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BACTERIAL INFECTION PATTERN AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ISOLATES FROM UNDER-5 CHILDREN IN GOVERNMENT APPROVED CRÈCHES AND NURSERY SCHOOLS IN ONITSHA NORTH LGA, NIGERIA

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ABSTRACT

Purpose: The objective of the study was to identify the bacteria causing infections in under-5 children in government approved crèches and nursery schools in Onitsha North LGA, Nigeria. **Research Methods and Procedure:** A descriptive cross-sectional study design was employed while multistage and systematic sampling techniques were used. The study population was under-5 children. Four hundred and thirty-two (432) swab stick samples consisting of 288 from the palms of the children and 144 from the floors of their crèches were collected, collated and analyzed. **Results and Conclusions:** Findings from the study revealed that the following pathogenic bacteria were responsible for sick conditions amongst under-5 children in Onitsha North LGA. They were; *Bacillus lichenformis, Bacillus megaterium, Escherichia coli, Enterobacter aerogenes, Pseudomonas cepacia, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Gentamicin, Ofloxacin and Ciprofloxacin antibiotics were effectively bactericidal showing the widest zones of inhibition against the bacteria isolates. **Recommendation:** It is expedient for every Crèche to implement in full the provisions and goals of the National School Health Policy (NSHP) which are; to enhance the quality of health in the school community, create an enabling environment for inter-sectoral partnership in the promotion of child friendly school environment, for teaching and learning and health development.

KEYWORDS: Bacterial infection pattern; Antimicrobial susceptibility profile; Crèches; Onitsha.

INTRODUCTION

Infections are the leading causes of diseases in children, and are especially common in infancy. All children get infectious diseases and, statistically, they are the most overwhelming reason for sick absence in school and day care.[1]

Bacterial infections are caused by bacteria, which enter the body and try to conquer living space by producing toxins. Bacteria are single cell organisms that resemble the structure of a human cell, with certain differences such as a hard outer shell, making them more resilient to the environment. There are a lot of bacteria living inside our body, our gut and on our skin. These are so called "good bacteria". There are also millions of bacteria that are caught on the mucous membranes of the nasal cavities or the respiratory tracts. This is the body's primary defense against invading micro-organisms, because bacteria have a hard shell that makes some of them able to survive even the toughest environments such as the acidity of the stomach. Some antibiotics such as penicillin target the bacterial shell, and damage the hard cellular wall of the bacteria, killing the bacteria. Some bacteria may transform themselves into a dormant state or spore, which can survive extreme environment, such as high temperature or long periods of drought and hatch when they find themselves in a good living environment; e.g. the human body.

Etiologic causes of community-acquired bacterial infection are usually due to those organism associated with respiratory diseases in children.[2] In addition, a variety of opportunistic pathogens present great challenges to the diagnostic laboratories in the developing country setting. Due to these challenges, treatment is usually based on clinical symptoms and knowledge of the epidemiologic pattern of local pathogens, often leading to inappropriate treatment as well as increased morbidity and mortality.[3] Increase in the incidence of bacteria resistant to several antimicrobial agents.[2] periodic bacteriologic surveillance of invasive bacteria disease from normally sterile sites (e.g. blood cultures and cerebrospinal fluid) where appropriate are important to confirm clinical

diagnoses, conduct accurate infectious disease surveillance and direct public health care policy.[4]

Microorganisms are ubiquitous in nature. Only a small proportion of microorganisms are associated with man, either as commensals or pathogens.[5,6] The commensals are found living on the skin, mucous membranes of the upper respiratory tract, intestinal tract and female genital canals, obtaining nourishment from the secretions and/or food residues. These therefore constitute the normal flora of the healthy body being well established on the external and internal surfaces of the body without causing harm.[6,7] The true pathogens on the other hand have developed mechanisms of overcoming the normal defenses of the healthy body, they invade the tissue, proliferate and produce toxins which often cause damage to the tissues and result in the manifestation of disease.[8]

Since the discovery of penicillin, more antibiotics have been produced with novel ingredients and mechanisms of action but, the more the antibiotics, the more the pathogens find means of circumventing the effects of the antibiotics,[7,9] leading to antibiotic resistance.[9] Resistance is observed when microorganisms continue to grow at an attainable concentration in the presence of antimicrobial agents.[10] Clinically important drug resistant bacterial strains can circumvent effect of antibiotics by change/alteration of the structural target site for the drug, reduction in cellular permeability, conversion of the active substance to an inert product (via production of destructive enzymes) or via increased production of a biochemical intermediate.[8]

