

ORIGINAL RESEARCH ARTICLE

Isolation, identification, and antibiotic susceptibility profile of bacterial isolates from unused diapers sold in major markets in Enugu Metropolis: Clinical and policy perspectives

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Abstract

Diapers, being one of the most used items for infant care, it is imperative that they are always free of contamination from microorganisms. The objective of this study is to determine the rate of bacterial contamination and the antibiotics profile of isolated bacteria from unused diapers sold in markets in Enugu Metropolis, southeast Nigeria. The study also investigated the effectiveness of the Nigerian Consumer protection laws towards maintaining the standard of care for infants and toddlers. Fifty pieces of different brands of diapers were sampled for bacterial contamination using standard bacteriological procedures. The results show that out of 50 samples analyzed, bacterial growths were identified in 9 (18%), of which five different bacterial species were isolated. The most prevalent being *Escherichia coli* and *Staphylococcus aureus* 3 (33.3%). Other bacteria isolated were *Lactobacillus* spp, *Klebsiella* spp, and *Streptococcus* spp 1 (11.1%). There was no statistically significant difference in the distribution of the bacterial contamination of diapers across the different brands ($p > 0.05$). We conclude that baby diapers sold in markets in Enugu metropolis are prone to contamination with bacteria. We recommend that appropriate measures should be taken during the manufacturing process to reduce or prevent the incidence of bacterial contamination of diapers. (*Afr J Reprod Health* 2024; 28 [8]: 133-139).

Keywords: Contamination; Bacteria; Unused Diapers; Antibiotic susceptibility; consumer; strict liability

Résumé

Les couches étant l'un des articles les plus utilisés pour les soins des nourrissons, il est impératif qu'elles soient toujours exemptes de contamination par des micro-organismes. L'objectif de cette étude est de déterminer le taux de contamination bactérienne et le profil antibiotique des bactéries isolées provenant de couches inutilisées vendues sur les marchés de la métropole d'Enugu, dans le sud-est du Nigeria. L'étude a également examiné l'efficacité des lois nigérianes sur la protection des consommateurs pour maintenir le niveau de soins pour les nourrissons et les jeunes enfants. Cinquante morceaux de couches de différentes marques ont été échantillonnés pour détecter toute contamination bactérienne en utilisant des procédures bactériologiques standard. Les résultats montrent que sur 50 échantillons analysés, des croissances bactériennes ont été identifiées dans 9 (18 %), parmi lesquels cinq espèces bactériennes différentes ont été isolées. Les plus répandues sont *Escherichia coli* et *Staphylococcus aureus* 3 (33,3 %). Les autres bactéries isolées étaient *Lactobacillus* spp, *Klebsiella* spp et *Streptococcus* spp 1 (11,1 %). Il n'y avait pas de différence statistiquement significative dans la répartition de la contamination bactérienne des couches entre les différentes marques ($p > 0,05$). Nous concluons que les couches pour bébés vendues sur les marchés de la métropole d'Enugu sont sujettes à la contamination bactérienne. Nous recommandons que des mesures appropriées soient prises pendant le processus de fabrication pour réduire ou prévenir l'incidence de la contamination bactérienne des couches. (*Afr J Reprod Health* 2024; 28 [8]: 133-139).

Mots-clés: : Contamination; Bactéries ; Couches inutilisées ; Sensibilité aux antibiotiques ; consommateur; responsabilité stricte

Introduction

Diapers, also regarded as nappies, are specially designed underpants for infants. They can be made from natural fibers like cotton, muslin, bamboo,

wool, linen, jute, as well as artificial fibers such as non-woven polypropylene, polyethylene, nylon, polyester and other materials. Diapers are constructed with antimicrobial agents in the top sheet (the part in contact with the infant's skin) to

inhibit bacterial growth and stop its harmful effect on the baby¹. Diapering has been a widespread custom observed across various cultures throughout history, spanning many centuries². Diapers are a necessity for babies, and they are in constant contact with the skin for extended periods. Therefore, they are likely to harbor different types of bacteria, some of which can cause infections. The presence of pathogenic bacteria in diapers can pose significant health risks to both infants and adults who handle them. Diaper whether cloth or disposable, allows the wearer to defecate or urinate without using a toilet. Disposable diapers typically consist of three layers: a permeable top layer made of non-woven fabric, a permeable pad in the middle, and an impermeable bottom layer³. When diapers get soiled, they require replacement with a new one by a parent or a caregiver⁴. Diapers are mostly worn by infants, toddlers, and children who are neophyte or experiencing bedwetting. However, they are also necessary for certain health conditions like incontinence, mobility impairment, severe diarrhea, or dementia⁵. In addition, adults with physical or mental disabilities and those working in extreme conditions, for example, astronauts, may also require diapers⁶.

