brien, 1992).

The pathogenesis of coliform-related gastro intestinal tract (GIT) infection involves two types of toxins, heat labile (LT) enterotoxin and a heat stable (ST) enterotoxin. The two toxins work in tandem to produce the disease state in a multistage process (Blanco *et al.*, 1993; Chapman *et al.*, 1993). Blanco *et al.* (1993) reported that where the heat stable enterotoxin is involved, there is first the colonization of the intestine, followed by elaboration of the enterotoxin which can then stimulate intestinal guanylate cyclase. The enzyme is able to convert guanosine 5'– triphosphate (GTP) to cyclic guanosine 5'-monophosphate (cGMP) causing an increase in the intracellular cGMP which inhibits intestinal fluid uptake, resulting in net fluid secretion. Heat labile (LT) enterotoxin in composed of two types of sub units, one type of sub unit (the B unit) binds the toxin to the target cells via a specific receptor that has been identified as GM1 ganglioside. The other type of sub unit (the A unit) its then activated by cleavage of a peptide bond and then internalized. It then catalyzes the ADP-ribosylation (transfer of ADP- ribose from nicotinamide adenine dinucleotide [NAD] of a regulatory sub unit of membrane-bond adenylate cyclase, the enzyme that converts ATP to cAMP. This activates the adenylate cyclase, which produces excess intracellular cAMP, which leads to hyper secretion of water and electrolytes (Winneras *et al*, 1992). The enterotoxins are antigenic proteins whose mechanism of action is similar to that of the *Vibrio cholerae* enterotoxin and shares antigenic determinants with cholera toxin and similarity in primary amino acid sequence (Evans and Evans, 1990).

Another factor associated with the pathogenesis of virulent coliforms is the

possession of specialized pili, antigenically unrelated to the common pili which acts as ligands to bind the bacterial cells to specific complex carbohydrate receptors on the epithelial cell surfaces of the small intestine (Giron *et al.*, 1994). The investigators reported that in *Escherichia coli* for example, such interaction results in the formation of colonization factor antigens (CFAs) because of the colonization of the intestine with the enterotoxigenic strains (ETEC) of the organism and subsequent multiplication on the gut surface. Most coliforms produce either CFA/I, CFA/II or CFA/IV whereas CFA/III and an undetermined number of other CFAs occur on other serogroups playing major roles in host specificity (Blanco *et al.*, 1993). Some CFAs are produced by *Escherichia coli* that cause acute diarrhoea in domestic animals (Chapman *et al.*, 1993). The pathogenesis of some coliform serogroups may also be through the intimate binding of epithelial cell surfaces via the adhesive bundle- forming pilus (BFP) (Giron *et al.*, 1991). The investigators reported that the lesion caused by these groups consists mainly of the destruction of the microvilli with no evidence of tissue invasion. Chapman *et al.* (1993) and Dytoc *et al.* (1993) reported that cell damage occurs in two steps, collectively termed attaching and effacing first is intimate contact, sometimes characterized as pedestal formation, second is loss of microrilli which is the result of re-arragement of the host cell cytoskeleton. Some strains though negative for ST and LT enterotoxins however, produce relatively small amounts of a potent shiga-like toxin that has both enterotoxin and cytotoxin activity (Tesh and O'Brien, 1992).

2.4.0 SOME COLIFORM RELATED DISEASES

Coliform-related diseases are varied and include cystitis, food poisoning, conjuctivities, dysentery, bubonic plague, watery diarrhoea, haemorhagic colitis, scarlet fever, haemolytic uraemic syndrome (HUS) toxic shock syndrome (TSS), travellers diarrhoea, pneumonia, gastroenteritis, meningitis, erysipelas and otitis media (Singleton, 1999). Some of these diseases are discussed further.

2.4.1 FOOD POISONING

Takeda (1997) defined food poisoning as an acute gastroenteritis resulting from the ingestion of food contaminated with certain pathogens and or/toxins. Traditionally, the term has also included food-borne cases of botulism. Prescott *et al.* (1990) reported the involvement of *Escherichia coli* in a mixed population of pathogens with the disease; The pathogens include *Shigella* spp. *Salmonella* spp. *Staphylococcus* spp. *Bacillus* spp. *Clostridium* spp. *Listeria monocytogenes. Campylobacter jejuni* and *Yersinia enterolitica.* These pathogens including *E.coli* may be present at the food source or at the point of consumption of the food where cross-contamination may have ensured adequate re-distribution of the pathogens. The pathogens must grow on or in the food to form enough cells or toxin to cause disease although this may not be necessary where rerlatively few cells of some pathogens are needed to initiate the disease (Prescott *et al.,* Singleton, 1999).

Germanii *et al.* (1997) reported that when food poisening involves an infection of the gut, it is referred to as food-borne infection and when it is through the action of toxins, it is known as food-borne intoxication. The investigators described a number of factors that are associated with the risk of food posening; these are the initial level of contamination on/in the food, the type of processing, storage and distribution as well as the preparations involved in its production and the dose/response relationship, particularly the smallest dose able to cause disease. Other factors reported by the investigators are the variation of pathogen susceptibility with age, acquired immunity, state of health, the existence of special risk groups in which the risk is greater and the disease may be far more serious which include infants, the very old, pregnant women and the immunocompromised (e.g. AIDS patients).

Prescott *et al.* (1990) reported two principal mechanisms of gastrointestinal tract disease resulting from food poisening; these are colonization and growth within the

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gastrointestinal tract (GIT), where the bacteria may invade the tissue of the host or secrete exotoxins, a process that requires the presence of reproducing bacteria and the secretion of an exotoxin that contaminates food and then is ingested by the host. The latter process does not involve the presence of living bacteria (Tuttle *et al.*, 1995). Cunin *et al.* (1991) reported cases of food poisening in the Northern hemisphere following an epidemic that involved 5000 cases described in infants that had ingested contaminated commercially prepared food. Sources of contamination identified by the investigators included meat in hamburgers, sandwiches, milk, drinking water, water absorbed during baths, non-pasteurized apple juice and salads. In a small number of cases reported by Judwig *et al.* (1997), cross-contamination had tended to favour intrafamilial transmission of the disease. This is particularly true of day-care centres and neonatal health institutions where negative sanitary conditions sustained for long periods and the tendency of children to ingest dirty, contaminated materials during periods of neglect by their handlers or nurses prevails continuously (Mitchel, 1991).

The most frequently incriminated *E.coli* serotype in food poisening aetiology is *E.coli*.0157: Hz and its presence in a mixed population with other causal agents of food poisening or intoxication provides an avenue for conjugative transposition of pathogenicity or the enhancement of an existing one (Cunin *et al.*, 1999). The implication of such a possibility is the acquisition by commensal *E.coli* of the abilities to elaborate the toxins of pathogens with which they share basic biochemical characteristics or the enhancement of the pathogencity of virulent strains of *E.coli* through the strengthening of colicin V production in addition to foreign acquired virulence factors (Chopra *et al.*, 1997). The initiation and establishment of the infection usually follows ingestion of the pathogen or toxin in contaminated food via the oral-faecal route (Prescott *et al.*, 1990). Takeda (1997) reported common symptoms of food poisoning to include acute gastroentiritis, abdominal pains, and discomfort, little or no diarrhoea and vomiting.

2.4.2 DYSENTERY (INFLAMMATORY DIARRHOEA)

A number of reports have implicated several *E.coli* serotypes together with *Shigella* spp.as the causative agents of dysentery (Lennette *et al.*, 1985; Cossart and Kocks,1994). Specifically mentioned were enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli* (EIEC) and *Shigella dysenteriae*. Lennette *et al.* (1985) reported that members of the genus *Shigella* have long been recognzied as causative agents for bacillary dysentery and incriminated these organism in the aetiology of gastro intestinal tract infections but very rarely other types of infections. Dysentery is the clinical condition usually brought about by the combined action of the Shiga- toxin and *Shigella*-like verotoxins elaborated by the etiologic agents of the disease (Singleton, 1999). Infant nurseries with low hygiene status, poorly kept and congested homesteads serve as reservoirs of the pathogens responsible for inflammatory diarrhoea. Shigellosis (an alternative term for dysentery) is reported to be one of the main causes of severe diarrhoea in Africa, accounting for 12% of all deaths in the Kibue sector in Burundi in 1992 and 19% of paediatric hospital deaths in Kwazulu-Natal in 1995 (Birmingham *et al.*, 1997; Chopra *et al.*, 1997).

Tesh and O'brien (1992) reported the transmission of dysentery via the faecal-oral route followed by the rapid colonization of the ileal mucosa by the pathogens' pili (or fimbriae). There are other reports (Seriwatana *et al.*,1983 Singleton 1999) that the eneterotoxins formed by some strains of enterohaemorrhagic *E.coli* and *Shigella dysenteriae* repectively have similar mechanism; they bind to sites in the gut and are taken up by receptor- mediated endocytosis. Singleton (1999) is of the view that the verotoxins apparently affect protein synthesis and inhibit NaCl absorption although the mechanism responsible for fluid loss in dysentery remains unclear but may involve tissue damage / inflammation ,with the toxin serving to exacerbate the sympoms by causing vascular damage and promoting the formation of bloody stools. Cytotonic enterotoxins,

encoded on plasmid or bacteriophage DNA, can induce the clinical state of the disease while plasmid- encoded invasion factors permit invasion of the mucosa, and plasmid- or bacteriohage-encoded cytotoxic enterotoxins induce tissue damage (Tesh and O'brien, 1992). The investigators reported that the presence of either of these factors can induce a host inflammatory reaction with an influx of lymphocytes and resulting dysentery.

Winneras *et al.* (1992) reported that the infection is most common where saintation is poor with both infants and susceptible adults in developing countries classified in the particularly high risk groups.The investigators observed that the disease is most serious in infants.

Gastric acid and intestinal transit time are important host defenses: specific intestinal immunoglobulin A (IgA) developes and appears to be protective (Evans and Evans, 1990; Dytoc *et al .,* 1993).

2.4.3 WATERY DIARRHOEA (NON INFLAMMATORY DIARRHOEA)

Depending on the virulence factor they possess, strains of virulent *Enterobacter aerogenes, Escherichia coli* and *Yersinia enterolitica* can cause non-inflammatory or watery diarrhoea (Lennette *et al.,* 1985). Strains that acquire bacteriophage or plasmid DNA encoding enterotoxins or invasion factor become virulent and can causes the plain, watery diarrhoea most familiar to Western Europeans as travellers diarrhoea (Evans and Evans, 1990). Spangler (1992) and Tesh O'Brien (1992) have identified three groups of *E.coli* strains that produce invasive factor (EIEC)and the enteropathgenic *E.coli* (EPEC).Prescott *et al.* (1990) reported the incrimination of these organisms and Several members of the Enterobacteriaceae in serious outbreaks of diarrhoea in newborn nurseries.

The initiation of the disease is usually at the superficial epithelial cell surface of the colon where microbial adherence is rapidly followed by invasion of the epithelial

cells which induces a non- inflammatory response and subsequent tissue damage (Aragon et al., 1995; Koutkia et al., 1997). Cunin et al. (1999) reported that the transmission in children which is via the faecal- oral route induces diarrhoea following ileal mucosal attachment and cytotonic enterotoxin production. The pathogenic strains of the diarrheal pathogens contain virulence plasmids that code for special cell wall antigens and other factors enabling them to enter and destroy the epithelial cells (Prescott et al., 1990). The investigators reported that when invasive factors are bacteriophage encoded, cytotoxic enterotoxins induce a host non-inflammatory reaction with an influx of lymphocytes and resulting diarrhoea. They are of the opinion that this is of special significance in the severity of the infection in newborn babies.

2.4.4 OTITIS MEDIA

Otitis media is described as an inflammatory reaction of the lining mucous membrane of the middle ear cleft or part of its extent from the Eustachian tube to the mastoid antrum and their cells (Birrel et al., 1977). The authors classified the disease as being generally related to the extent and degree of the inflammatory reaction in which there is either no formation of pus in the middle ear (non -suppurative otitis media) or one in which there is exudation of pus (suppurative otitis media).

Strains of pathogenic *Escherichia coli, Streptococcus Pneumoniae* are among the variously reported etiologic agents of otitis media in addition to *Staphylococcus aureus, Streptococcus haemolyticus* and *Haemophilus influenzae* (Birrel, et al., 1979; Lennette et al., 1985; Reichler et al., 1992). The severity of infection by these agents is enhanced by the production of hyaluronate lyase (a spreading factor) which is an enzyme that cleaves hyaluronic acid, a component of the intercellular cement in animal tissues and assists bacterial penetration of infected sites (Singleton, 1999).

Acute non-suppurative otitis media, characterized by a non-purulent diffusion in the middle ear, commonly follows Eustachian tube obstruction, and the predisposition of

this condition among children is related in part to their susceptibility to respiratory pathogens in close contact areas such as day-care centres (Collet *et al.*, 1991). The symptons of non-suppurative otitis media in children include earache that is associated with Eustachian tube obstruction and deafness, autophony and tinnitus which is sometimes present and may be a troublesome aftermath in cases involving barotrauma (Reichler *et al.*, 1992).