Bacteria pattern in children are the diverse strains of bacteria that causes infectious diseases in them. It is hypothesized that urinary tract infection (UTI) is caused by an ascending infection via the urethra. Colonic bacteria, especially *Enterobacteriaceae*, are the commonest organisms isolated from children with uncomplicated UTI. Infection with *Staphylococcus aureus* was thought to be rare in children without indwelling catheters or other sources of infection.[10] However, recent Nigerian studies had observed it as a common cause of UTI in otherwise well children. In 75 to 90% of female children with UTI, the incriminating organism is usually *Escherichia coli* followed by *Klebsiella* and *Proteus* while in the males older than 1 year, *Proteus* is as common as *E. coli* as a bacterial cause of UTI. The diagnosis of UTI in young children is important as it can be a marker for urinary tract abnormalities and, in the newborn, may be associated with bacteremia.[10] Early diagnosis is critical to preserve renal function of the growing kidney. Delay in initiation of the antibacterial therapy is associated with an increased risk of renal scarring, hypertension, and progression to end-stage kidney disease.

An antimicrobial agent is a chemotherapeutic agent used to treat the underlying cause of infectious diseases that is, by inhibiting microbial growth and microbial survival.[11] Bacterial infections are usually treated with antibiotics. However, the antibiogram or antimicrobial resistance of different strains of microorganisms varies widely. The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria.[12] Horizontal transfer of resistance among bacterial strains potentially poses serious danger in the community and hospital generally.

The commonest causes of under-5 morbidity in Southern Nigeria are malaria infections, acute-respiratory infections and skin infections. It is therefore a matter of renewed concern in the public to safe guard the health of children as they seem to be endangered and highly vulnerable due to their unintentional childhood habits and developing immune system which can be susceptible to cheap compromise from bacterial infections. Due to the varying nature of bacterial agents and their sensitivity to antimicrobials from place to place and time to time in the same environment, it is important to continually monitor bacterial isolates and their antimicrobial sensitivity pattern in infants, so that appropriate antibiotics are selected in time for the treatment of infections to avert possible sequelae.

The general objective of this study is to identify the bacteria causing infections in under-5 children in government approved crèches and nursery schools in Onitsha North LGA. Specifically, **t**his study ascertained the prevalence and drug resistance patterns of bacterial isolates; the anti-microbial susceptibility patterns of bacterial isolates and determined multidrug resistance profile of bacterial isolates.

MATERIALS AND METHODS

The study area is Onitsha North local government area in Anambra state, south-eastern Nigeria. Onitsha is the only town in the local government and is located on the eastern bank of the Niger River, in Nigeria's Anambra state. The town is a metropolitan and the commercial nerve centre of the South-East and South-South regions of Nigeria and is host to the Onitsha Main Market, the largest market in Africa in terms of geographical size and volume of goods.

Onitsha had an estimated city proper population of over quarter a million people. Its urban area occupies a land mass of 5030.8/km^2 and has been projected to reach around 1,500,000 inhabitants in 2021. Onitsha traditionally consists of nine villages namely Isiokwe, Olosi, Umuezearoli, Okebunabo, Obikporo, Ogbeotu, Awada (Ogbeozoma), Obamkpa and Ogbeotu. Also, the town lies at a major East-West crossing point of the Niger River and occupies the northernmost point of the river regularly navigable by large vessels. Rapid urbanization in recent years negatively affects natural vegetation and local landscape. Onitsha has also

transformed into a modern urban society in the present-day Anambra State. It has a federal government college, an army cantonment and a school of metallurgy, to mention a few landmark institutions.[13]

Study Design: was descriptive cross-sectional and the duration of the study was twelve (12) weeks.

Study Population was specifically under-5 children and their care givers in the local government.
Source of data: The primary source of data was obtained from the care givers and under-5 children.
Inclusion Criteria were Children who zero (0) to fifty-nine (59) months old and their Care givers. Exclusion Criteria were Children not present in school at the time of sample collection.

Sample Size Determination:
The sample size was determined using Cochran formula.[14]

$$N = \frac{Z^2PQ}{d^2} = \frac{z^2p\,(1-p)}{d^2}$$

Where: N = Desired sample size z = standard normal deviate at confidence level of 95% or 1.96
p = prevalence rate of bacteria is 50% [15], q = complementary prevalence (1-p), d = degree of precision/accuracy (0.05)
Therefore,

$$N = \frac{1.96^2 \times 0.5 \times (1-0.5)}{0.05^2} = \frac{0.9604}{0.0025} = 384.16 = 384$$

To minimize errors arising from non-response; n / (1-f) = 384 / (1-0.1)
Therefore, final sample size is 426.
Sampling Technique: Multistage and systematic sampling techniques were employed as follows: *Stage 1*: All communities in Onitsha North LGA were listed. A bottle was spun at the centre to determine the community to start with in a clockwise direction.
Stage 2: There are twenty-four (24) government approved crèches and nursery schools in Onitsha North LGA. From each, swab samples were collected from the palms of a minimum of twelve (12) children and six (6) different points on the floor. *Stage 3*: Every second male and female name respectively was selected from the class register for their palms to be swabbed.

