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Microbial Contamination of Infant Diapers

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Abstract *This study investigated the microbial contamination of infant diapers. Five (5) different samples of infant diapers were used for this study. The results revealed that samples were contaminated by microorganisms. The highest bacterial count was recorded in sample 1 with a total bacterial count of 2.0 x 10^3 CFU/g, followed by sample 3 with a total bacterial count of 1.4 x 10^3 CFU/g. Sample 4 had a bacterial count of 1.3 x 10^3 CFU/g, sample 5 had a total bacterial count of 1.2 x 10^3 CFU/g while the least bacterial count was recorded in sample 2 with a total bacterial count of 1.0 x 10^3 CFU/g. The bacterial genera identified and their percentage frequency of occurrence were Bacillus sp.(29%), Staphylococcus sp.(35%), Pseudomonas sp.(12 %), Streptococcus sp.(24 %),while ,E.coli and Proteus sp. had 26 % percentage frequency of occurrence each respectively. The fungal count ranged between 1.0 x 10^3 to 1.6 x 10^3 CFU/g. The fungal isolates identified from this study and their percentage frequency of occurrence were Candida albicans (24 %), Penicillium sp.(19 %), Rhizopus sp.(10 %), Aspergillus sp.(14 %) and Mucor sp.(8 %). Susceptibility results indicated that 95 % of that bacterial isolates were sensitive to Ciprofloxacin, Gentamycin, Levofloxacin, Oflaxacin, Ampicillin, Septrin, Ceprorex, Amoxil, Streptomycin, and Rifampicin. Five bacterial isolates were resistant to more than one class of antibiotics. E.coli. was resistant to Pefloxacin, Ceprorex, and Ampicillin. Pseudomonas sp. was resistant to pefloxacin and gentamycin. Proteus sp. was resistant to septrin and pefloxacin while Bacillus sp was resistant to amoxil, rifampicin and chloramphenicol. The presence of these microbial contaminants in diapers could be attributed to existing of microorganisms in the raw materials during processing and manufacturing, packaging, storage, handling and transportation of this product. Hence, a routine microbial study is thus suggested.*

Keywords: *Microorganisms, contamination, diapers, bacterial isolates, fungal isolates*

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1.0 Introduction

Diapers, also known as nappies are the underpants consisting of the absorbent materials especially designed for the infants so as to prevent the leakage and elimination of waste products from babies at inapt places and maintain hygiene. Diapers can be prepared from a number of natural fibers such as cotton, muslin, bamboo, wool, linen, jute, etc and synthetic fibers such as non-woven polypropylene, polyethylene, nylon, polyester, etc. Diapers are designed in such a way that the top sheet (facing to the infant's skin) of

the diaper comprises of the antimicrobial agents to encumber or inhibit the growth of bacteria and cease its action on the baby.

A diaper (nappy) is a type of underwear that allows the wearer to defecate or urinate without the use of a toilet, by absorbing or withholding waste products to prevent soiling of outer clothing or the external environment. A disposable diaper by definition consists of three layers, permeable top layer made of nonwoven fabric, an absorbent pad in the middle layer and an impermeable bottom layer (Stamatas and Tierney, 2014).

Diapers are made of cloth or synthetic disposable materials. Cloth diapers are composed of layers of fabric such as cotton, hemp, bamboo, microfiber, or even plastic fibers, and can be washed and reused multiple times. Disposable diapers contain absorbent chemicals and are thrown away after use (Odio and Friedlander, 2000).

The use of cloth diapers has been practiced from ages. Till date, many mothers prefer using cloth during the night and outings. Factors including, diaper material, financial status, belief, and child's comfort influence the use of diapers (Thaman and Eichenfield, 2014).