Acute suppurative otitis media is described as a common disease of childhood occuring frequently as a result of acute upper respiratory tract infection of viral origin (Lennette *et al.*, 1985; McCracken and Nelson, 1990). The disease manifests clinically as an acute inflammation of the middle ear cleft with pus formation as a result of the invasion of the mucoperoisteal lining by pyogenic organisms (Teele *et al.*, 1990). The investigators reported that in children, pain is a variable sympton at the early stage of the infection with the patient complaining more of fullness in the ear, dullness of hearing and excessive loudness of the patient's own voice in the affected ear and sometimes high-pitched tinnitus. As the inflammatory reaction spreads from the Eustachian tube to involve the typmpanic cavity,resulting in increasing vascular dilation and tension, pain becomes the most prominent symptom and deafness more pronounced.Ear ache becomes sharp, throbbing or lancinating which is initially confined to the depths of the ear but later radiates over the affected sites of the head and is intensified by any activity which increases intra tympanic pressure (Birrel *et al.*, 1977). Generalized symptoms occurring in children include persistent restlessness, fever (with temperature running up to 39^0C), thirst, vomitting, rubbing of the affected ear, boring into the pillow on the affected side and sudden wakening and screaming with pain (Blusestone *et al.*, 1983).

In chronic suppurative otitis media, the suppuration of the middle ear results from infection of the mucosal lining of the middle ear cleft which in the vast majority of neonatal cases, had been preceeded by an acute suppuration that had either been untreated

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or inadequately treated (Stool and Field,1989). Birrel *et al.*, (1977) reported the principal symptoms as including chronic purulent discharge from the ear, perforation of the tympanic membrane and deafness The advanced stages of otitis media in children has been correllated in some studies with delays in speech, language and cognitive development and in some severe cases had usually led to hearing loss and intracranial suppurative sequelae (Reichler *et al.*, 1992).

2.5   ANTIBIOTIC RESISTANCE IN COLIFORMS

Almost every major resistance mechanism has been described in coliforms or other Enterobacteriaceae. However, the prevalence and significance varies with time, location and type of infection (Bryan, 1982). Outbreaks of infection with multiple-resistance strains have been a major problem with many of the bacteria (Levine, 1997). This has been particularly so with *Klebsiella, Serratia, Proteus, Providencia* and *Enterobacter* isolates (Hassani *et al.*, 1999). Prior to the antibiotic era, resistant bacteria were rare. With the use of antibiotics in clinical medicine and as animal feed additives, the situation has changed dramatically (Jacoby, 1985). Bacteria appear to have known how to coexist with antibiotics for a long time. Spores of penicillinase-producing *Bacillus* spp. have been found to attach to plant specimens collected in the seventeenth century and a strain of *Escherichia coli* carrying a plasmid-determining resistance to streptomycin and tetracycline has been recovered from a culture stored in the 1930s before these antibiotics were discovered (Johnson, 1991). Basset *et al.*, (1980) reported that antibiotics as natural products accumulated in some environments in sufficient quantity to act as selective agents.

Attempts in the past to understand how resistance arose focused on mutation; Plasmids as transmissible agents of resistance were discovered in Japan in 1959, and by the 1960s were accepted as the major cause of antibiotic resistance in clinical isolates (Jacoby, 1985). Infection outbreaks often involve the spread of single multiple-resistant

strains through the environment. However, outbreaks can involve more than a single strain or species or involve both strain and plasmid spread (Mezrioui and Echab, 1995). The discovery that individual resistance genes on plasmids were transposable added new impetus to the study of plasmids and their epidemiology (Harjai et al., 1996). Coliform bacteria show high levels of antibotic resistance which is usually carried by plasmids called R factors (Bell et al., 1983). These organisms have the potential to transfer their resistance to pathogenic bacteria resulting in reduced efficacy of antimicobal chemotherapy in the event of an infection (Hassani et al., 1999). The investigators reported that in Mexico for instance, a large water- borne outbreak involving R- plasmid bacteria (R^+) led to a large number of deaths, due partly to the failure of the patients to respond to antibiotics of choice.

In general, emergence of resistance problems among coliforms requires a resistant strain usually due to resistance plasmids, selective pressure of antibiotic use, a strain capable of colonization and some virulence and a group of susceptible patients (Lamikanra and Okeke, 1997; Hart and Kariuki, 1998). Situations where these factors may be operative include intensive care, urology, burn and neurosurgical units as well as neonatal institutions (Bryan, 1982; Iruka et al., 2000). Increased resistance to virtually any antibiotic can be achieved by successive transfer into increasing sub-inhibtory concentrations of the drug. Such derivatives generally contain multiple mutations, each providing in increment in resistance (Jacoby, 1985). With some agents such as streptomycin or rifampicin, a high level of an alteration in the target site to which the antibiotic binds, respectively the 30s sub unit of the ribosomes or DNA-dependent RNA polymerase (Greenwood and O'Grady, 1985).

Several coliforms and other members of the Enterobacteriaceae are intrinsically resistant to drugs frequently active on gram-negative bacteria (Flemming et al., 1988),for example Enterobacter spp, are often resistant to cefazolin and cephalothin in part due to a

cephalosporinase; *Klebsiella Pneumoniae* often show low-level ampicillin resistance, *Proteus* spp. are resistant to polymyxin; *Providencia* are resistant to cephalothin (Bryan, 1982, Hart and Kariuki, 1998). Hatha *et al.* (1993) observed rapid increases in the prevalence of resistance in commensal Enterobacteriaceae to most of the older, less expensive antimicrobial drugs used in the management of infections in developing countries.

The antibiotic susceptibility patterns of coliforms producing heat labile (LT) or heat stable (ST) or both toxins have varied from different geographical locations. Iruka *et al.* (2000) reported a high increase in the prevalence of resistance to most drugs tested on *E.coli* isolates from apparently healthy Nigerian students from 1980 to 1998. However, a previous report by Bryan (1982) stated that the prevalence of antibiotic resistance in toxigenic *E.coli* isolated was not significant although this contrasted sharply with toxigenic strains recovered during the same period from the far east which showed a high degree of multiple-antibiotic resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracyclines and cephaloridine.

Klebsiella Pneumoniae is an important cause of respiratory tract and urinary tract infections and bacteriamias in young adults and children. In many instances, multiple resistant strains possessing plasmids produce these infections following initial intestinal colonization (Bryan, 1982). However, index cases initiating outbreaks may be individuals within a susceptible population with overt. Infection. Outbreaks of gentamicin-resistant *Klebsiella* spp. have been relatively common. Such resistance is usually due to R-factor specified enzymes which confers transmissibility of resistance to other drugs such as penicillins, cephalosporins, chloramphenicol and sulfonamides (platt *et al.*, 1984).

Serratia Marcescens is frequently involved in outbreaks similar to *Klebsiella.* This is proven by studies that have shown that *S.marcescens, Klebsiella* spp, and *Enterobacter aerogenes* isolated from one such outbreak as possessing an identical R-

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plasmid specifying aminoglycoside and β-lactam resistance which had spread among the strains (Bergstrom *et al.*, 1982). Other investigations have shown that such 'plasmid epidemics' can involve various Enterbacteriaceae including *E.coli* and non-Enterobacteriaceae such as *Citrobacter, Morganella* and *Acinetobacter* (Richaume *et al.*, 1992). These organisms represent a mixture of intrinsic resistance and plasmid-mediated resistance (Levy, *et al.*, 1988).

Several of the Enterobacteriaceae are intrinsically resistant to drugs frequently active on gram-negative bacteria (Spratt, 1983) *Enterobacter, Escherichia coli, Klebsiella* spp., *Proteus, Salmonella, Serratia* and *Shigella* carry R Plasmids that enable and sustain transmissibility of resistance within the group (Datta and Hughes, 1983). Plasmid-determined resistance when amplified by gene duplication, could lead to the production of higher levels of resistance or it can be carried on a segment of DNA that can transpose from one replicon to another, allowing greater flexibility in resistance dissemination (Flemming *et al.*, 1988). In addition, resistance to multiple antibiotics can be packaged on a single plasmid having a very broad host range (Hensche *et al.*, 1991). Such plasmids can act as 'shuttle' vectors permitting the flow of resistance determinants to organisms within their host range (Ryder, 1994).

Levin *et al.* (1997) reported that ingestion of antibiotics provides selective pressure which ultimately leads to a higher prevalence of resistance bacteria even among persons who have not taken antibiotics. The sources of such resistance organisms may include food, water, and person-to-person transfer (Iruka *et al.*, 2000). The investigators observed that sub-optimal sanitary conditions and overcrowding may facilitate the spread of these organisms. Such conditions have been known to persist in some child day-care centres (Reichler *et al.*, 1992).

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Media and reagents used:

Standard media and reagents were used in the present study.

3.2   Description of study areas:

The study areas were New Secretariat Day-Care Centre (NDC), Unguwan –daji Day-care centre (UDC) and Government Day-care Centre (GDC), all in Minna, Niger State. The NDC located within Abdulkareem Lafene New Secretariat in Tunga area of Minna, was established in 1998. It is made up of an office for the staff supervisor and a larger room for the children. The UDC is located in Unguwan-daji area, a short distance from the Minna Central Mosque. It was established in 1999 and shares a smiliar arrangement with the NDC. It has an average children population of 12. The GDC is situated in the central area of 1-2-3-quarters about a hundred yards from the Islamic Education trust (IET),Minna, The centre was established in 1981, and it is made up of two inner rooms in addition and a to larger one for the children with an average children population of 10.The NDC and the UDC are cleaned about 2-3 times a week while the GDC gets a daily cleaning depending on the level of accumulated dirt. With the exception of the GDC that is cleaned with a combination of disinfectants and detergents the NDC and the UDC are cleaned with either of the two depending on availability.

3.3   Collection and processing of samples:

Six hundred and ninety-three (693) samples were obtained from seven categories of environmental surfaces in the three day-care centres. The environmental surfaces sampled were wall, furnitures, door handles, toys, floor, utensils and toilet seats. The samples were collected by rubbing sterile swabs on the surfaces. Sampling periods ranged from mid-morning to late afternoon hours of each sampling day. The swabs were

inserted into 5ml sterile lactose broth medium and transported to the laboratory where they were incubated for 18-24 hours before analysis. The primary incubation was to enhance the growth of any organism(s) present on the swab and to dilute the effect of residual disinfectant the surface, although it was not possible to determine how and when each surface was last cleaned. Two hundred and thirty one (231) samples were collected from each centre over a six-month (mid-November 2000-early may, 2001) sampling period. Staff of the three day care centres were not informed of the day or week that sampling would take place so that day to day conditions in the centres would be preserved at the time of sampling.

3.4   Isolation of *Escherichia coli* and other coliforms:

Escherichia coli and other coliforms were isolated using the technique of Peterson *et al.*, (1970) Sterile swabs were inoculated into Lactose fermentation broth (LFB) and incubated for 18-24 hours at 37^0C (for quick lactose fermenters) and 18-48 hours (for late lactose fermenters). Production of gas was considered presumptive evidence of coliform bacteria (American Public Health Association, APHA, 1975). Sterile wire loop was used to inoculate Endo agar from the gas positive tubes which were then incubated at 37^0C for 24 hours. Suspected coliform colonies were transferred onto Eosin methylene Blue (EMB) agar, incubated overnight at 37^0C and observed for pinkish purple pigmentation with a metallic sheen (a characteristic of *E.coli*). Presumptive *E.coli* isolates were sub-cultured repeatedly on fresh EMB agar to obtain pure isolates. Colonies of other suspected coliforms were similarly sub-cultured on fresh media (MacConkey and Blood agar) to obtain pure isolates. Isolated *E. coli* cells and other coliforms were maintained on EMB slants in McCartney bottles and stored in refrigerator (Thermocool 400, thermocool Engineering Company Limited, Ikeja, Nigeria) at 4^0C for further characterization and identification.

3.5 Characterization and identification of bacterial isolates:

The bacterial isolates were characterized based on gram staining, colonial morphology and biochemical tests. The biochemical tests carried out included production of catalase, coagulase, urease, citrate utilization, indole, nitrate reduction, methyl red, voges proskauer and sugar fermentation. Bacterial isolates were identified by comparing their characteristics with those of known taxa using the scheme of Buchanan and Gibbons (1974) and Lennette et al., (1985).

3.6 Antibiotic Susceptibility testing:

Escherichia coli isolates obtained from each of the three day-care centres were tested for their susceptibility or resistance to eight antibiotics commonly used for the treatment of human infections. The antibiotics tested, (Standard concentrations for disc diffusion test) were: Nalidixic acid (Na: 30 (µg/ml, Gentamicin (GM: 10(µg/ml), Chloramphenicol (Chl: 30(µg/ml), Erythromycin (Ert: 20 (µg/ml) Tetracycline (TC:30(µg/ml), Sulphamethoxazole-trimethroprim (Tpm-smx): 25(µg/ml), Penicillin (pn:300(µg/ml),and Ampiclox (Apc:25(µg/ml): Susceptibility or resistance was determined using the disc diffusion method of (Bauer et al., 1966).