Sample Collection, Transportation, Preservation and Handling: Swab samples were collected from the floors of the crèches and palms of the children using swab sticks and transported at 25^0c in tightly sealed, leak-proof plastic bags within an hour of collection to St. Joseph's Hospital Medical Laboratory, Adazi-Nnukwu. Streaking on media (agar in petridishes) commenced within one (1) hour of collection. All streaked plates or petridishes were placed in the incubator overnight at 37^0C to allow bacterial growth. The following plates characteristics were observed and documented; growth pattern, color, odour and appearance. Pure cultures of bacterial growths were preserved in nutrient agar slant bottles for subsequent use if needed. **Research Assistants** Three (3) research assistants who are graduates of Microbiology were trained on the research proceedings and deployed for questionnaire administration; oral interview of care givers and report writing; and swab samples collection.

Study Instruction and Data collection: A structured interviewer-administered questionnaire was used to collect data on socio demographic characteristics of the children and the classrooms.

All laboratory procedures were carried out aseptically wherein airflow was controlled to prevent contamination and import of unwanted microbes during the processes. Gram staining and biochemical tests were applied in identification and the Kirby Bauer method[16] was employed in antibiotic sensitivity testing, all as explained below;

Cultivation media for bacteria: Isolation of bacteria was done by culturing or growing them on a solid nutrient media surface. Also, a medium may be enriched by adding blood or serum. A selective media contains ingredients that inhibit the growth of some organisms but allow other to grow. An example; Mannitol salt agar has high concentration of sodium chloride which inhibits the growth of most organisms but permits staphylococci to grow. A differential media contains compounds that allow groups of micro-organisms to be visually distinguished by the appearance of the colony or the surrounding media mostly on the basis of some biochemical difference between the two.

Isolation of organisms: All pure isolated colonies were sub-cultured on blood agar plates (for growth of heterotrophic bacteria), chocolate and MacConkey agar plates (for coliforms) for 24hrs at 37^oC for colony isolation and morphological identification.

Gram staining: Gram staining was performed according to the method described in Cheesbrough.[16]

Identification of organisms: Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase and Urease tests.

Antibiotic susceptibility test: Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion technique[16] using antibiotic discs corresponding to the drugs most commonly used in the treatment of under-5 infections caused by bacteria.

Culture media used are: *Chocolate agar* for cultivating fastidious organisms like *Neisseria* or *Haemophilus sp*. *MacConkey agar*, a selective and differential medium that identifies non-fastidious gram-negative enteric bacteria. Red colonies indicate fermentation of lactose and white indicates no fermentation of lactose. *Blood agar* an enriched medium which encourages the growth

of fastidious bacteria like *Streptococci* that do not grow on ordinary growth media.

Each smear was properly prepared, labeled and fixed correctly before being stained using the gram staining technique. Biochemical tests like *Indole test* was used in the identification of enterobacteria. Most strains of *E. coli*, *P. vulgaris*, *P. rettgeri*, *M. morganii* and *Providencia* species break down the amino acid tryptophan with the release of indole. *Oxidase test* helped to identify *Neisseria*, *Pasteurella*, *Vibrio*, *Brucella* and *Pseudomonas* species which all produce cytochrome oxidase enzyme. Moreover *Catalase test* was used to differentiate staphylococci from streptococci by the production of the enzyme catalase. Furthermore, *Citrate utilization test* differentiated enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon. *Coagulase test* identified *Staphylococcus aureus* which produces the enzyme coagulase while the *Urease test* helped to identify *Proteus*, *Morganella*, *Y. enterocolitica* and *H. pylori*.

Data Processing and Analysis: Questionnaire was assigned serial numbers for entry identification of data collected. Thereafter, the data collected was coded, entered and cleaned. The analysis was done using the software – IBM Statistical Package for Social Sciences (SPSS) version 21. Descriptive statistics such as frequencies, percentages and standard deviation was used to summarize the independent and dependent variables.

Ethical Consideration: Ethical clearance to carry out the study was obtained from Nnamdi Azikwe University Teaching Hospital Health Research Ethics committee. Also, the local government's authorities were duly notified. The purpose of the study was explained to the proprietors and/or proprietresses of the crèches and nursery schools. Also, the non-inclusion or anonymity of their care giving facility/institution identified in the results was assured. The entire participants were treated with dignity and respect. Taking part in this study was voluntary.