When diapers become soiled, they require changing, generally by a second person such as a parent or care giver (Evans *et al.,* 2014). Diapers are primarily worn by infants, toddlers who are not yet potty trained, and by children who experience bed wetting. However, some health conditions such as incontinence, mobility impermanent, severe diarrhea or dementia have also made it necessary for diaper to be available for adult (Helmes *et al.*, 2014). Other conditions include some physical or mental disability, diaper fetishists, and people working in extreme condition, such as astronauts (Clark –Greuel *et al.,* 2014).

Diaper dermatitis is an irritant contact dermatitis that is seen between the ages of 6 and 12 months. Factors that are involved in the pathophysiology of diaper dermatitis are excessive wetness, friction, high pH, high enzymatic activity due to faeces, urine that compromise the epidermal barrier function (Erasala *et al.,* 2007). Dermatitis prevalence is estimated at 7–35 %, and incidence is highest in infants between 9 and 12 months of age (Klunk *et al*., 2014; Kazaks and Lane, 2000;Tuzun *et al*., 2105; Adalat *et al*., 2007). he water absorbing capacity of cloth napkin is very low as compared to modern disposable diaper which increases the chances of leakage and irritant contact dermatitis. Due to the use of cloth diapers, the frequency of diaper change also increases. There is a belief that cotton diapers give more breathability (Akin *et al.,* 2001). However, with the advanced designs and function of diapers, many parents are opting for modern disposable diaper than cloth diapers (Counts *et al.,* 2014).

The skin microflora of young children is varied and is influenced by age, hygiene, pH, moisture, soiling and other factors. Variations in skin moisture and the availability of nutrients are cited as the major reason for differences in skin flora. Diapers can influence the skin microflora by affecting skin moisture and by maintaining urine and faeces in close proximity to the skin. For example, the flora on skin under diapers is known to be increased in number, as compared to skin not covered by a diaper (Odio *et al.,* 2000).

It has been found that diapers used for babies could leads to some health-related complications through the interaction of baby skin and the unhygienic status of the diaper. This can also could trigger colonization of microorganisms on the baby diaper. Therefore, this study is aimed at investigating the microbial contamination of infant diapers.

2.0 Materials and Method

2.1 Study area

The study was carried out in Ikot Ekpene Local Government of Akwa Ibom State, Nigeria. Ikot Ekpene Local Government Area has an area of 125 km^2 and a population of about 254,806; with 5.18^o North latitude and 7.71 East longitude and 159 meters elevation above the sea level.

2.2 Sample collection / preparations

Different samples were purchased at pharmaceutical stores and supermarkets/ superstores within in Ikot Ekpene metropolis. Samples were packaged in a polyethylene bag and transported to the Microbiology Laboratory for microbiological analysis.

2.3 Microbiological Analysis

2.4 Sterilization of Materials and Media Used

Sterilization of the materials was done according to the methods described by Cheesbrough, (2010). All the glass wares used in this analysis were washed, dried and sterilized in a hot air oven at 160 °C or one hour. Culture media were sterilized at 121 °C for 15 minutes by moist heat using an autoclave. Inculcating loop was sterilized by flaming, using dry heat, while the laboratory

working bench was disinfected using cotton wool soaked in 70% ethanol.

2.5 ***Media composition***

The media used in this analysis were nutrient agar and Sabouraud Dextrose Agar (SDA) and were prepared according to the manufacturer instruction and specification, and were sterilized in an autoclave at 121 °C for 15 minutes, then they were transferred to the laboratory working bench and allowed to cool to about 45 °C before use.

2.6 Inoculation of samples

These were carried out by streak plate technique of inoculation. About 15 ml of an already prepared medium (nutrient agar and Sabouraud dextrose Agar) was aseptically poured into sterile petri dishes respectively, swirled gently and allowed on the laboratory bench to solidify. Thereafter, the swab sticks that were used to swab the sample were streak on the surface of the agar on the respective plates. The plates were wrapped with foil paper and labelled respectively, and the plates which contained the nutrient agar were incubated invertedly at 37^0C for 24 hours for bacterial growth and the plates which contained Sabouraud Dextrose Agar were kept in the inoculating chamber for 5 – 7 days for fungal growth.