Each test organism was inoculated in nutrient broth and incubated at 37^0C for four hours to ensure that the organism was at the logarithmic phase of growth. It was then inoculated on EMB agar using sterile swab to ensure near confluent growth on incubation. Before incubation eight filter paper discs, each impregnated with different antibiotics were placed at different locations on the inoculated medium. The plates were incubated at 37^0C for 24 hours to ensure maximum diffusion of antibiotic from each disc. A control plate containing empty paper discs was similarly prepared for means of comparison. After incubation, the diameters of the zone of inhibition was measured and recorded. Antimicrobial activity was expressed as the average diameter of the zone inhibition calculated as the difference in diameter of the disc and the cleared zone around

the disc. Zones of inhibition less than or equal to 14mm (< 14mm) for the least susceptible isolate were regarded as resistant while values above 14mm were regarded as susceptible. Zones of inhibition greater than 14mm (or equal to or higher than intermediate values) were regarded as susceptible (National Committee for Clinical Laboratory Standards, NCCLS, 1988).

3.7 Determination of the minimum inhibitory concentration (MIC):

The same number and type of antibiotics used for the disc diffusion test were used in the MIC studies for each *E.coli* isolate: Standard initial concentrations were; Nalidixic acid (Na:500mg), Gentamicin (Gm: 80mg), Chloramphenicol (Chl:250mg), Erythromycin (Ert:250mg), Tetracycline (Tc:250mg), Sulphamethoxazole-trimethroprim(Tpm-smx:480mg),Penicillin (Pn:4000,000 1.V) and Ampiclox (Apc:250mg).

Two milliliters (2ml) of nutrient broth was dispensed in each of a set of test tubes, by autoclaving at 121^0C and allowed to cool to 45^0C. To each set of test tubes, test antibiotics were added by serially diluting from their standard initial concentrations beyond their lowest achievable concentration in blood and serum. A loopful of test organisms previously adjusted to a concentration of 10^7 cells/ml was introduced. The test tubes were incubated at 37^0C for 24 hours.

For each of the *E.coli* isolate and standard antibiotic, the same procedure was followed. Another set of test tubes containing broth only were seeded with the test organism (as described above) for comparison. After overnight incubation at 37^0C, the highest dilution of the antibiotic that prevented visible growth of the test isolates was taken as the minimum inhibitory concentration (MIC) of the antibiotic for that strain (Lennette *et al.*, 1985; Baron and Finegold, 1990).

3.8 Statistical Analysis:

Data generated were assessed using analysis of variance (ANOVA) for the treatments. Tukey's test (Steele and Torie, 1968) was used for means comparison. Multi-factor analysis of variance was used to evaluate variation due to the factors (prevalence, sample site, and susceptibility of isolates and potency of test antibiotics) and their interactions. Probability level was maintained at 0.05 (95% confidence limits) and was used for the significant test of the variations. Percentage data were transformed by arc-sin transformation according to Zar (1984). Statistical software statgraphic (version 5.0)was used for the analysis.

CHAPTER FOUR

RESULTS

4.1 Occurrence of coliforms on environmental surface in Day-care Centres in Minna:

A total of 693 samples were collected and 95 samples (representing 13.7%) were positive for coliforms while 598 samples (representing 86.3%) were not positive for coliforms in three day- care centres in minna (Table 1). New secretariat day- care center (NDC) had the highest number (48.4%) of environmental surfaces that were positive for coliforms followed by unguwan-daji day- care center (30.5%) while Government day-care center (GDC) had 21.1% of the environmental surface that haboured coliforms.

4.2 Characterization and Identification of bacteria:

The coliforms isolated from the various environmental surfaces were identified as *Escherichia coli, Enterobacter aerogenes, Klebsiella* spp, and *Serratia* spp. (Table 2). The non- coliforms were similarly characterized and identified as *Shigella* spp. *Bacillus* spp. *Staphyloccus* spp. *Lactobacillus* spp. *Diploccus* spp. *Streptococcus* spp. *Salmonella* spp. and *Proteus* spp.(Table3).

4.3 Prevalence of coliforms on environmental surfaces in day- care Centres:

The results (Table 4) revealed that, at the New Secretariat day- care center (NDC), *Enterobacter aerogenres* had the highest prevalence rate (39.1%) followed by *E. coli* (26.1%) while *serratia* spp. had the lowest prevalence rate (17.4%). *Klebsiella* spp. had a prevalence rate of 19.6%. It was observed that the walls were highly contaminated with the organisms followed by utensils while the least contaminated environmental surfaces were the toys (Table 4).

For Unguwan- daji day- care Centre (UDC), *E. coli* was more prevalent (31.6%)

followed by *E. aerogenes* (24.1%). *Klebsiella* spp. and *Serratia* spp. had prevalence rates of 20.7% and 13.8% respectively (Table 5). Of the environmental surfaces sampled, toys were highly contaminated followed by utensils and door handles (Table 5). The extent of bacterial contamination of floor, furniture, toilet seat and wall was comparable.

The results (Table 6) showed that *E.coli* was the predominant bacterial contaminant on the environmental surfaces in the Government day- care Centre (GDC) followed by *E.aerogones*, *Klebsiella* spp. and *Serratia* spp. in that order. On site- specificity, furnitiures and children toys had the highest (25.0%) contamination rate followed by floor (20.0%). Door handles and utensils respectively had contamination rates of 10.0% and 5.0% while no coliforms were recovered from toilet seat (Table 6).

Statistical analysis of data collected on the prevalence of organisms and sample site showed that there were significant differences at 5% probability level between *E.erogenes Klebsiella* spp., and *Serratia* spp. at the NDC (Appendix 1) but no significant difference existed for *Escherichia coli* at the same probability level(P< 0.05). This was similar for data generated from the UDC (Appendix 2). At the GDC however, analysis of data showed significant differences in terms of prevalence of organisms but to a less extent in terms of sample site (Appendix 3).

TABLE 1: Occurrence of coliforms on environmental surfaces in 3 day-care centres.

Day-care centre	Number of samples		
	Collected	Positive for coliforms	Positive for non coliforms
NDC	231	46(48.4)	185(30.9)
UDC	231	29(30.5)	202(33.8)
GDC	231	20(21.1)	211(35.3)
Total	693	95(13.7)	598(86.3)

Number in parenthesis represents percentage sample Positive for Coliforms.

NDC: New Secretariat Day-care Centre

UDC: Unguwan-daji Day-care Centre

GDC: Government Day-care Centre

Table 2:Characterization and identification of coliforms isolated from environmental
        surfaces in day - care centres.

Organism	Gram Reaction	Methyl Red (M-R)	Voges Proskauer	Indole	Nitrate Reduction	Citrate Utilization	Catalase Reaction	Sugar fermentation					
								Lactose	Glucose	Arabinose	Mannitol	Galactose	Sucrose
Escherichia coli	-Rods	+	_	+	+	_	+	AG	AG	AG	AG	A	AG
Enterobacter Aerogenes	-Rods	_	+	_	+	+	+	AG	AG	AG	AG	AG	AG
Klebsiella Spp.	-Rods	_	+	+	+	+	+	AG	AG	A	AG	A	AG
Serratia Spp.	-Rods	_	+	_	+	+	+	AG	AG	AG	AG	AG	AG

+: Positive, -: Negative, A: Acid, AG: Acid and Gas

Table 3: Characterization and identification of coliforms isolated from environmental surfaces in day – care centres.

Organism	Gram Reaction	Catalase Reaction	Citrate Utilization	Nitrate Reduction	Indole	Presence of spores	Sugar fermentation					
							Lactose	Glucose	Arabinose	Mannitol	Galactose	Sucrose
Shigella spp.	-Rods	+	-	+	-	NA	A	A	AG	AG	A G	A
Bacillus spp.	+-Rods	+	+	-	-	+	A	A	A	AG	AG	AG
Staphyloccus spp.	+Cocci	+	-	+	-	NA	A	A	A	A	A	A
Lactobacillus spp.	+Rods	-	-	+	-	-	A	AG	A	A	AG	AG
Diplococcus spp.	-Cocci	-	-	+	-	NA	AG	A	A	A	A	A
Streptococcus spp.	+-Cocci	-	-	+	-	NA	A	AG	A	A	A	AG
Salmonella spp.	-Rods	+	+	+	-	NA	A	A	A	AG	A	A
Proteus spp.	-Rods	+	-	+	-	NA	A	AG	A	A	AG	AG

+: Positive, -: Negative, A: Acid, AG: Acid and Gas, NA: Not Applicable

TABLE 4: Prevalence coliforms on environmental surfaces in New Secretariat Day-care Centre (NDC), Minna.

Environmental Surfaces	Number of isolates obtained				
	E.coli	*Enterobacter aerogenes*	*Klebsiella* spp.	*Serratia* spp	Total
Wall	2(4.3)	7(15.2)	2(4.3)	1(2.2)	12(26.1)
Furnitures	5(10.7)	0(0.0)	2(4.3)	0(0.0)	7(15.2)
Door handle	1(2.2)	4(8.7)	1(2.2)	0(0.0)	7(15.2)
Toys	0(0.0)	0(0.0)	2(4.3)	0.(0.0)	2(4.3)
Floor	1(2.2)	0(0.0)	0(0.0)	5(10.7)	6(13.0)
Utensils	2(4.3)	0(0.0)	1(2.2)	0(0.0)	8(17.4)
Toilet seat	2(4.3)	0(0.0)	1(2.2)	1(2.2)	4(8.7)
Total	12(26.1)	18(39.1)	9(19.6)	8(17.4)	46(100.0)

Number in parenthesis represents percentage sample positive for *E.coli* and other Coliforms.

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TABLE 5: Prevalence of *E.coli* and other coliforms on environmental surfaces in Unguwan-daji Day-care Centre (UDC), Minna.

Environmental Surfaces	Number of isolates obtained				
	E.coli	*Enterobacter aerogenes*	*Klebsiella* spp.	*Serratia* spp.	Total
Wall	0(0.0)	2(6.9)	1(3.1)	0(0.0)	3(10.3)
Furnitures	0(0.0)	2(6.9)	0(0.0)	0(0.0)	2(6.9)
Door handle	2(6.9)	0(0.0)	1(3.1)	1(3.1)	4(13.8)
Toys	7(24.1)	0(0.0)	2(6.9)	0(0.0)	9(31.0)
Floor	0(0.0)	0(0.0)	0(0.0)	2(6.9)	2(6.9)
Utensils	2(6.9)	1(3.1)	2(6.9)	1(3.1)	6(20.7)
Toilet seat	0(0.0)	2(6.9)	0(0.0)	0(0.0)	2(6.9)
Total	9(31.0)	7(24.1)	6(20.7)	4(13.8)	29(100.0)

Number in parenthesis represents percentage sample positive for *E.coli* and other Coliforms.

TABLE 6: Prevalence of *E.coli* and other coliforms on environmental surfaces in Government Day-care Centre (GDC), Minna.

Environmental Surfaces	Number of isolates obtained				
	E.coli	*Enterobacter aerogenes*	*Klebsiella* spp.	*Serratia* spp.	*Total*
Wall	0(0.0)	2(10.0)	1(5.0)	0(0.0)	3(15.0)
Furnitures	0(0.0)	3(15.0)	1(5.0)	1(5.0)	5(25.0)
Door handle	2(10.0)	0(0.0)	0(0.0)	0(0.0)	2(10.0)
Toys	5(25.0)	0(0.0)	0(0.0)	0(0.0)	5(25.0)
Floor	4(20.0)	0(0.0)	0(0.0)	0(0.0)	4(20.0)
Utensils	1(5.0)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
Toilet seat	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	**12(60.0)**	**5(25.0)**	**2(10.0)**	**1(5.0)**	**20(100.0)**

Number in parenthesis represents percentage sample positive for *E.coli* and other Coliforms.

4.4 Prevalence of non-coliforms on environmental surfaces in a day-care centres:

Of the non-coliform bacteria isolated from the environmental surfaces in NDC, *Staphylococcus* recorded the highest prevalence rate (41.6%) followed by *Bacillus* spp. (26.5%). The least (1.1%) prevalent of the organism on the environmental surfaces was *Salmonella* spp. while *Shigella* spp. *Lactobacillus* spp. *Diplococcus* spp., and *Streptococcus* spp had the prevalence rates % 11.9.10, 4.7%, 10.3% and 3.0% respectively (Table 7). Site-specific prevalence showed furnitures with 18.4%, toilet seat and wall with 16.8% and 8.6% respectively (Table 7). The organisms occurred on the door handle and floor to the same extent (14.6%).

Table 8 shows the prevalence of non-coliforms one environmental surfaces in UDC.The results revealed that *Staphylococcus* spp. were more predominantly encountered while Proteus spp. were least predominant (1.0%) on the environmental surfaces. *Diplococcus* spp., and *Streptococcus* spp. had the same prevalence rate of (2.3%). *Bacillius* spp., *Lactobacillus* spp., *Shigella* spp., and *Salmonella* spp.. Similarly occurred on the environmental surfaces sampled (Table 8). It was observed that the organisms occurred highly on furnitures (19.3%), the floor (16.3%) and utensils (15.8%) (Table 8).

Staphylococcus spp. and *Bacillus* spp. were more prevalent on the environmental surfaces at GDC than other non-coliform bacteria *(Lactobacillus, Diplococcus, Shigella, Streptococcus, Salmonella, Proteus)* isolated (Table 9). The results (Table 9) revealed that prevalence rates of the organisms on the environmental surfaces ranged between 13.7% and 19.9%. The highest value (19.9%) being obtained for door handle (Table 9).