RESULT

Four hundred and thirty-two (432) swab stick samples were collected and analyzed, 288 from the palms of the children and 144 from the floors of their crèches respectively. All questionnaires were adequately filled giving a response rate of one hundred percent (100%).

Table 1: Socio demographic characteristics of the Children and Classrooms.

Socio-demographic Characteristics	Frequency	Percent
Age (months)		
0-12	199	23.4
13-24	182	21.4
25-36	214	25.2
37-48	146	17.2
49-60	108	12.8
Total	**849**	100
Gender		
Male	426	50.2
Female	423	49.8
Total	849	100
Number of Classrooms		
1	9	37.5
2	9	37.5
3	6	25
Total	24	100
Types of Classrooms		
Single	22	91.7
Others	2	8.3
Total	24	100
Type of Floor		
Cemented	19	79.2
Tiled	5	20.8
Total	24	100

Table1 highlights the age and gender distribution of the children. The age distribution was classified into twelve months successively. Also shows the number of classrooms used across the twenty-four schools. Nine schools used one classroom for the children, another set of nine schools used two classrooms and six schools used three classrooms to accommodate the pupils. Also, the type of classroom and the type of floor in each classroom are shown on the table.

Table 2: Confirmation tests to identify bacteria isolates.

Bacteria / Biochemical tests	*Bl*	*Bm*	*Ea*	*Ec*	*Lb*	*Lc*	*Pa*	*Pc*	*Pf*	*Sa*	*Se*	*Ss*
Gram staining	+	+	-	-	+	+	-	-	-	+	-	+
Indole	-	-	+	+	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	-	-	+	+	+	+	+	+
Citrate	-	+	+	-	+	+	+	+	+	+	+	-
Oxidase	+	+	-	-	-	-	+	+	+	-	+	-
Coagulase	-	-	+	+	-	-	-	+	+	+	-	-
Urease	-	-	-	-	-	-	-	+	-	+	-	-

KEYS: Bl- *Bacillus lichenform;* Pa- *Pseudomonas aeruginosa;* Bm- *Bacillus megaterium*; Pc- *Pseudomonas cepacia*; Ea- *Enterobacter aerogenes* Pf- *Pseudomonas fluorescence*, Ec- *Escherichia coli*; Sa- *Staphylococcus aureus*; Se- *Staphylococcus epidermidis*; Lc- *Lactobacillus casei* Ss- *Staphylococcus saprophyticus*

Table 2 shows results of gram staining and biochemical tests carried out to differentiate and identify the bacteria isolates. A total of twelve (12) types of bacteria were isolated. Biochemical tests were carried out after culturing to identify pathogens with the use of substrates and by their enzymatic and fermentation reactions.

Table 3: Bacteria isolated from schools.

Sample School	Floor	Palm
1	*Staphylococcus saprophyticus*	*Staphylococcus aureus and Bacillus megaterium*
2	*S. saprophyticus and Pseudomonas cepacia*	*S. aureus*
3	*Staphylococcus epidermidis and P. cepacia*	*S. aureus*
4	*P. cepacia and Staphylococcus aureus*	*Lactobacillus casei and Bacillus lichenformis*
5	*S. saprophyticus and S. aureus*	*S. aureus*
6	*S. saprophyticus*	*Pseudomonas aeruginosa*
7	*Enterobacter aerogenes and S. aureus*	*Escherichia coli and Pseudomonas fluorescence*
8	*E. aerogenes*	*Lactobacillus brevis*
9	*S. aureus and E. aerogenes*	*Lactobacillus casei and S. aureus*
10	*S. aureus and S. saprophyticus*	*S. aureus and S. saprophyticus*
11	*S. saprophyticus and S. aureus*	*Pseudomonas fluorescence and S. saprophyticus*
12	*P. cepacia, S. aureus and S. saprophyticus*	*Escherichia coli and S. aureus*
13	*S. epidermidis and S. saprophyticus*	*Lactobacillus brevis and S. saprophyticus*
14	*S. epidermidis and P. cepacia*	*Bacillus megaterium and S. saprophyticus*
15	*S. aureus and E. aerogenes*	*Escherichia coli and L. casei*
16	*S. saprophyticus and S. aureus*	*Pseudomonas fluorescence and S. saprophyticus*
17	*P. cepacia and S. aureus*	*Bacillus megaterium*
18	*E. aerogenes*	*S. aureus and Pseudomonas aeruginosa*
19	*E. aerogenes and P. cepacia*	*Escherichia coli and Bacillus megaterium*
20	*S. saprophyticus and S. epidermidis*	*S. aureus and S. saprophyticus*
21	*S. aureus and S. saprophyticus*	*Escherichia coli and L. casei*
22	*S. epidermidis and P. cepacia*	*Lactobacillus brevis and Bacillus megaterium*
23	*S. saprophyticus*	*Lactobacillus brevis and S. epidermidis*
24	*P. cepacia and S. saprophyticus*	*Escherichia coli, Bacillus lichenformis and E. aerogenes*