2.7 Characterization of bacterial and fungal isolates

Characterization of bacterial isolates was carried out using the method reported elsewhere (Cheesbrough, 2000). The main procedures carried on the bacterial isolates were gram staining, coagulase, catalase, oxidase, motility, citrate, urease, spore test and sugar fermentation (glucose, sucrose, lactose, maltose). And that of fungal isolates were macroscopic and microscopic examination of the fungi isolates.

2.8. Purification of bacterial colonies

Sub-culturing of all the grown colonies were done by streak plate method on fresh prepared agar plates. They were incubated for 24 hours at 37^0C.

2.9 Identification of bacterial and fungal isolates

Visual inspections for the colonial appearance of all the isolates were carried out in order to identify the gross characteristics. This inspection of the isolates was done by holding the plates in one hand and observing for surfaces, growth shape, edge, elevation, consistency and pigmentation. Then, that of fungal isolates were also done based on macroscopic examination of fungi based on the following characteristics; surface colour, reverse colour and appearance of the fungi isolate.

2.10. Antibiotic susceptibility test

This test determines which antibiotics will kill the bacteria that have been isolated from the culture. Mueller Hinton agar was prepared according to manufacturer's recommendation. After sterilization, the agars were dispensed into desired petri dishes and were allowed to solidify. Using disc diffusion method, the test organisms were aseptically picked with a sterile wire loop and were streaked uniformly over the entire surface of the medium. A commercial sensitivity disc was aseptically transferred to the centre of the inoculated plates using a forceps. Both gram positive disc and gram-negative disc were place differently based on the test organism. All the inoculated plates were incubated invertedly at 37 ^{0}C for 24 hours. After incubation, observations of the zone of inhibition around the sensitivity disc on the plates were measured with ruler in mm while no zone of inhibition showed negative result. Interpretation of the result was done using the zone of inhibition sizes according to standard of NCCL (2007).

2.11 Statistical analysis.

All results were analysed statistically using SPSS mini tab (version 2.0)

3.0 Results and Discussion

Table 1 shows the total bacterial counts of colonies isolated from different samples obtained. The highest bacterial count was recorded in sample 1 with a total bacterial count of 2.0 x 10^3 followed by sample 3 with a total bacterial count of 1.4 x 10^3. Sample 4 had a bacterial count of 1.3 x 10^3, sample 5 had a total bacterial count of 1.2 x 10^3 while the least bacterial count was recorded in sample 2 with a bacterial count of 1.0 x 10^3.

Fig. 1 show the percentage frequency occurrence of the bacterial isolates obtained from various diapers sample. The highest percentage frequency of occurrence of 35 % was recorded in *Staphylococcus aureus,* Bacillus *sp.* recorded the percentage frequency of 29 %.

Table 1: Total Bacterial Count

Samples	Total bacterial Count (CFU/)g.
1	2.0 x 10^3
2	1.0 x 10^3
3	1.4 x 10^3
4	1.3 x 10^3
5	1.2 x 10^3

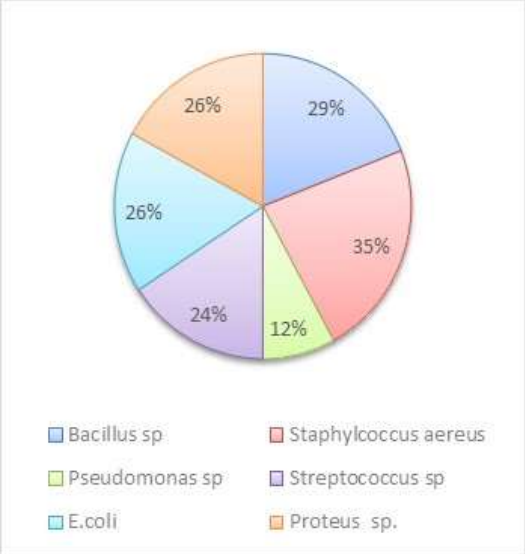

Fig. 1. Percentage frequency of bacterial isolates.