4.5 Resistance of *Escherichia coli* to antibiotics:

The number and percentage of resistant isolates to test antibiotics are presented in Table 10. All *E.coli* isolates obtained at the NDC were resistant to penicillin and

ampiclox while 27.3% of the isolates were resistant to gentamicin, tetracycline and chloramphenicol. The antibiotic resistant strains to nalidixic acid, erythromycin and sulphamethoxazole-smx were 72%, 81.8% and 18.2% respectively (Table 10).

At the UDC,18.2% of the isolates were resistant to gentamicin and tetracycline while 100% were resistant to erythromycin, penicillin and ampiclox (Table 10).

Ten percent (10%) of the isolates obtained from GDC were resistant to gentamicin, Chloramphenicol and sulphamethoxazole- trimethnoprim while 90% were resistant to nalidixic acid and erythromycin. 80% of the isolates were resistant to ampiclox (Table 10). In general, *E.coli* isolates were highly resistant to ampiclox, penicillin, erythromycin and nalidixic acid.

4.6   Antibiotic susceptibility of *Escherichia coli:*

The mean diameter of zones of inhibition of *E.coli* from the three day-care centres are presented in Figure 1. It was observed that diameters of zones of inhibition (18.27-22.60mm) were obtained for tetracycline and chloramphenicol in the three centres meaning that the antibiotics are more effective than the rest of the antibiotics tested. (Figure 1). Gentamicin followed closely in activity with 15.0-16.0mm diameter zone of inhibition.

Moderately susceptible *E.coli* isolates to test antibiotics in the three day-care centres were also recorded (Table 11). 18.2% of *E.coli* isolates from the NDC were moderately susceptible to sulphamethoxazole- trimethrophrim (Table 11). At the UDC, 9.1% of the isolates were moderately susceptible to tetracycline, chloramphenicol and sulphamethoxazole-trimethroprim while 18.2% of the isolates were moderately susceptible to nalidixic acid (Table 11) 50.0% of isolates in GDC were moderately susceptible to sulphamethoxazole-trimethrophrim while 10.0% of the isolates were moderately susceptible to nalidixic acid gentamicin erythromycin and chloramphenicol (Table 11).

The number and percentage of *E.coli* isolates susceptible to test antibiotics in the three day-care centres are presented in Table 12. 72.0% of isolates from NDC were susceptible to tetracycline and gentamicin while erythromycin, chloramphenicol and sulphamethoxazole-smx-susceptible isolates were 9.1%, 54.5% and 18.2% respectively (Table 12). At the UDC 8(12.0%) 7(63.6%) and 5(45.5%) isolates were susceptible to tetracycline, gentamicin and sulphamethoxazole-trimethroprim respectively (Table 12), whereas at the GDC, 80.0% and 100.0% of *E.coli* isolates were susceptible to gentamicin and tetracycline respectively (Table 12). Chloramphenicol, ampiclox and sulphamethoxazole-trimethroprim susceptible *E.coli* at the same day-care centre were 60.0%, 20.0% and 30.0% respectively.

Statistical analysis of the data from the three day-care centres based on the susceptibility of isolates to antibiotics showed that erythromycin differed significantly ($P<0.05$) from among the rest of the antibiotics (Appendix 8). Statgraphic (Version 5.0) analysis of data showed *E.coli* isolates from GDC as being most susceptible to tetracycline while isolates from NDC were most susceptible to chloramphenicol (Figure 2). Isolates from both NDC and UDC were least susceptible to penicillin (Figure 2). It was observed that there were significant differences in potency among the eight antibiotics tested (Appendix 9).Tetracycline was the most potent antibiotic for isolates from GDC while chloramphenicol was the most potent for isolates from NDC and UDC (Figure 3).

4.7 Minimum inhibitory concentration (MIC) of antibiotics on *E.coli* in three day-care centres:

The range of MIC values for each antibiotics is shown in Table 13 while the raw data of minimum inhibitory concentration (MIC) of eight antibiotics tested on *E.coli* isolates from the three day-care centres are presented in Appendices 10,11 and 12.The

widest range (0.04 – 48.0(μg/ml) of MIC values was obtained with chloramphenicol isolates from NDC and the shortest range (12.0-24.0(μg/ml) was shown by ampiclox. MIC values recorded for nalidixic acid, gentamicin, erythromycin, tetracycline, sulphamethoxazole-trimethroprim, and penicillin in the same centre were 4.0-24.0, 0.2-48.0, 1.5-24.0, 0.75-48.0 3.0-24.0 and 5.0-24.0μg/ml respectively (Table 13). The MIC values for erythromycin (12.0-48.0(μg/ml) was low, followed by nalidixic acid (8.0-48.0(μg/ml) and Ampiclox (8.0-48.0(μg/ml) while those of chloramphenicol (0.25-24.0(μg/ml) and tetracycline (1.5-24.0(μg/ml) were high (Table 13) in respect to *E.coli* isolates obtained from UDC. For *E.coli* isolates obtained from GDC, the MIC values showed no appreciable variation from values obtained for NDC and UDC (Table 13).

Statistical analysis of data based on study area, antibiotic concentration and susceptibility of isolates showed no significant difference at 5% probability levels (P>0.05) among the day-care centres (Appendix 13). Statgraphic analysis of data however, showed that *E.coli* were more susceptible to tetracycline at the GDC than at the NDC (Figure 4). Isolates from UDC were highly susceptible to chloramphenicol (Figure 4). This means that tetracycline and chloramphenicol are very active in checking the growth of the isolates tested (Figure 5).
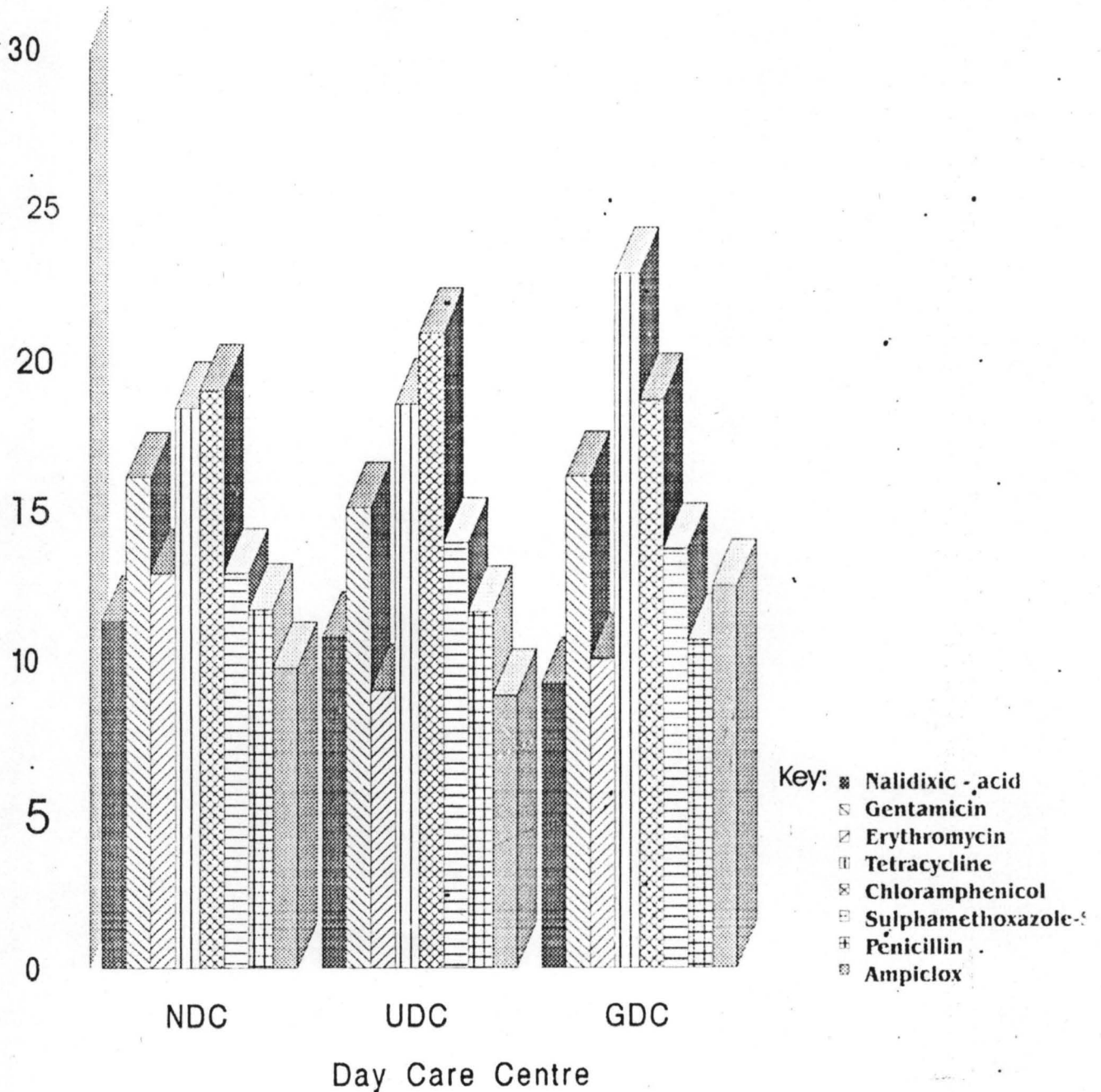
Figure 1:Susceptibility of *E.coli* to antibiotics

NDC : New Secretariat Day-care Centre.
UDC : Unguwan-daji Day-care Centre.
GDC : Government Day-care Centre.

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TABLE 7: Prevalence of non-coliforms on environmental surfaces in the New Secretariat Day Care Centre, Minna.

Bacteria	Number of isolates on environmental surface							
	wall	Furniture's	Door handle	Toys	Floor	Utensils	Toilet seat	Total
Shigella spp.	0(0.0)	3(1.6)	3(1.6)	0(0.0)	0(0.0)	8(4.3)	8(4.3)	22(11.9)
Bacillus spp.	8(4.3)	6(3.2)	10(5.4)	9(4.7)	10(5.4)	6(3.2)	0(0.0)	49(26.5)
Staphylococcus Spp.	8(4.3)	8(4.3)	14(7.6)	10(5.4)	13(7.0)	5(2.7)	19(10.3)	77(41.6)
Lactobacillus spp.	0(0.0)	5(2.7)	0(0.0)	4(2.3)	0(0.0)	0(0.0)	0(0.0)	9(4.7)
Diplococcus spp.	0(0.0)	11(5.9)	0(0.0)	3(1.6)	0(0.0)	5(2.7)	0(0.0)	19(10.3)
Streptococcus spp.	0(0.0)	1(0.5)	0(0.0)	0(0.0)	4(2.3)	0(0.0)	2(1.1)	7(3.8)
Samonella spp.	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.1)	2(1.1)
Total	16(8.6)	34(18.4)	27(14.6)	26(14.1)	27(14.6)	24(13.0)	31(16.8	185(100.0)

Number ion parenthesis represents percentage sample positive for non-coliforms.

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Key:
- ▨ Nalidixic - acid
- ◩ Gentamicin
- ▧ Erythromycin
- ▥ Tetracycline
- ▨ Chloramphenicol.
- ▤ Sulphamethoxazole-SMX
- ▦ Penicillin
- ▨ Ampiclox
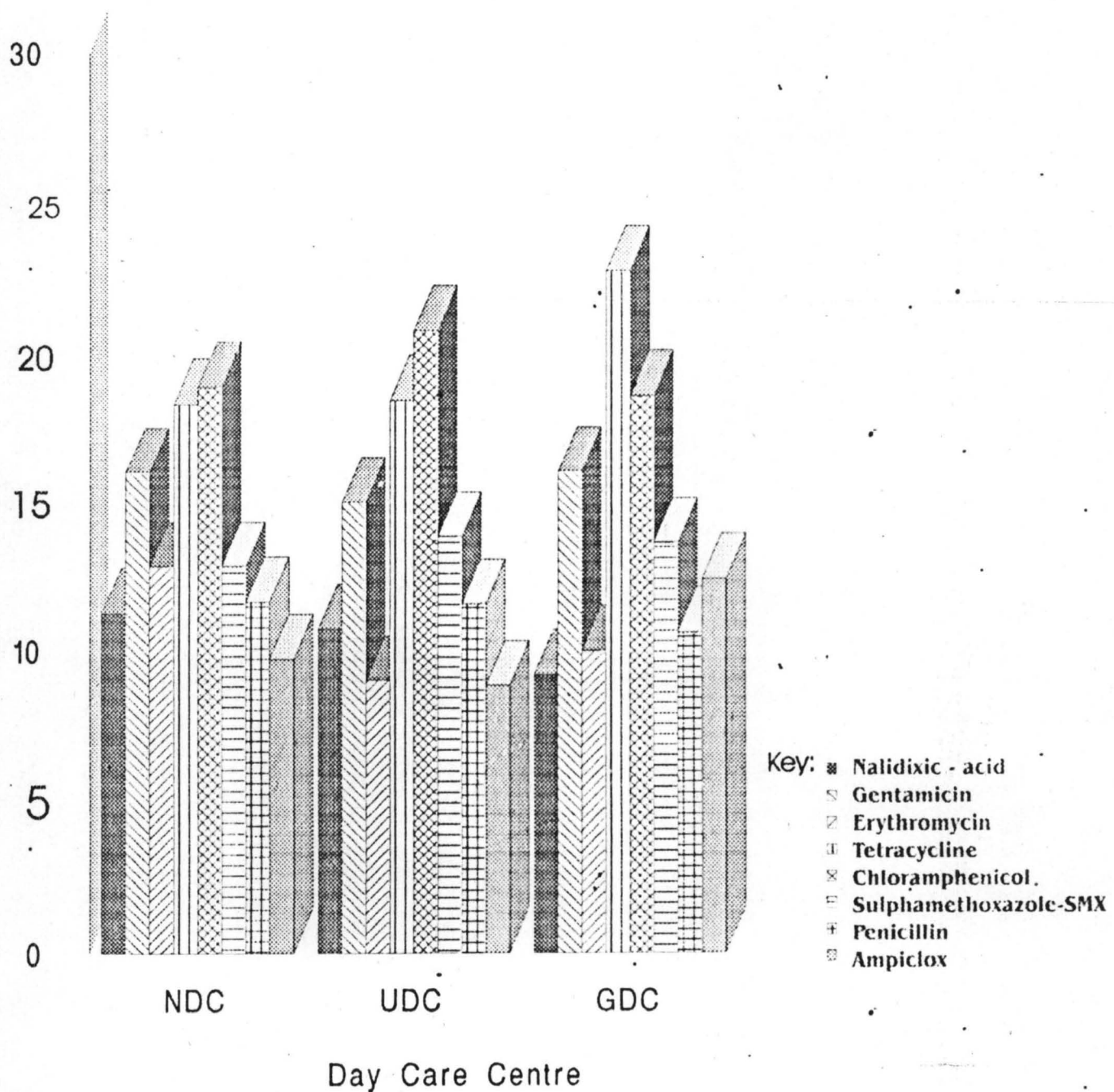
Day Care Centre