Several studies have been carried out both internationally and locally on microbial contamination of unused diapers sold in various locations, both in supermarkets and in general community markets. A study by Akin *et al.*⁷ to demonstrate the presence of bacteria on baby diapers in Istanbul, Turkey showed that all the 10 sampled diapers had bacterial growth on them with their prevalence as follows: *Escherichia coli* 5 (50%), *Proteus*spp 4 (40%) and *Pseudomonas*spp 1 (10%). These isolated organisms were also demonstrated to be highly resistant to common conventional antibiotics. Another study by Odio *et al.*⁸ on microbial contamination of adult diapers in Ethiopia revealed that 17 (85%) out of 20 samples of diapers purchased across a city were contaminated by pathogenic bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*. Uko *et al.*¹ also conducted a study on microbial contamination of infant diapers at Ikot Ekpene, Akwa Ibom State, Nigeria, and reported that five infant diapers designated samples 1 to 5 used in the study were contaminated with the following organisms:

*Bacillus*spp (29%), *Staphylococcus* spp (35%), *Pseudomonas* spp (12%), *Streptococcus* spp (24%), *Escherichia coli* (26%) and *Proteus* spp (26). The highest bacterial count was recorded in sample 1 with a total bacterial count of 2.0×10^3 CFU/ml followed by sample 3 with a total bacterial count of 1.4×10^3 CFU/ml. Sample 4 had a total bacterial count of 1.3×10^3 CFU/ml, sample 5 had a total bacterial count of 1.2×10^3 CFU/ml while the least bacterial count was recorded in sample 2 with a bacterial count of 1.0×10^3 CFU/ml.

This study on the bacterial contamination of unused diapers sold in Enugu metropolis is driven by the significant public health concerns associated with such contamination. Diapers are a vital component of infant and toddler care, used extensively daily. However, if these diapers are contaminated with bacteria, they can pose serious health risks to the vulnerable population they serve. Therefore, one of the primary objectives of this study is to ensure consumer safety by evaluating the quality and safety standards of diapers available in the market. Consumers rely on the assumption that the products they purchase, especially those used for personal hygiene, meet appropriate safety regulations. This discourse transcends several areas of law spanning from child's rights, consumer protection, torts, and commercial law.

By examining the levels of bacterial contamination in unused diapers sold in markets and supermarkets, crucial insights on whether manufacturers and suppliers adhere to adequate quality control measures can be obtained. This study therefore aimed at isolating, identifying and antibiotic susceptibility profile of bacterial isolates from unused diapers sold in major supermarkets/markets in Enugu metropolis.

Methods

Research designs and setting

The study was conducted in Enugu city in Nigeria's south-east. Enugu is the capital city of Enugu state, one of Nigeria's 36 federating states. The population of Enugu according to the 2006 Nigerian census was 722,664. The city is mostly covered on open grassland, with occasional woods and clumps of oil palm trees. The Igbo ethnic group comprises the majority of the state's population. Agriculture is crucial to the state's economy, with the primary

crops being yams, oil palm products, taro (ede), maize (corn), rice, and cassava.

Study sample

Different brands of diapers were bought from different locations within Enugu metropolis for sampling. They were bought from the following places: Shoprite, Roban stores, Market square, Spar, Garriki market, New market, Artisan market, Ogbete Main market and Kenyatta market.

Sampling and data collection procedure

A total of 50 diapers were purchased from the above-mentioned locations. 5 pieces of different brands (BM, MX, KD, HG, LB YR, SC and AG) were obtained from each location with the exception of Ogbete Market and Four Market Days supermarket where 6 and 4 pieces were purchased respectively. The study was carried out from 18th July to 11th of August 2023 in the Microbiological laboratory of Medical Laboratory Science, University of Nigeria Nsukka, Enugu Campus.

Sterile swab sticks were moistened with 2ml of peptone water aseptically added into the jacket. Each diaper was then swabbed with a swab stick and cultured into the appropriate culture media. The sterile swab sticks were also incubated for 24 hours and plated in the appropriate culture media.

Standard microbiological analyses were carried out using modified method of⁹. Nutrient agar (NA), blood agar (BA), MacConkey agar (MA), and peptone broth were all prepared using manufacturer's instructions and were sterilized in an autoclave at 121°C for 15 minutes. Sterile swab sticks were moistened with 2ml of peptone water aseptically added into the jackets. Each diaper was then swabbed with the moistened swab stick and cultured into the appropriate culture media and incubated for 24 hours at 37°C. All isolation and inoculations were carried out under aseptic condition. After incubation, the culture plates were examined for the presence of growth and their colonial characteristics were noted. Gram stain and biochemical tests were carried out for further identification.