Proteus sp. and *E.coli* recorded 26 % each. *Streptococcus sp* had a percentage frequency of 24 % while the least percentage frequency of occurrence of 12 % was recorded in of *Pseudomonas sp*.

Table 2 presents the total fungal count obtained from the samples. Sample 3 had the highest fungal count of 1.6 x 10^3 followed by sample 5 with 1.3 x 10^3, while sample 1 had a total fungal count of 1.1 x 10^3. Sample 2 had the least fungal count of 1.0 x 10^3. Sample 4 recorded no fungal count.

Fig. 2 shows the percentage frequency of occurrence of fungal isolates. The highest frequency occurrence of 24 % was recorded for *Candida albicans, sp.*, followed by *Peniccilium sp* with 19 %, *Aspergillus sp. had 14* % , *Rhizopus sp.* had 10 % frequency of occurrence and the least was recorded in *Mucor sp.* with 8% of occurrence.

Table 2: Total Fungal Count

Samples	Total bacterial Count (CFU/)g.
1	1.1 × 10^3
2	1.0 × 10^3
3	1.6 × 10^3
4	-
5	1.3 × 10^3

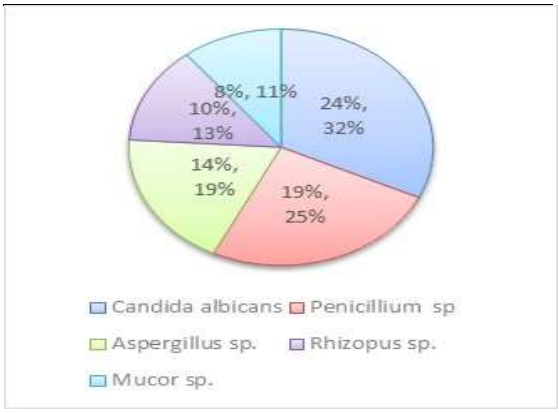

Fig. 2. Percentage frequency occurrence of fungal isolates.

Table 3 shows the antibiotic susceptibility profile of the bacteria isolates obtained from the samples. From the table, *Bacillus sp.* was highly sensitive to chloramphenicol, ciprofloxacin, streptomycin, erythromycin, ampiclox and gentamycin. *Micrococcus sp.* was highly sensitive to norfloxacin, levofloxacin, ciprofloxacin, gentamycin, amoxil, streptomycin, erythromycin and chloramphenicol. *Staphylococcus sp.* was highly sensitive to ciprofloxacin, streptomycin, rifampicin and chloramphenicol. *Pseudomonas sp.* was highly sensitive to ofloxacin, pefloxacin, sceptrin, streptomycin and nalidixic acid

Table 3.3: Antimicrobial susceptibility patterns of the Bacterial Isolates

No of Bacterial Isolates	**Antibiotics for Gram Positive Organisms**									
	CPX	**NB**	**CN**	**AML**	**S**	**RD**	**E**	**CH**	**ATX**	**LEV**
Streptococcus sp.	14mm	18mm	20mm	22mm	30mm	18mm	24mm	28mm	26mm	30mm
Bacillus Sp.	32mm	24mm	27mm	10mm	22mm	10mm	18mm	12mm	18mm	20mm
Staphylococcus aureus.	24mm	12mm	28mm	18mm	27mm	-	30mm	18mm	20mm	28mm

Antibiotics for Gram Negative Organisms

	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
Proteus sp.	20mm	12mm	30mm	18mm	10mm	11mm	28mm	-	10mm	26mm
*Pseudomonas s*p.	26mm	12mm	22mm	-	10mm	16mm	14mm	18mm	26mm	19mm
E.coli	28mm	12mm	20mm	26mm	17mm	22mm	10mm	-	18mm	10mm

NCCL,2007.