Figure 2:Susceptibility of *E.coli* to antibiotics based on diameters of zone of inhibition

NDC : New Secretariat Day-care Centre.
UDC : Unguwan-daji Day-care Centre.
GDC : Government Day-care Centre.

TABLE 8: Prevalence of non-coliforms on environmental surfaces in the Unguwan Daji Day-care Centre, Minna.

Bacteria	Number of isolates on environmental surface							
	Wall	Furnitures	Door handle	Toys	Floor	Utensils	Toilet seat	Total
Shigella spp.	0(0.0)	2(1.0)	0(0.0)	0(0.0)	5(2.3)	0(0.0)	2(1.0)	9(4.5)
Bacillus spp.	16(7.9)	12(5.9)	10(5.0)	8(4.0)	11(5.5)	15(7.4)	5(2.3)	77(38.1)
Staphylococcus spp.	11(5.5)	16(7.9)	8(4.0)	9(4.5)	13(6.4)	11(5.5)	13(6.4)	81(40.1)
Lactobacillus spp.	0(0.0)	6(3.0)	4(2.0)	2(1.0)	3(1.5)	5(2.3)	0(0.0)	20(9.9)
Diplococcus spp.	0(0.0)	2(1.0)	0(0.0)	3(1.5)	0(0.0)	0(0.0)	0(0.0)	5(2.3)
Streptococcus spp.	1(0.5)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	5(2.3)
Salmonella spp.	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	2(1.0)	3(1.5)
Proteus spp.	1(0.5)	0(0.0)	0(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.0)
Total	**29(14.4)**	**39(19.3)**	**23 (11.4)**	**22(10.9)**	**33(16.3)**	**32(15.8)**	**24(11.8)**	**202(100.0)**

Number in parenthesis represents percentage sample positive for non -coliforms.

Figure 3:Potency of antibiotics on *E.coli* based
on diameters of zone of inhibition.

NDC :New Secretariat day-care centre.
UDC :Unguwan-daji day-care centre.
GDC :Government day-care centre.

TABLE 9: Prevalence of Non-coliforms on environmental surfaces in Government Day-care Centre, Minna.

Bacteria			Number of isolates on environmental surface					
	Wall	Furniture(s)	Door handle	Toys	Floor	Utensils	Toilet seat	Total
Shigella spp.	12(5.7)	2(0.9)	1(0.5)	0(0.0)	2(0.9)	0(0.0)	N.A	67(31.8)
Bacillus spp.	9(4.3)	7(3.3)	9(4.3)	13(6.1)	11(5.2)	15(7.1)	N.A	86(40.8)
Staphylococcus spp.	6(2.8)	12(5.7)	18(8.5)	16(7.6)	14(6.6)	17(18.1)	N.A	34(16.1)
Lactobacillus spp.	3(1.4)	7(3.3)	8(3.8)	2(0.9)	8(3.8)	3(1.4)	N.A	10(4.7)
Diplococcus spp.	0(0.0)	0(0.0)	3(1.4)	4(1.9)	0(0.0)	0(0.0)	N.A	3(1.4)
Streptococcus spp.	0(0.0)	0(0.0)	1(0.5)	0(0.0)	2(0.9)	0(0.0)	N.A	2(2.3)
Salmonella spp.	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	N.A	2(0.9)
Proteus spp.	0(0.0)	0(0.1)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	N.A	2(0.9)
Unidentified spp.	0(0.0)	0(0.0)	01(0.0)	1(0.5)	0(0.0)	0(0.0)	N.A	2(0.9)
Total	**30(14.2)**	**29(13.7)**	**42 (19.9)**	**37(17.5)**	**38(18.0)**	**35(16.6)**		**211(100.0)**

Number in parenthesis represents percentage sample positive for non -coliforms.   NA: non-applicable.

TABLE 10   Resistance of *E.coli* to antibiotics.

ANTIBIOTIC	Number of *E.coli* resistant to antibiotics		
	NDC(N-11)	UDC(N-11)	GDC(N-10)
Nalidixic acid	8(72.0)	8(72.0)	9(90.0)
Gentamicin	3(27.3)	2(18.2)	1(10.0)
Erythomycin	9(81.8)	11(100.0)	9(90.0)
Tetracycline	3(27.3)	2(18.2)	0(0.0)
Chloramphenicol	3(27.3)	1(9.1)	1(10.0)
Sulphamethoxaole Trimethroprim	2(18.2)	3(27.3)	1(10.0)
pencillin	11(100.0)	11(100.0)	10(100.0)
Ampiclox	11(100.0)	11(100.0)	8(80.0)

UDC: New Secretariat Day-care Centres

UDC: Unguwan Daji Day-care Centre

GDC: Government Day-care Centre.
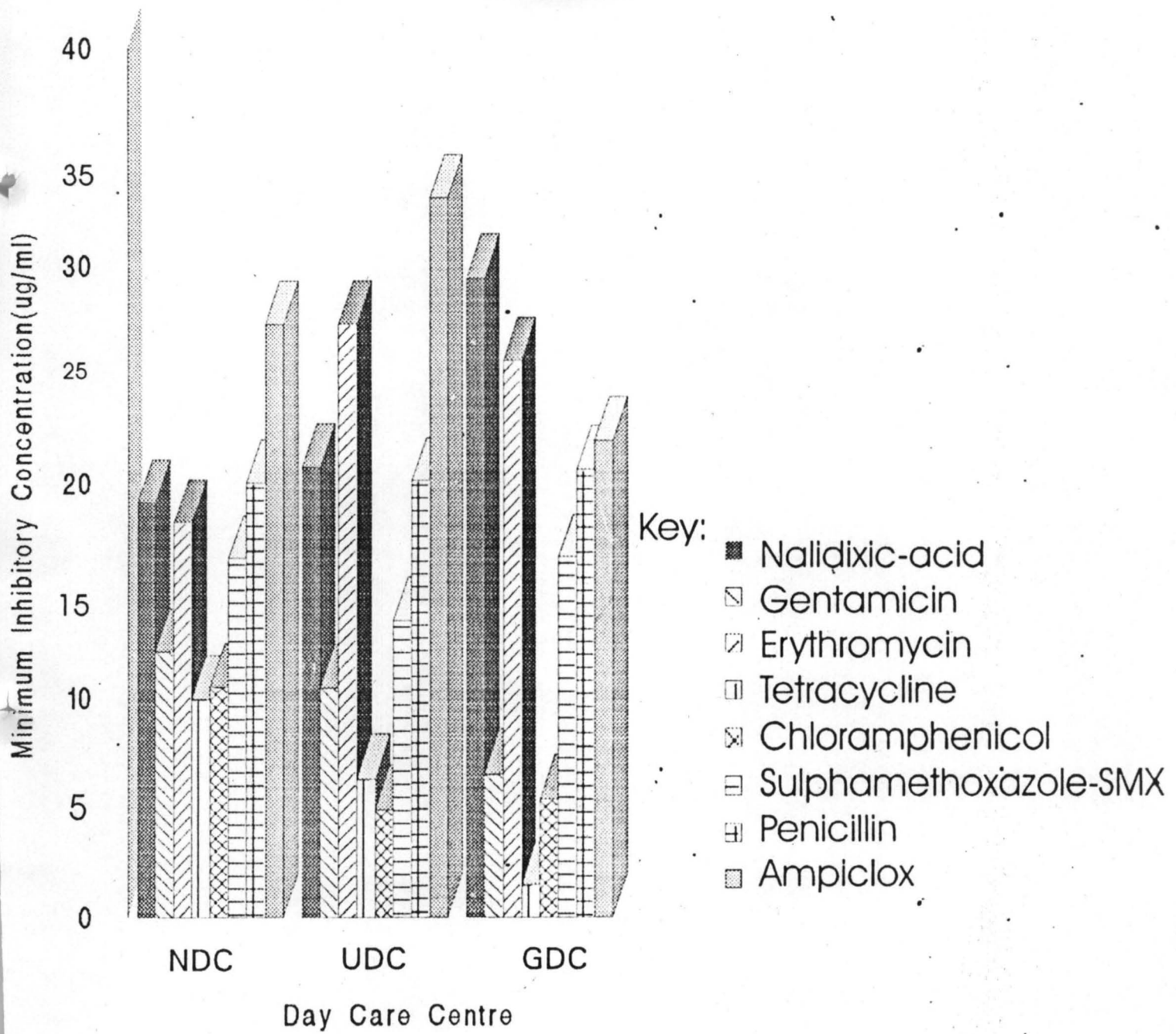
Figure 4: Minimum inhibitory concentration of antibiotics on *E.coli*

NDC :New Secretariat day-care centre.
UDC :Unguwan-daji day-care centre.
GDC :Government day-care centre.

TABLE 11: *E.coli* moderately susceptible to antibiotics per day-care centre.

ANTIBIOTIC	Number of *E.coli* moderately susceptible to antibiotics		
	NDC	UDC	GDC
Nalidixic acid	2(18.2)	3(27.3)	1(10.0)
Gentamicin	0(0.0)	2(18.2)	1(10.0)
Erythromycin	2(18.2)	0(0.0)	1(10.0)
Tetracycline	0(0.0)	1(9.1)	0(0.0)
Chloramphenicol	0(0.0)	1(9.1)	0(10.0)
Sulphamethoxazole-Trimethroprim	3(27.3)	1(9.1)	5(50.0)
Pencillin	0(0.0)	0(0.0)	0(0.0)
Ampiclox	0(0.0)	2(18.2)	0(0.0)

11, 11, and 10 *E.coli* isolates were tested from NDC, UDC, and GDC respectively.