Table 3 shows the specific bacteria isolates identified in the twenty-four (24) schools used in this study. In terms of the bacteria spread, *Staphylococcus aureus* was the most isolated followed by *Staphylococcus saprophyticus*

and *Staphylococcus epidermidis*. The percentage occurrence of bacteria isolates on the palms of the children was derived from table 3 which shows all the isolates from each crèche. *Staphylococcus aureus* (20.9%) and *Staphylococcus epidermidis* (14%) were the most frequently isolated. On the whole, 11 isolates were made from their palms.

Figures 1 and 2 show the percentage occurrence of bacteria isolates on the palm of the children and floor of the crèches with the later derived from Table 3 which shows all the isolates from each crèche. *Enterobacter aerogenes* (13.6%), *Pseudomonas cepacia* (20.5%), *Staphylococcus aureus* (25%), *Staphylococcus epidermidis* (11.4%) and *Staphylococcus saprophyticus* (29.5%). There are a total of five isolates from the floor of the crèches.

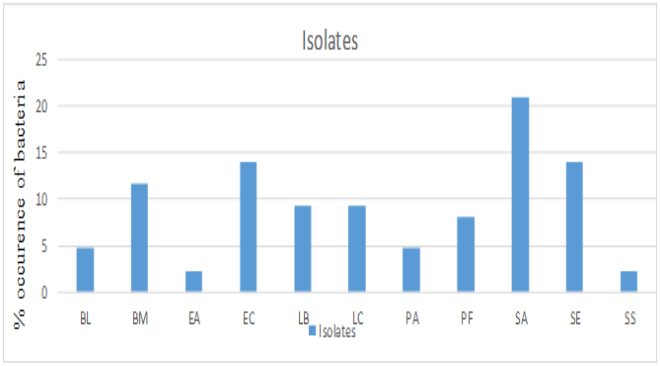

Figure 1: Percentage occurrence of bacteria isolates on the palms of the children.

KEYS: Bl- *Bacillus lichenform*; Pa- *Pseudomonas aeruginosa*; Bm- *Bacillus megaterium*; Pf- *Pseudomonas fluorescence*; Ea- *Enterobacter aerogenes*; Sa- *Staphylococcus aureus*; Ec- *Escherichia coli*; Se- *Staphylococcus epidermidis*; Lb- *Lactobacillus brevis*; Ss- *Staphylococcus saprophyticus*; Lc- *Lactobacillus casei*

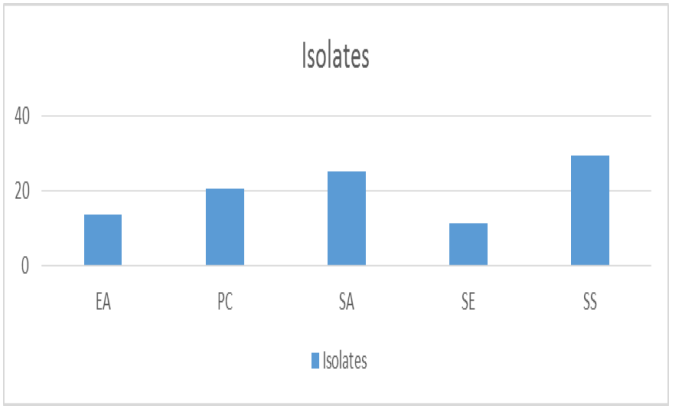

Figure 2: Percentage occurrence of bacteria isolates on the floor.

KEYS: Ea- *Enterobacter aerogenes*; Pc- *Pseudomonas cepacia*; Sa- *Staphylococcus aureus*; Se- *Staphylococcus epidermidis* Ss- *Staphylococcus saprophyticus*

Table 4 presents the levels of reactions of gram positive bacteria isolates to the antibiotics disc. Some isolates were resistant (had no zone of inhibition around them) to some antibiotics and those sensitive to any antibiotic showed varying diameters of zone of inhibition around the antibiotics. The widest zone of inhibition represents the most effective antibiotic (s) against the bacteria isolate. The graph (Figure 3) gives a clearer view of the action of each antibiotic on gram positive bacteria isolates. Starting from the most effective to the least; Gentamicin (100%), Ofloxacin (50%), Cefuroxime (50%), Erythromycin (50%), Ceftriaxone (33.3%), Augmentin (16.7%), Ceftazidime (16.7%) and Cloxacillin (0%).