KEYS: S → Sensitive, I → Intermediate, **R** → Resistant, > 18 and above was considered sensitive, >13 – 17mm was intermediate and 0 – 12mm was resistant

Antibiotics disc for gram positive and negative organisms

Gram Positive Antibiotics			Gram Negative Antibiotics		
CPX	**-**	**Ciproflox (10mcg)**	**OFX**	**-**	**Tarivid (10mcg)**
NB	**-**	**Norfloxacin (10mcg)**	**PEF**	**-**	**Reflacin (10mcg)**
CN	**-**	**Gentamycin (10mcg)**	**CPX**	**-**	**Ciproflox (10mcg)**
AML	**-**	**Amoxil (20mcg)**	**AU**	**-**	**Augmentin (10mcg)**
S	**-**	**Streptomycin (30mcg)**	**CN**	**-**	**Gentamycin (10mcg)**
RD	**-**	**Rifampicin (20mcg)**	**S**	**-**	**Streptomycin (30mcg)**
E	**-**	**Erythromycin (30mcg)**	**CEP**	**-**	**Ceprorex (10mcg)**
CH	**-**	**Chloramphenicol (30mcg)**	**NA**	**-**	**Nalidixic Acid (30mcg)**
APX	**-**	**Ampiclox (20mcg)**	**SXT**	**-**	**Septrin (30mcg)**
LEV	**-**	**Levofloxacin (20mcg)**	**PN**	**-**	**Ampicillin (30mcg)**

The microbiological analysis and antibiogram of infant diapers were studied. Results obtained showed that the total bacterial count ranged between 1.0 x 10^3 CFU/g to 2.0 x 10^3 CFU/g. The bacterial genera isolated from samples were *Staphylococcus aureus, Streptococcus sp,. Bacillus sp*, *Proteus sp., E.coli, and Pseudomonas sp.* The percentage frequency of occurrence of bacterial isolates were *Staphylococcus aureus* (35 %), *Bacillus sp* (29 %), *Proteus sp* (26 %), *E. coli* (26 %), *Streptococcus sp.* (24 %), while *Pseudomonas sp*. had the least percentage of occurrence of (12 %).

The fungal count ranged between 1.0 x 10^3 to 1.6 x 10^3 CFU/g. The percentage frequency of occurrence of fungal isolates were *Candida albican* (24 %), *Penicillium sp* (19 %), *Aspergillus sp*. (14 %), *Rhizopus sp*. (10 %). *Mucor sp* had the least percentage of occurrence of (8%). This finding correlates with the work of Keswick, (1983), Akin *et al.,* (2001) and Odio *et al.,* (2000), who also reported the presence of some aforementioned bacterial and fungal genera in baby diapers and skin care products. The high microbial count in some samples could be attributed to the existent of microorganisms in the raw materials during manufacturing, low amounts of antimicrobial agents embedded in the diaper, handling, transportations and other environmental and intrinsic factors. The presence of these microorganisms could cause a serious infection to infants. *Staphylococcus sp* had the highest frequency of occurrence of 35%. This bacterium can secrete toxins that can cause gastrointestinal disorders (Kotch *et al*., 2007). Its presence maybe attributed to human handling and other factors since it resides normally on the skin and mucous membrane of human and other organisms.

Bacillus sp. was found to be present during the course of the work. *Bacillus sp.* are aerobic sporulating rod shaped bacteria that are ubiquitous in nature. Some species of this genus exhibit a wide range of physiologic abilities that allow them to live every natural environment. The spores are resistance to heat, cold, radiation, desiccation and disinfectants (Prescott *et al.,* 2005).