NDC: New Secretariat Day-care Centre

UDC: Unguwan Daji Day-care Centre
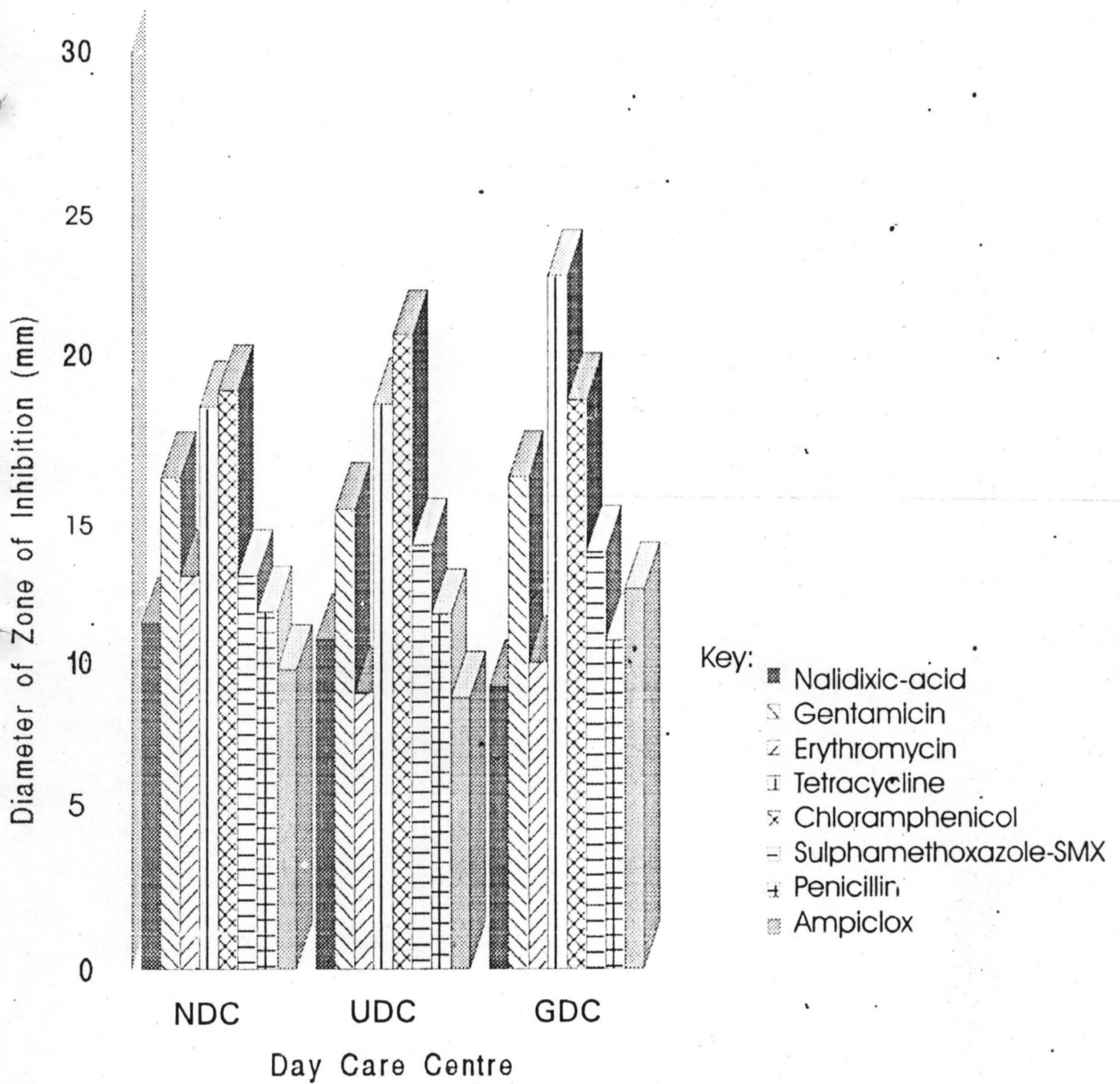
GDC: Government Day-care Centre.

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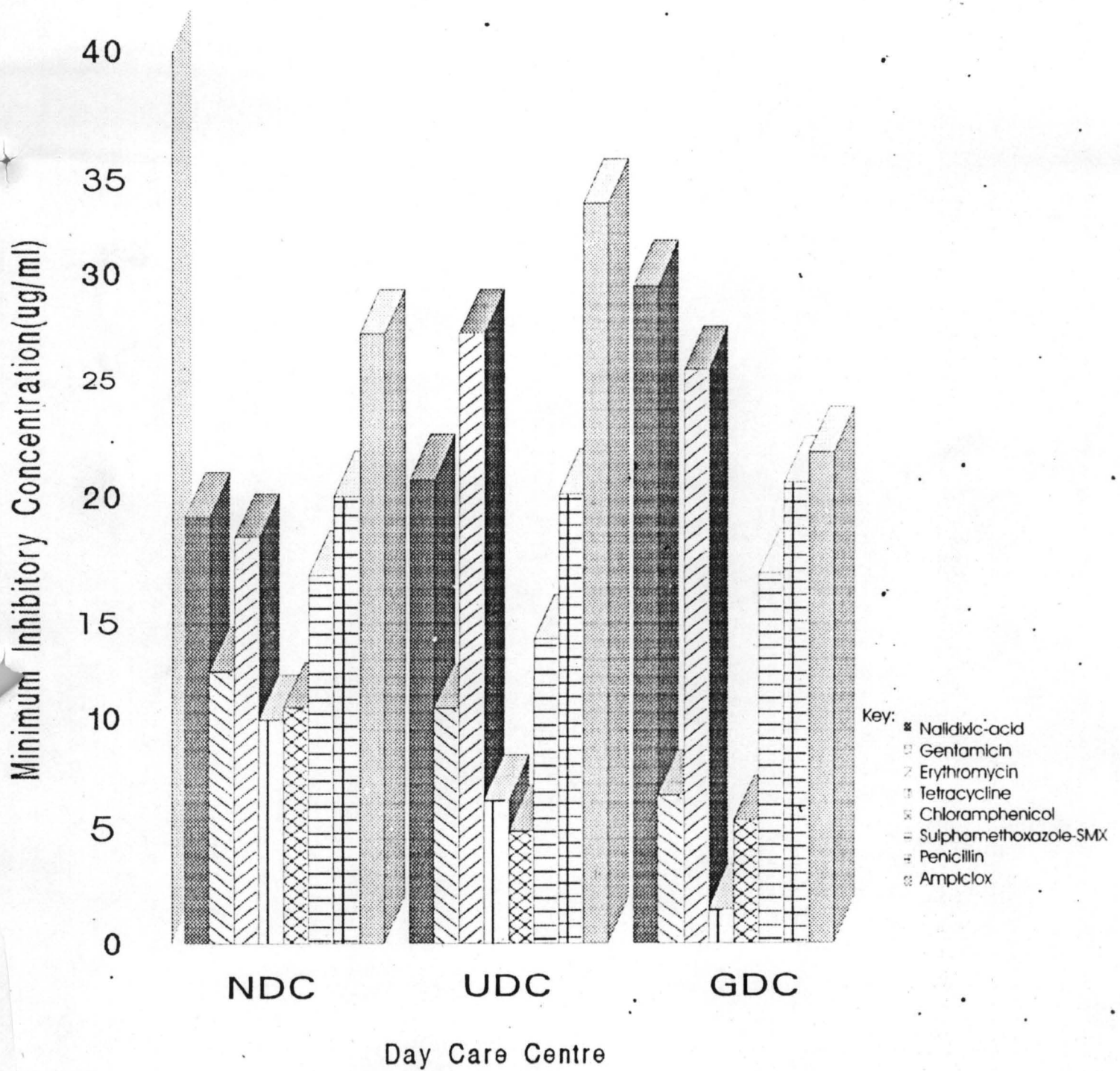
Figure 5: Potency of antibiotics on *E.coli* based
on minimum inhibitory concentration.

NDC :New Secretariat day-care centre.
UDC :Unguwan-daji day-care centre.
GDC :Government day-care centre.

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TABLE 12: Number of *E.coli* susceptible to antibiotics per day-care centre.

ANTIBIOTIC	Day -care centres		
	NDC	UDC	GDC
Nalidixic acid	0(0.0)	0(0.0)	0(0.0)
Gentamicin	8(72.0)	7(63.6)	8(80.0)
Erythromycin	1(9.1)	0(0.0)	0(0.0)
Tetracycline	8(72.0)	8(72.0)	10(100.0)
Chloramphenicol	6(54.5)	7(63.6)	6(60.0)
Sulphamethoxazole-Trimethroprim	2(18.2)	5(45.5)	3(30.0)
Pencillin	0(0.0)	0(0.0)	0(0.0)
Ampiclox	0(0.0)	0(0.0)	2(20.0)

11, 11, and 10 *E.coli* isolates were tested from NDC, UDC, and GDC respectively.

NDC: New Secretariat Day-care Centres

UDC: Unguwan Daji Day-care Centre

GDC: Government Day-care Centre.

TABLE 13: Minimum inhibitory concentration (ug\ml) of antibiotics on *E. Coli*.

| ANTIBIOTIC | Range of MIC values | | |
	NDC	UDC	GDC
Nalidixic acid	4.0-24.0	8.0-48.0	4.0-24.0
Gentamicin	0.2-48.0	2.0-24.0	0.2-48.0
Erythromycin	1.5-24.0	12.0-48.0	1.5-24.0
Tetracycline	0.75-48.0	1.5-24.0	0.75-48.0
Chloramphenicol	0.04-48.0	0.25-24.0	0.04-48.0
Sulphamethoxiazole-Trimethroprim	3.0-24.0	2.0-24.0	3.0-24.0
Pencillin	8.0-24.0	3.0-48.0	8.0-24.0
Ampiclox	12.0-24.0	8.0-48.0	12.0-48.0

11, 11, and 10 *E.coli* isolates were tested from NDC, UDC, and GDC respectively.

NDC: New Secretariat Day-care Centre

UDC: Unguwan Daji Day-care Centre

GDC: Government Day-care Centre.

CHAPTER FIVE

## 5.0   DISCUSSION

The study has shown that environmental surfaces in day- care centre in minna, Niger state harbour different species of coliforms and other bacterial contaminants. The coliforms included *Escherichia coli, Enterobacter aerogenes, Klebsiella* spp. and *Serratia* spp. while the non- coliform bacteria included species of *Shigella, Bacillus, Staphylococus, Lactobacillus, Streptococus, Salmonella* and *Proteus*. The frequency of occurrence (13.7%) of coliforms in the three day- care centres studied was much higher than that (7.8%) reported for a similar establishment in Porharcourt, Nigeria by Antai. and Ogbonna (1988).

The highest prevalence rate (19.9%) of coliforms recorded for the New Secretarial day- care centre (NDC) should be of particular concern because when compared with Unguwan- daji day- care centre (UDC) and Government day- care centre (GDC), the NDC is the cleanest of the three centres. The GDC with a prevalence rate of 8.3% is the oldest of the three centres and not as well kept as the NDC and the UDC with 12.6% prevalence rate. It is possible that the large number of children in the NDC which favour easier interfamilial contact may have been responsible for the high prevalence of coliforms in the centre.

Escherichia coli was the most prevalent coliform bacterial contaminant in the UDC and the GDC, while *Enterobacter aerogenes* was the most prevalent coliform in NDC. The high (60.0%) prevalence rate of *E coli* in GDC and UDC may be due to the absence of poorly managed toilet facilities in the centre. The passage of stool by children is therefore likely to be poorly managed by staff who may have little inclination to clean thoroughly areas where faeces of children may have been deposited or feel the need to disinfect their hands after washing off faeces from the children. It is possible, therefore that they contaminate their hands and then transfer the organism to environmental

surfaces when they touch the surfaces and objects around them (Feachem *et al.*, 1983). This may have been responsible for the high prevalence of coliforms particularly *E. coli* and *Enterobacter aerogenes* on toys in UDC and GDC and on walls in NDC. The relatively high prevalence of coliforms on wall, furnitures, toys and utensils in the three centres suggests that these items may serve as vehicles of infection by the organisms (Antai and Ogbonna, 1988). This should be a cause for concern because many strains of these organisms have been implicated in food poisening, urinary tract infections (UTI), particularly cystitis (Lennette *et al.*, 1985) and infantile diarrhoea (Ijar and Sar, 1996).

Other infections that could arise as a consequence of contamination of environmental surfaces of day- care centres by the organisms include wound, respiratory, blood stream and central nervous system infections (Lennette *et al.*,1985). *Serratia* and *Klebsiella* spp. had low prevalence rates in the three centres. Both organisms are mainly associated with nosocomial infections. Ijar and Sar (1996) reported strains of *Klebsiella* spp. as being apparently well adapted to cause endogenous infections, for example, in the urinary tract or be transmitted to other susceptible persons. Their recovery from the centres indicates the potential health hazard they pose to the children and staff of the centres.

Non- coliform bacteria were also isloated in this study. The organisms that had high prevalence in the three centres were species of *Bacillus* and *Staphylococcus*. Lower prevalence rates were recorded for *Lactobacillus, Shigella, Diplococus*, and *Salmonella* spp.. The prevalence of *Bacillus* species could be due to the widespread distribution of the organism in nature. Besides, the organisms form spores which help them to withstand disinfection (Ijah and Mohammed, 2001). Some species of the organism (particularly *Bacillus subtilis*) are aerial contaminants and have been isolated from environmental surfaces (Ijah and Mohammed, 2001). The high prevalence of *Staphylococcus* recorded in the three day–care centres may be attributed to the predominance of the organisms in

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nature. *Staphylococcus* are widely distributed in the environment and their primary habitats are the anterior body and skin of man from where these organisms are easily transfered to environmental surfaces (Talaro and Talaro, 1993).In the course of their mingling and playing, children and staff of the centres may shed the organisms from their skin and nose through sneezing and coughing in to the environment. The high prevalence of *Staphylococcus* in the three centres should be viewed seriously as this organism has been incriminated in many food poisoning cases (Antai and Ogbonna, 1988), nosocomial diseases and urinary tract infections (Baron and Finegold, 1990). If some strains of these organisms are enterotoxigenic, then these centres do present some health hazard to the children. At present, little has been documented about the possible prevalence of *Lactobacillus, Diplococcus* and *Salmonella* spp. in day- care centres. However, *Shigella* have been associated with diarrheal illness in day- care centres (Hadler *et al*.,1985). The isolation of the other organism in the centres should draw attention to the possibilities of infections arising from them. For instance, some *Lactobacillus* spp. have been incriminated in plueropulmonary infections and other miscellaneous infections. (Lennette *et al*, 1985). Some *Salmonella* spp. (*S.typhi* for example) are well known agents of typhoid fever. The possibility of occurrence of this disease among children or staff of the centers based on the generally poor sanitary conditions of the centres where even drinking water supplies may be seriously contaminated. Diplococcus spp. have the potential to cause meningitis or congenital herpes or other sexually transmitted diseases (Prescott *et al*., 1990; Talaro and Talaro, 1993).