Table 4: Antibiotic sensitivity of gram positive isolates.

Antibiotics / Isolates	GEN	OFL	AUG	CRX	CTR	CXO	ERY	CAZ
Bacillus lichenformis	S	S	R	R	R	R	R	R
Bacillus megaterium	S	R	R	R	R	R	S	R

Lactobacillus brevis	S	R	R	I	I	R	S	R
Lactobacillus casei	S	R	R	I	I	R	S	S
Staphylococcus aureus	S	S	S	I	R	R	R	R
Staphylococcus saprophyticus	S	S	R	R	R	R	R	R

KEYS: R- Resistant; I- Intermediate; S- Susceptible; GEN- Gentamicin; OFL- Ofloxacin; AUG- Augmentin; CRX- Cefuroxime; CTR- Ceftriaxone; CXO- Cloxacillin; ERY- Erythromycin; CAZ- Ceftazidime

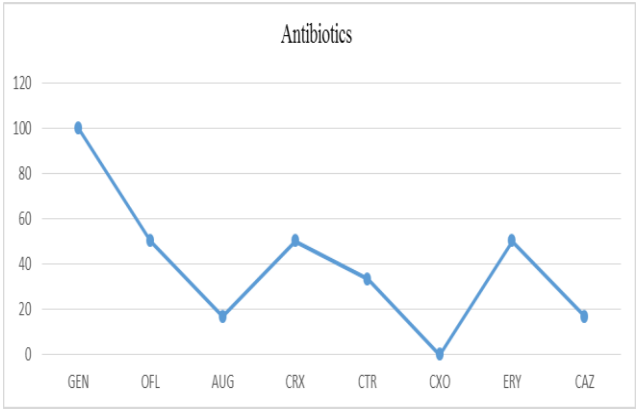

Figure 3: Percentage resistance of gram positive isolates to antibiotics.

Table 5 presents the levels of reactions of gram negative bacteria isolates to the antibiotics disc. Some isolates were resistant (had no zone of inhibition around them) to some antibiotics and those sensitive to any antibiotic showed varying diameters of zone of inhibition around the antibiotics. The widest zone of inhibition represents the most effective antibiotic (s) against the bacteria isolate. The graph (Figure 4) gives a clearer view of the action of each antibiotic on gram negative bacteria isolates. Starting from the most effective to the least; Ciprofloxacin (100%), Ofloxacin (100%), Gentamicin (75%), Cefuroxime (66.7%), Nitrofurantoin (58.3%), Cefixime (33.3%), Ceftriaxone (33.3%) and Augmentin (8.3%).

Table 5: Antibiotic sensitivity of gram negative isolates

Antibiotics / Isolates	**GEN**	**OFL**	**AUG**	**CRX**	**CTR**	**CPR**	**NIT**	**CXM**
Enterobacter aerogenes	R	S	R	R	R	S	R	S
Escherichia coli	S	S	I	S	R	S	S	S
Pseudomonas aeruginosa	S	S	R	S	R	S	I	R
Pseudomonas cepacia	S	S	R	I	I	S	I	R
Pseudomonas fluorescence	S	S	R	I	I	S	I	R
Staphylococcus epidermidis	I	S	R	S	S	S	S	R

KEYS: R- Resistant; I- Intermediate; S- Susceptible; GEN- Gentamicin; OFL- Ofloxacin; AUG- Augmentin; CRX- Cefuroxime; CTR- Ceftriaxone; CPR- Ciprofloxacin; NIT- Nitrofurantoin; CXM- Cefixime

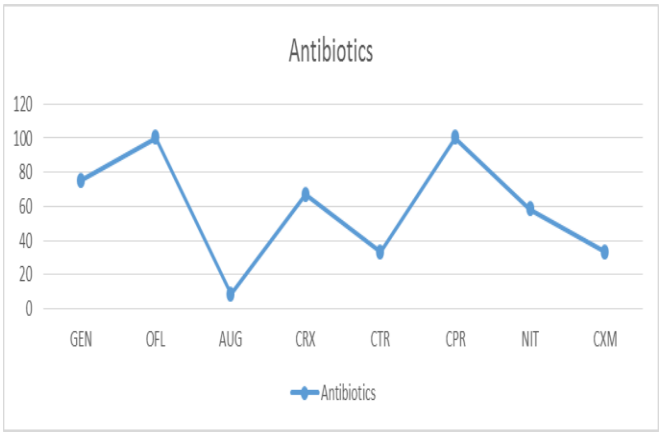

Figure 4: Percentage resistance of gram negative isolates to antibiotics.