In addition, the presence of *Escherichia coli* is a good indicator of faecal contamination probably from the water used during manufacturing. *Proteus sp* is Gram-negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water, and fecal matter. The presence of *Pseudomonas sp.* in diapers maybe associated with poor hygienic condition during manufacturing, storing, packaging, transportation etc. The USP (2007)

pointed out that *Pseudomonas, E.coli, Staphylococcus aureus* and *Candida albicans* are indicators of pathogenic contamination. According to Kotch *et al.,* (2007), diapers surface that are porous, cracked, or damaged increases the likelihood that pathogens will escape disinfection or anti-microbial agents and allow transmission of fungi and bacteria which could cause side effect of diapers to babies.

The USP (2007) recommends *Salmonella sp*., *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus*, and Candida albicans as indicators of pathogenic microbial contamination of The USP (2007) recommends *Salmonella* sp., *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, and *Candida albicans* as indicators of *pathogenic microbial* contamination of The USP (2007) recommends Salmonella sp., Pseudomonas aeruginosa, Escherichia coli, *Staphylococcus aureus* and *Candida albicans* as indicators of pathogenic microbial contamination.

The presence of this bacterial and fungi genius in diapers analysed in this study – indicates that diapers may cause allergic reactions, skin rashes (Diapers rash) toxicity and chances of infection. *Aspergillus* and *Penicillium* are possible allergic and toxin producers (Prescot *et a*l., 2005). Similarly, NAFDAC Handbook (2000), stipulates that fungi count must not exceed 1.0x102 cfc/ml, but the results on fungi count far exceeded this value in all the 5 samples with fungi growth. Some of the bacterial isolates were sensitive to more than one class of antibiotics. Six drug resistant isolates (*Staphylococcus aereus, Bacillus sp, Proteus sp, E.coli, Streptococus sp, Pseudomonas sp.)* were subjected antibiotic susceptibility testing. *Streptococcus sp* was sensitive ciproflox, gentamycin, amoxil, streptomycin, erythromycin and chloramphenicol and levofloxacin. *Bacillus sp* was sensitive to ciproflox, norfloxacin, gentamycin, streptomycin and lefloxacin. but resistant to amoxil, rifampicin and chloramphenicol. *Staphylococcus aureus* was sensitive ciproflox, gentamycin, amoxil, streptomycin and levofloxacin. Proteus sp. was sensitive to ofloxacin ciproflox, gentamycin, ceprorex, septrin and ampicillin but resistant to reflacin. *Pseudomonas sp* was sensitive to ofloxacin, ciproflox, ceprorex, and ampicillin but resistant to reflaxcin and gentamycin. *E. coli* was sensitive to ofloxacin , ciproflox, augmentin, streptomycin and septrin, but resistant to reflaxcin, ceprorex and ampicillin. It was observed that that, some organisms were sensitive to some antibiotics, while some were resistant according to NCCLS (2007).

The result obtained agrees with the work of Adalat *et al.,* (2007), who isolated *Pseudomonas sp.* from wound infection and was susceptible to ofloxacin, ampicillin, perfloxacin and ceprorex.. This work also correlates with the finding of Al- kaf *et al.,* (2015), who reported susceptibility pattern of clinical isolates to ciprofloxacin, amoxil, erythromycin and streptomycin. The antibiotic resistance is as a result of natural resistance in certain types of bacteria, genetic mutation or by species acquiring resistance from another. Moreover, the misuse of antibiotic promotes the development of antibiotic-resistance bacteria.

4.0 Conclusion

The results and findings if the present study indicated that there is higher microbial load in the investigated diaper samples, which indicate that they are contaminated. Microorganism identified in the samples were S*taphylococcus aureus, Streptococcus sp,. Bacillus sp*, *Proteus sp., E.coli, Streptococcus sp., and Pseudomonas sp.* Therefore, diapers have the potential to be contaminated and cause skin infections on babies that use them. Hence, there is need for proper handling of diapers meant to be used on infants.

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Conflict of Interest

The authors declare no conflict of interest