The prevalence of non-coiform bacteria was higher in UDC and GDC than the NDC. The possible explanation for their difference may be the quality of sanitary facilities and the level of supervision by the staff of the centres. The relatively high population of workers and visitors to the Niger State Secretariat where NDC is located

and the higher number of children, compared to the UDC and GDC is likely to favour a higher nasal-carrier state of commensals and pathogens that could cause infections, transmissible among the people in the secretariat and staff in the center. Further more, children of ages 2 to5 years are still likely to be undergoing toilet training and therefore, may be expected to have poorly developed hygiene and toilet habits. In addition, the poorly motivated attendants of the centres may not have paid adequate attention to children during visits to the toilet and where such facilities do not exist as in GDC, defaecation and passage of urine is indiscriminate.

Streptococcus and *proteus* spp. was isolated from the centers in this study. The implication of these is the potentials of some species of *Streptococcus* (*S. pneumoniae*) to cause pneumoccal infection as well as otitis media in young children. This possibility is supported by the findings of Reichler *et al.* (1992) that reported the involvement of *Streptococcus pneumoniae* in an outbreak of otitis media in day-care centers in Ohio, United State of America.

All *E.coli* isolates in the three day-care centres were resistant to at least two antibiotics particularly, penicillin and ampicillin. Two isolates (18.2%) in NDC and (10.0%) in UDC were resistant to all eight antibiotics used in the study while1 (9.1%) in GDC was resistant to seven antibiotics. These findings agree with the report of Iruka *et al.* (2000) who observed rapid increases in the prevalence of resistance in commensal as well as pathogenic *E.coli* to antibiotics commonly used in the treatment of infections in Nigeria. It is probable that resistance to the two β-lactams (penicillin and Ampicillin) may have been due to the clustered population of high risk persons in the day-care centers which could favour the spread of resistance strains of *E.coli* among susceptible individuals in the centres. This view is shared by Haley *et al.* (1982) who reported that outbreak of resistance to antibiotics (particularly the β-lactams) generally involves areas with large clusters of people. The frequency of prescription of the two drugs by

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physicians and other medical practitioners coupled with the easy availability of the drugs may also have contributed to the development of resistance by *E.coli* to the drugs. Resistance to the two antibiotics could also be alttributed to the virtual ineffectiveness of this class of antibiotics on Gram negative bacteria and the transmission of chromosomally-acquired plasmids determining resistance to the several different analogues of β-lactam antibiotics. Such transmission has been known to occur through secretion of β-lactamases by resistant organisms into growth media (Bryan,1982).

Over 80.0% of *E.coli* isolates were resistant to erythromycin in all three centres. Resistance to the antibiotic (a macrolide) could develop because the drug is usually used in the treatment of chest and respiratory tract infections in children and young adults. Thus, there in the possibility of indiscriminate use of the drug by illiterate parents. Besides, the bacteriostatic (instead of bacteriocidal) effect of the drug may be effective in the elimination of vegetative cells while selecting for resistance in the remaining cells (Roberts, 1996). Seventy-two percent (72.0%) of *E.coli* isolates were resistant to nalidixic-acid in NDC and UDC while 90.0% of the *E.coli* in GDC were resistant to the drug. Nalidixic-acid is a fluoroquinoline antibiotic known to inhibit bacterial growth greatly when consumed in low dosages (Green and Tillotson, 1999). It however, appears from this study to have developed reduced efficiency in the course of time on pathogens such as *E.coli*.This supports the assertion of Green and Tillotson, (1997) and Hoge *et al.* (1998) that resistance to drugs by microorganisms seems to spread rapidly once a drug is introduced into clinical practice. An additional factor contributing to therapeutic failures of nalidixic-acid may be the multiplicity of sites at which resistance to the drug could arise (Jacoby, 1985).

Resistance of *E.coli* to sulphamethoxazole-trimethroprim was also observed. Resistance to the broad-spectrum bacteriostatic derivative of sulphonamide-diaminopyrimidine by *E.coli* may be caused by the ineffective penetration of the Gram-

negative envelope by the drug. Other resistance mechanisms are the use of external source of folate (since resistance is principally through the inhibition of dihydrofolic acid (DHF) synthesis) or the synthesis of the organisms' own folate or direct engagement in the production of high levels of paraminobenzoic acid (PABA) (Singleton, 1999.)

More than 60.0% of *E.coli* isolates in the three centres were susceptible to gentamicin. The relatively low resistance (<27.0%) recorded for the antibiotic could be attributed to the reluctance of clinicians to prescribe the drug for patients for fear of the side effects the overuse of the drug may induce. Such side effect include dose-dependent damage to the 8th cranial nerve which could cause impairment of hearing (Singleton, 1999).

Less than 30.0% of *E.coli* isolates showed resistance to chloramphenicol. Apart from the usual means of acquisition of resistance (through resistance plasmids) by synthesis of the enzyme chloramphenicol acetyltransferase (CAT) ,overuse of the drug (mainly through self medication in the treatment of enteric fevers) may be another means by which resistance could develop. The relatively higher susceptibility level (>70.0%) recorded for the drug in all three centres may be due to the toxicity of the drug in addition to the reluctance of most patients to go through the rigorous drug regimes usually prescribed by physicians in the treatment of diseases such as typhoid fever. The high susceptibility recorded may be misleading because, inspite of the broad-spectrum activity of the drug, it exerts bacterostatic effect mainly on growing cells (Singleton, 1999). The absence of safer analogues (Hart and Kariuki, 1998) may have contributed to the low preference shown by would- be consumes.

Over 70.0% susceptibility to tetracycline was recorded in the study. The low resistance recorded should not be taken lightly because of the findings of Hart and Kariuki (1998) as well as Okeke *et al.* (1999) that a considerable rise in resistance to the drug had been observed over a 12 year period. Reasons responsible for this trend cold be

the relative cheapness of the drug coupled with its wide availability without prescription from authorized health institutions and pharmacies as well as from unauthorized patent medicine shops and other distributors, a situation typical of most developing countries.

Low MIC values were observed for chloramphenicol (0.04- 48.0μg/ml) and tetracycline (0.75- 48.0 μg/ml) in the three day- care centres while high MIC values were recorded for penicillin (3- 48.0μg/ml). This means that chloramphenicol and tetracyline are more effective in the treatment of infections caused by *E. coli* than penicillin and ampiclox. Differences in constituents of the antibiotics may have influenced this and other MIC results obtained. The broad spectrum activity of chloramphenicol and tetracyline on growing (vegetative) cells with the resultant lower MIC values compared to other drugs used in the stuy. Secretion of β-lactamase into the broth meduim (used to determine the MIC) by resistant *E. coli* may have neutralized the effect of the two (- lactams (penicillin and ampiclox) thereby resulting in the higher MIC values observed for the drugs (Bryan, 1982).

5.1 CONCLUSION AND RECOMMENDATIONS

This study has drawn attention to the presence of coliforms and other bacterial pathogens in day-care centres operating in Minna metropolis. *Enterobacter aerogenes* was the most frequently occurring coliform organism in the NDC while *Escherichia coli* was most prevalent in UDC and GDC. Among the non-coliform bacteria isolated, *Staphylococcus* and *Bacillus* spp. were most prevalent in all three day-care centres while *Klebsiella* and *Proteus* spp. were the least prevalent. The proliferation of coliforms and non-coliforms reflects the poor sanitary conditions of the day-care centres. The centres could then serve as potential reservoirs of infectious agents that could cause disease or oven initiate epidemics.

The study also established the prevalence of multiple-antibiotic resistance in *Escherichia coli* isolated from environmental surfaces in some day-care centres in Minna. The organisms were observed to be particularly resistant to penicillin, ampiclox and erythromycin and to some extent, gentamicin. These drugs belong to the class of older, less expensive antimicrobial drugs used in the management of infections in Nigeria. Not only are these strains of *E.coli* potential causes of infection, they are also potential reservoirs of resistance genes that could be transferred to other pathogens in the environment. For this reason, trends seen with commersal *E.coli* may also occcur with pathogenic organisms. Most of the *E.coli* isolates were however, susceptible to chloramphenicol and tetracycline. Low MIC values were also recorded for the two antibiotics. This suggests that chloramphenicol and tetracycline may be useful in treating infections caused by pathogenic *E.coli* and other related bacteria in Nigeria. Based on the results of the present study the following recommendations are made:

1. Operators of day-care centres should regularly and thoroughly clean environmental surfaces with which children come in direct contact. Certain approved germicidal detergents recommended for hospital disinfection can be

appropriately applied on day-care centres.

2. Regular sampling of these surfaces should be carried out to determine the level of microbial contamination and thereby advise on appropriate measures to be taken to check disease transmission.

3. It is also important to enlighten people on the health implications of close-contact environments such as day-care centres which can harbour pathogenic bacteria. Emphasis of such enlightenment should be on handwashing by day-care centre employees and children to reduce the contamination of the environmental surfaces.

4. There is also the need for effective isolation (quarantining) of convalescent children until they are no longer able to act as sources of pathogenic organisms.

5. There is the need for a nation-wide survivallance of antibiotic resistance with special emphasis on day-care institutions and other close-contact environments.

6. The trend in enteric pathogens is toward increasing antibiotic resistance. There is the need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment.

7. A programme to improve the public's understanding of the implications of antibiotic misuse should be encouraged. This could assist in reducing pressure on prescriptions of drugs without the necessary sensitivity testing in the laboratory.

8. Regulations and policies on the manufacture and use, as well as banning 'over the counter' sales of antibiotics should be strictly enforced. These will help to reduce resistance among bacteria.

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Appendix 1: Statistical analysis of prevalence of *Escherichia coli* and other coliforms on environmental surfaces at the New Secretariat Day-care Centre (NDC) Minna.

Organism	Wall	Furnitures	Door handle	Toys	Floor	Utensils	Toilet seat	±SEM
				Sample site				
Escherichia coli	8.34 ± 8.34^a	12.49 ± 4.19	8.30 ± 0.009	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	8.34 ± 8.34	±4.73
Enterobacter aerogenes	19.40 ± 1.00^c	0.00 ± 0.00^a	11.10 ± 1.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a	19.40 ± 0.40^c	0.00 ± 0.00^a	±0.56
Klebsiella spp	5.60 ± 0.90^b	5.60 ± 1.00^b	2.70 ± 0.20^{ab}	5.60 ± 0.20^b	0.00 ± 0.00^a	2.70 ± 0.10^{ab}	2.70 ± 0.90^{ab}	±0.62
Serratia spp	2.70 ± 0.00^b	0.00 ± 0.00^a	2.70 ± 0.10^b	0.00 ± 0.00^a	13.70 ± 0.00^c	0.00 ± 0.00^a	2.70 ± 0.30^b	±0.12

Data on the same row carrying different superscripts differ significantly from each other (P< 0.05).

Appendix 2: Statistical analysis of prevalence of *Escherichia coli* and other coliforms on environmental surfaces at the Uguwan-daji Day-care Centre (UDC) Minna.

Organism	Sample site							±SEM
	Wall	Furnitures	Door handle	Toys	Floor	Utensils	Toilet seat	
Escherichia coli	0.00 ± 0.00^a	0.00 ± 0.00^a	4.15 ± 4.15^a	12.50 ± 12.50^a	0.00 ± 0.00^a	8.35 ± 8.36^a	0.00 ± 0.00	± 5.89
Enterobacter aerogenes	5.60 ± 0.00^c	5.60 ± 0.06^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	2.70 ± 0.20^b	5.60 ± 0.30^c	± 0.26
Klebsiella spp	0.00 ± 0.00^a	0.00 ± 0.00^a	2.70 ± 0.70^{ab}	0.00 ± 0.00^a	5.60 ± 0.60^c	2.70 ± 0.60^b	0.00 ± 0.00^a	± 0.42
Serratia spp	2.45 ± 0.45^b	0.00 ± 0.00^a	2.70 ± 0.00^b	5.60 ± 1.00^c	0.00 ± 0.00^a	5.60 ± 0.20^c	0.00 ± 0.00^a	± 0.42

Data on the same row carrying different superscript differ significantly from each other ($P > 0.05$).

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Appendix 3: Statistical analysis of prevalence of *Escherichia coli* and other coliforms on environmental surfaces at the Government Day-care Centre (UDC) Minna.

Organism	Sample site							±SEM
	Wall	Furnitures	Door handle	Toys	Floor	Utensils	Toilet seat	
Escherichia coli	8.34 ± 8.34^a	0.00 ± 0.00^a	0.00 ± 0.00^a	8.34 ± 8.34^a	12.50 ± 0.70^a	8.34 ± 8.34^a	0.00 ± 0.00^a	± 4.89
Enterobacteraerogenes	5.50 ± 1.00^b	8.30 ± 0.90^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	± 0.51
Klebsiella spp	2.70 ± 0.00^b	2.70 ± 0.20^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	± 0.08
Serratia spp	0.00 ± 0.00^a	2.70 ± 1.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	± 0.38

Data on the same row carrying different superscript differ significantly from each other ($P < 0.05$).

APPENDIX. 4.