DISCUSSION

The highest occurring bacteria isolates were *Staphylococcus saprophyticus* (43.5%), *Staphylococcus aureus* (41.4%) and *Pseudomonas cepacia* (20.5%). The isolation of *Lactobacillus brevis, Lactobacillus casei* and *Pseudomonas fluorescence* which are non-pathogenic bacteria from the palms of the children can be attributed to its presence in baby feed formula. According to FDA, the standard regulators stipulated that *B. cereus* must be less than and/or equal 100 cfu/g in infant formula.[17] In another investigation, 10% of samples infant formula was contaminated with *B. cereus* with counts ranged from $4.0X10^1$ to $2.1X10^2$ and average $1.45X10^2$.[18] The marked differences in percentage and range of *B. cereus* may be due to the different formulas, different types of samples and different tests.[19] New nontoxic and high stable bioactive materials were developed for controlling of the pathogens. In addition to food grade, natural materials may be the best chance to control of the pathogens.[20]

Serious infections caused by *Bacillus* species include ocular infections, endocarditis, bacteremia and septicemia. Also pneumonia, meningitis, musculoskeletal infections[21] and infections associated with injuries from motor vehicle accidents associated with road trauma.[22] Systemic antibiotic therapy is usually required in the treatment of most serious *Bacillus* infections. From the study, Gentamicin was a highly active bactericidal antibiotic against both *Bacillus* species. There was high resistance to all other antibiotics. The significant increase in both multi-resistance and range of bacterial pathogens displaying resistance to a growing number of clinically important drugs is threatening the success of medical therapy.[23] The incidence of bacteria which are resistant to one or to multiple antibiotics has been traditionally studied by plating on antibiotic-containing nutrient media, or by screening bacterial isolates for their antibiotic resistance patterns.[24]

Staphylococcus spp. has the highest occurrence on both the palms of the children and their classrooms. The microbe is usually found on the human skin and mucous membranes.[25] This indicates that staphylococcal infections are common in crèches within the study site. Staphylococcal infections can be superficial, affecting the skin and subcutaneous tissue or deep into the skin. *Staphylococcus aureus* remains one of the most common and troublesome of bacteria causing disease in humans, despite the development of effective antibacterials.[25] The organism is responsible for over 70% of all skin and soft tissue infections in children and accounts for up to one-fifth of all visits to pediatric clinics.[25]

There is an increasing trend towards the isolation of *Staphylococcus saprophyticus* in urinary tract infections in children. The risk of a UTI during the first decade of life is 1% in males and 3% in females. It has been suggested that 5% of school girls and up to 0.5% of school boys undergo at least one episode of UTI during their school life.[26] *S. epidermidis* is a common commensal bacterium of the human skin and mucosa. While *S. epidermidis* has long been considered nonpathogenic, it is now recognized as a relevant opportunistic pathogen.[27,28] Most *S. epidermidis*-related not only are associated with intravascular devices (prosthetic heart valves, shunts, etc.) but also commonly occur in prosthetic joints, catheters and large wounds. However, recently published data revealed the high prevalence of *S. epidermidis* in the cases of human clinical infections.[27-29] Cefuroxime, Ceftriaxone and Nitrofurantoin are effective antibiotic options to treat specifically *S. epidermidis* infections.

Consequences of *Staphylococcus spp.* can be serious because the pathogens are considered the main cause of surgical and skin infections being also responsible for the toxic shock syndrome and severe illnesses including pneumonia, meningitis, endocarditis and septicaemia.[30] Furthermore, it is considered an important pathogen in children that commonly causes pharyngitis because of its pathogenic mechanisms such as the production of virulence factors.[30] High level of sensitivity to Gentamicin, Ciprofloxacin and Ofloxacin was seen in the present study. Cefuroxime was effective against both *S. aureus* and *S. epidermidis*. The resistance of the isolates generally to Ceftriaxone, Augmentin, Ceftazidime and Cloxacillin could be linked to common use of these

antibiotics. It's indicated in another study that single drug treatment can lead to cross-resistance to other unrelated antibiotics.[31]

Enterobacter aerogenes as well as others in its genus are known to be resistant to antibiotics, especially *E. aerogenes* and *E. cloacae*.[31] Selection of enteric bacteria like *E. aerogenes* is opportunistic and only infects those who already have suppressed host immunity defenses.[31] Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections.[31] *Enterobacter aerogenes* has shown to display multidrug resistance due largely to mutations that encode porins (protein channels) and membrane efflux pumps that pump out antibiotics before they can harm the organism. These have been shown to be non-specific which accounts for their multiple drug resistance.[32] Also, in this study, *Enterobacter aerogenes* was only susceptible to three antibiotics which are Ofloxacin, Ciprofloxacin and Cefixime.