Zone diameter(s) of inhibition (mm) of *E.coli* from the New Secretariat Day-care Centre (NDC).

Coded Isolates	Nalidixic-acid	Gentamicin	Erythromycin.	Tetracycline	Chloramphenicol.	Sulphamethoxazole-t.m.p	Penicillin	Ampiclox
NDC-I	08	16	10	25	15	09	12	09
NDC-2	16	19	22	08	15	12	11	08
NDC-3	14	30	13	22	34	15	14	13
NDC-4	11	16	11	20	30	09	10	07
NDC-5	10	12	12	22	07	14	12	07
NDC-6	08	16	12	07	22	14	11	12
NDC-7	17	07	09	21	25	13	12	11
NDC-8	09	15	16	23	10	12	14	08
NDC-9	10	12	12	19	18	12	10	12
NDC10	09	17	13	11	11	12	13	12
NDC11	12	16	11	23	20	19	09	08

APPENDIX 5.

Zone diameter(s) of iInhibition (mm) *E.coli* from the Unguwan daji Day-care Centre (UDC).

Coded Isolates	Nalidixic-acid	Genta-micin	Erythr-omycin	Tetrac-ycline	Chlora-mphenicol	Sulphamet hoxazole-t.m.p	Penicill-in	Ampiclo-x
UDC-1	10	17	13	20	25	18	11	06
UDC-2	12	15	08	19	26	12	15	14
UDC-3	14	19	12	12	13	12	14	13
UDC-4	14	15	08	16	16	17	17	06
UDC-5	10	12	08	22	11	16	18	07
UDC-6	08	14	07	08	16	09	08	09
UDC-7	14	19	08	21	27	10	08	14
UDC-8	13	16	11	22	25	20	10	06
UDC-9	08	08	07	19	26	08	12	07
UDC-10	07	17	08	20	19	16	07	07
UDC-11	08	13	09	23	23	14	07	08

APPENDIX 6.

Zone diameter(s) of inhibition (mm) *E.coli* from the government Day-care Çentre (GDC).

Coded Isolates	Nalidixi-cacid	Gentam-icin	Erythr-omycin	Tetrac-ycline	Chlora-mphenicol	Sulphamet hoxazole - t.m.p	Penicill-in	Ampiclo-x
GDC-1	15	17	10	19	22	18	15	19
GDC-2	07	08	12	28	26	12	08	09
GDC-3	08	15	09	22	14	12	11	10
GDC-4	08	14	10	25	12	19	09	12
GDC-5	09	16	08	21	15	12	09	08
GDC-6	07	18	07	26	21.	18	11	09
GDC-7	07	17	08	19	17	08	09	07
GDC-8	12	20	14	22	21	12	10	30
GDC-9	11	16	11	20	18	12	11	08
GDC10	09	19	11	24	19	13	14	12

APPENDIX 7.

Mean zone diameter(s) of inhibition (mm) of *E.coli* per day-care centre.

Antibiotic(s)	NDC	UDC	GDC
Nalidixic-acid	11.27	10.73	9.20
Gentamicin	16.00	15.00	16.00
Erythromycin	12.82	9.00	10.00
Tetracyline	18.27	18.36	22.60
Chloramhenicol	18.82	20.64	18.50
Sulpha methoxazole-tmp	12.82	13.82	13.60
Penicillin	11.64	11.55	10.60
Ampiclox	9.73	8.82	12.40

APPENDIX 8.

Statistical Analysis of Susceptibility of *E.coli* to antibiotics in relation to their zone diameter(s).

ANTIBIOTIC	NDC	UDC	UDC	± SEM
Nalidixic acid	11.27 ± 0.94^a	10.73 ± 0.83^a	9.20 ± 0.84^a	± 2.63
Gentamicin	16.00 ± 01.71^a	15.00 ± 0.97^a	17.00 ± 0.58^a	± 3.64
Erythromycin	$12.82 \pm 1.07b$	9.00 ± 0.62^a	10.00 ± 0.67^{ab}	± 2.46
Tetracycline	18.27 ± 1.94^a	18.36 ± 1.40^a	22.60 ± 0.97^a	± 4.56
Chlorampenicol	18.82 ± 2.55^a	20.64 ± 1.75^a	18.50 ± 1.33^a	± 5.94
Sulphamethoxazole-Trimethophrim	12.82 ± 0.84^a	13.82 ± 1.18^a	13.60 ± 1.12^a	± 3.16
Penicillin	11.64 ± 0.49^a	11.55 ± 1.20^a	10.70 ± 72^a	± 2.60
Ampiclox	$9.73 \pm 0.6.9^a$	8.82 ± 0.98^a	12.40 ± 2.24^a	± 4.21

Data on the same row carrying different superscript(s) differ significantly from each other $(P < 0.05)$.

Appendix 9: Statistical analysis of potency of test antibioticson *Ecoli* in relation to their zonediameter(s) of inhibition per Day-care Centre (UDC) Minna.

Day-care centre	Nalixidic. acid	Gentamicin	Erythromycin	Tetracycline	Chloramphenicol	Sulphamethoxazol e t.m.p	Penicillin	Ampiclox	± SEM
NDC	11.27 ± 0.94^a	16.00 ± 1.71^{ab}	12.82 ± 1.07^{ab}	18.27 ± 1.94^b	18.81 ± 2.55^b	12.82 ± 0.84^{ab}	11.64 ± 0.49^a	9.72 ± 0.69^a	±1.44
UDC	10.73 ± 0.83^{ab}	15.00 ± 0.97^{bc}	9.00 ± 0.82^a	18.36 ± 1.40^a	20.64 ± 1.75^d	13.82 ± 1.19^{abc}	11.55 ± 1.20^{ab}	8.82 ± 0.98^{ab}	±1.16
GDC	9.20 ± 0.84^a	16.00 ± 0.05^{bc}	10.00 ± 0.67^a	22.60 ± 0.97^d	18.50 ± 1.33^{cd}	13.60 ± 1.12^{abc}	10.70 ± 0.72^{ab}	12.40 ± 2.24^{ab}	±1.21

KEY: NDC   -   New Secretariat Day-care Centre

UDC   -   Unguwan Daji Day-care Centre

GDC   -   Government Day-care Centre

Data on the same row carrying the same superscripts do not differ significantly from each other (P> 0.05).

APPENDIX 10.

Minimum inhibitory concentration of antibiotic on *E.coli* from the New Secretariat day-care centre (NDC), Minna.

Coded isolates	Nalidixi-cacid	Gentam-icin	Erythr-omycin	Tetracycl in-e	Chloram phe-nicol	Sulphameth oxazole t.m.p	Penicilli n	Ampiclo-x
NDC -1	≥ 24.0	6.0	≤24.0	0.75	≥6.0	≥24.0	≤24.0	≥24.0
NDC -2	6.0	3.0	≥1.5	≥24.0	≥6.0	≤24.0	≤24.0	≥24.0
NDC -3	8.0	≤0.2	≤12.0	≥1.5	≤0.04	≥6.0	8.0	≤12.0 .
NDC -4	≤24.0	6.0	≤24.0	≤2.0	≤0.2	≥24.0	≤24.0	≥48.0
NDC -5	≤24.0	≤24.0	≤24.0	≥1.5	≥48.0	8.0	≤24.0	≥48.0
NDC -6	≥24.0	6.0	≤24.0	≥48.0	≥1.5	8.0	≤24.0	≤24.0
NDC -7	4.0	≥48.0	≥24.0	≥1.5	0.75	≤12.0	≤24.0	≤24.0
NDC -8	≥24.0	≥6.0	6.0	≤1.5	≤24.0	≤24.0	8.0	≥24.0
NDC -9	≤24.0	≤24.0	≤24.0	3.0	3.0	≤24.0	≤24.0	≤24.0
NDC-10.	≥24.0	4.0	≤12.0	≤24.0	≤24.0	≤24.0	≤12.0	≤24.0
NDC-11	≤24.0	6.0	≤24.0	≤1.5	≤2.0	3.0	≤24.0	≥24.0

APPENDIX 11.

Minimum inhibitory concentration(μg/ml) of antibiotic on *E.coli* from the Unguwan-daji day-care centre (UDC), Minna.

Coded Isolates	Nalidixi-c-acid	Gentam-icin	Erythr-omycin	Tetracycl in-e	Chloram phe-nicol	Sulphameth oxazole t.m.p	Penicilli n	Ampiclo-x
UDC- 1	≤ 24.0	4.0	≤12.0	2.0	≥0.75	3.0	≤24.0	≥48.0
UDC -2	≤24.0	≤6.0	≥24.0	3.0	0.5	≤24.0	≤24.0	8.0
UDC -3	8.0	≤2.0	≤24.0	≥24.0	≤12.0	≥24.0	8.0	≤12.0
UDC- 4	8.0	≥6.0	≤24.0	≤2.0	≤0.2	≥24.0	≤24.0	≥48.0
UDC- 5	≤24.0	≤24.0	≤24.0	≥1.5	≥24.0	6.0	3.0	≥48.0
UDC- 6	≥24.0	≤24.0	≤48.0	≥24.0	6.0	≥24.0	≥24.0	≥24.0
UDC- 7	8.0	3.0	≥24.0	≥1.5	0.25	≤12.0	≤24.0	≤24.0
UDC- 8	≤12.0	6.0	≤24.0	≥1.5	≥0.38	2.0	≥24.0	>48.0
UDC- 9	≥24.0	≥24.0	≥48.0	3.0	0.5	≥24.0	≤24.0	≤48.0
UDC-10	≥48.0	4.0	≥24.0	2.0	3.0	6.0	≥48.0	≥48.0
UDC-1 1	≥24.0	≤12.0	≥24.0	≤1.5	≤1.5	8.0	≥48.0	≥24.0

APPENDIX 12.

Minimum inhibitory concentration of antibiotic on *E.coli* from the Government Day-care Centre (GDC), Minna.

Coded Isolates	Nalidixic-acid	Gentamicin	Erythromycin	Tetracycline	Chloramphenicol	Sulphamethoxazole t.m.p	Penicillin	Ampiclox
GDC-1	≥ 6.0	4.0	≤24.0	3.0	≥1.5	3.0	≤6.0	3.0
GDC-2	≥48.0	≥24.0	≥24.0	>0.25	≤0.5	≤24.0	≥24.0	≥24.0
GDC-3.	≥24.0	≥6.0	≥32.0	≥1.5	8.0	≤24.0	≤24.0	≤24.0
GDC-4.	≥24.0	8.0	≤24.0	≤0.75	≤24.0	3.0	≥24.0	≥24.0
GDC-5	≥24.0	6.0	≥24.0	≥1.5	≥6.0	≤24.0	>24.0	≥24.0
GDC-6	≥98.0	3.0	≥48.0	≤0.5	≤3.0	3.0	≥24.0	≥24.0
GDC-7	≥48.0	4.0	≥24.0	3.0	4.0	≥24.0	≥24.0	≥48.0
GDC-8	≤24.0	2.0	8.0	≥1.5	≥1.5	≤24.0	≤24.0	≥0.25
GDC-9	≤24.0	6.0	≤24.0	≤2.0	3.0	≤24.0	≤24.0	≥24.0
GDC-10	≥24.0	≥3.0	≤24.0	>0.75	3.0	≥12.0	8.0	≤24.0

APPENDIX 13.

Statistical analysis of susceptibility of *E.coli* isolates to test antibiotics in relation to their minimum inhibitory concentration (MIC) per Day-care Centre.

ANTIBIOTIC	NDC	UDC	GDC	+ SEM
Nalidixic acid	$19.09+2.55^a$	$20.73+3.53^a$	$29.40+4.42^a$	$+10.59$
Gentamicin	$12.11+4.31^a$	$10.45+4.31^a$	$6.60+2.02^a$	$+9.71$
Erythromycin	$18.14+2.59^a$	$27.27+3.27^a$	$25.60+3.11^a$	$+9.01$
Tetracycline	$9.93+4.67^a$	$6.36+2.66^a$	$1.48+0.31^a$	$+9.63$
Chloramphenicol	$10.50+4.60^a$	$4.99+2.20^a$	$5.54+2.18^a$	$+9.79$
Sulphamethoxazole-Trimethophrim	$16.45+2.69^a$	$13.55+3.06^a$	$16.50+2.18^a$	$+8.91$
Penicillin	$20.00+3.27^a$	$22.09+4.62^a$	$20.60+2.27^a$	$+9.80$

APPENDIX 21 b:

ANOVA Table- Susceptibility of *E.coli* determined from values of minimum of inhibitory concentration (MIC).

Source Of Variation	Chloromphenicol Between groups	Within groups	Total	Sulphamethoxazole-smx Between groups	Within groups	Penicillin Between groups	Within groups	Total	Ampiclox Between groups	Within groups	Total
Sum of	28.5369	1210.6818	1239.2188	6.0460	343.6788	5.5023	231.3727	236.8750	71.7818	603.2182	680.0000
Df	2	29	31	2	29	31	2	29	2	29	31
Ms	14.2685	41.7476		3.0230	11.8508	2.7511	7.9784		35.8909	20.9730	
F-ratio	.342			.255		.345			1.711		
Sign. level	.7133			.7766		.7112			.1984		

ZDI: Zone Diameter of Inhibition
Range test: Tukey
Confidence level: 95