E. coli is a type of bacteria that normally live in the intestines of people and animals. It can cause intestinal infection. The symptoms are diarrhea (can be bloody when severe), abdominal pain and cramps, fatigue, fever, loss of appetite or nausea and dehydration (healthline.com). Children with weakened immune system are at increased risk for developing these complications. Causes include; improper food handling, poorly processed food, contaminated water, animal to person and person to person (especially amongst children) (healthline.com). Diarrhoea is a frequent illness in developing countries and contributes to the deaths of 4.6 million to 6 million children annually in Asia, Africa and South America.[33] It has been estimated that in the very poor countries of these regions each child suffers up to 15 to 19 episodes of diarrhoea per year.[34] In Nigeria, available reports indicate that more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease.[35,36] Nevertheless, despite the public health implications and enormous burden imposed on the primary health care delivery system by infantile diarrhoea illness in the country, there is still paucity of information on the epidemiology and aetiology of infantile diarrhoea in many regions including the South-eastern Nigeria.[34]

Although the spectrum of bacterial isolate implicated in acute childhood diarrhoea varies from one region to the other, *E. coli* was the commonest bacteria pathogen isolated in this investigation and this was in conformity with findings from Abakaliki, Ebonyi state.[37] Both *E. coli* and *Salmonella spp.* are reported to be very commonly associated with enteric diseases in developing countries and are more important to the epidemiology of diarrhoea in poorer areas.[38] In this study, a number of the children could come down with diarrhoea due to the possibility they forget to wash their hands after eating. *Pseudomonas spp.* were all susceptible to five (5) antibiotics; Gentamicin, Ofloxacin, Cefuroxime, Ciprofloxacin and Nitrofurantoin. They showed 100% resistance to Augmentin and Cefixime while *Pseudomonas aeruginosa* was the only isolate resistant to Ceftriaxone.

Regulation of antibiotics usage is poor with their easy and over the counter availability without prescription. This could be linked to the high level of resistance observed in the study. Some health care workers and pharmacists are often paid incentives by the pharmaceutical companies to prescribe or sell irrelevant antibiotics.[39] Medical practice by unqualified personnel, who often prescribe irrelevant antibiotics, is yet another common problem in Nigeria. Locally produced antibiotics are of questionable quality and compliance of the patients is also considerate.[39] Gentamicin (89%) and Ofloxacin (75%) have a combined highest sensitivity on both gram positive and negative bacteria isolates. The study also demonstrates that Ciprofloxacin (100%) has the highest sensitivity on gram negative isolates. These show that they will be appropriate antibiotics for treatment in under-5 children. From the anti-susceptibility profile, Cloxacillin, Ceftazidime and Augmentin were the least sensitive to gram positive bacterial isolates. The percentage of resistance are; 0% and 19% each respectively. Augmentin showed 10% sensitivity against gram negative bacterial isolates. Deducing from the high rate of resistance of both gram positive and negative isolates to these antibiotics, administering them to treat sick children will yield inconsequential result as the sickness has a high tendency to persist.

In conclusion, Pathogenic bacteria isolated in this research are causes of opportunistic infections in humans from under-5 children to the aged. Therefore, child care services and their service providers must be optimal in hygiene and preventing conditions that can lead to disease outbreak in their crèches. Under-5 children are prone to infectious diseases and are more amenable to immediate care because some of the sicknesses suffered are environmental in origin. These infections contribute to the health problems of under-5 children in their early years. The sicknesses can be contained and prevented by effective care from home and care givers at school who have acquired relevant hygiene skills coupled with high maintenance of a neat environment.

Recommendations

Based on the findings, the following recommendations are given;

1). Consistent teaching and demonstration classes on personal hygiene with efficient educational teaching aids should be done for the children. Simple tasks and class activities that will help them develop a healthy lifestyle should also be incorporated. 2). Advocacy groups for clean environment in academic spheres should regularly train caregivers on the need to keep their learning environs neat. 3). Research on new and effective anti-

bacterial substances will go a long way to combat widespread microbial against existing ones. 4). Inter-ministry interaction and collaborative efforts between education and health are key to sharing knowledge capital aimed at eradicating the spread of infections in schools amongst under-5 children. This should also involve other non-governmental organizations that have the capacity to help develop a safer learning environment.

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