Sensitivity test was done using modified Kirby Bauer disc diffusion method⁹. Using a sterile wire loop, one colony of the organism was aseptically streaked uniformly over the entire

surface of the medium. A commercial sensitivity disc was transferred aseptically to the center of the inoculated plates using sterile forceps. Gram positive and Gram-negative disc were placed differently based on the test organism and the plates were incubated at 37°C for 24 hours.

Data analysis techniques

All data analysis was done using SPSS statistical package for Windows version 22. One-way Analysis of variance (ANOVA) where $p < 0.05$ was accepted as being statistically significant and $p > 0.05$ was accepted as being statistically insignificant.

Results

Out of the 50 diaper samples analyzed for bacterial contamination, growth was recorded on 9 samples only (18% of the sample had growth while 82% had no growth). The study revealed the prevalence of the different bacteria species isolated from diapers. *Escherichia coli* and *Staphylococcus aureus* have a prevalence of 3 (33.3%) each while *Streptococcus spp*, *Klebsiella spp* and *Lactobacillus* has a prevalence of 1 (11.1%) each. It further showed the frequency distribution of bacterial isolates according to different brands of diapers in their order of prevalence. The bacterial isolates were gotten from the following brands: BM 2 (22.2%), MX 2 (22.2%), KD 2 (22.2%), HG 1 (11.1%), LB 1 (11.1%) and YR 1 (11.1%) SC and AG registered zero growth. Results also revealed the frequency distribution of the bacterial isolates according to their locations of purchase. Ogbete Market had the highest occurrence rate at 22.2 %, while Artisan, Kenyatta Market, Roban stores, New Market, Four Market Days and Shoprite had an occurrence rate of 11.1% each. No growth was recorded for diapers bought from Garriki Market and Spar supermarket.

Table 1 shows the bacterial count (CFU/g) of the organisms. *Staphylococcus aureus* isolated from brand KD bought from Ogbete market had the highest bacterial count (2.9×10^3 CFU/ml) followed by *Escherichia coli* isolated from brand MX that was purchased from new market (2.4×10^3 CFU/ml), *Streptococcus spp*. recorded the least amount of bacterial count (1.0×10^3 CFU/ml).

Table 2 presented the susceptibility pattern for isolated Gram-negative organisms. *Escherichia*

Table 1: Total bacterial Count of the Isolated Organisms for different brands of diapers

Brand	Location	Organism	Total bacterial count (CFU/ml)
BM	Artisan	<i>Escherichia coli</i>	1.3 x 10 ³
BM	Market square	<i>Lactobacillus</i> spp	2.3 x 10 ³
KD	Roban stores	<i>Klebsiella</i> spp	1.6 x 10 ³
MX	Four marketdays	<i>Escherichia coli</i>	2.0 x 10 ³
MX	New market	<i>Escherichia coli</i>	2.4 x 10 ³
HG	ShopRite	<i>Staphylococcus aureus</i>	1.3 x 10 ³
YR	Ogbete Market	<i>Streptococcus</i> spp	1.0 x 10 ³
KD	Ogbete Market	<i>Staphylococcus aureus</i>	2.9 x 10 ³
LB	Kenyatta Market	<i>Staphylococcus aureus</i>	1.1 x 10 ³

Table 2: Susceptibility pattern for isolated Gram negative organisms

Organisms	SP	CPX	AM	AU	CN	PEF	OFX	S	SXT	CH
<i>Escherichia coli</i>	19	20	7	5	8	19	20	8	11	7
<i>Klebsiella</i> spp	17	20	14	20	20	20	19	18	17	10
<i>Escherichia coli</i>	19	20	10	7	20	20	20	20	11	16
<i>Escherichia coli</i>	12	13	10	-	9	12	12	11	-	-

Key: SP=Sparfloxacin CPX=Ciprofloxacin AM=Amoxicillin AU=Augmentin CN=Gentamycin PEF=Pefloxacin OFX=Tarivid S=Streptomycin SXT=Septrin CH=Chloramphenicol

Table 3: Susceptibility pattern for isolated Gram-positive organisms

Organisms	AS	BA	PR	TE	CF	CP	LE	LZ	CX	RF	LM	GM
<i>Lactobacillus</i> spp	8	7	26	19	8	28	27	23	30	20	24	21
<i>Staphylococcus aureus</i>	-	-	7	8	-	10	10	13	-	8	-	10
<i>Streptococcus</i> spp	-	7	7	14	6	12	11	-	15	10	12	19
<i>Staphylococcus aureus</i>	-	3	5	8	4	13	11	15	-	11	-	11
<i>Staphylococcus aureus</i>	-	-	6	10	-	13	12	17	-	9	3	11

Key: AS=Ampicillin/Sulbactam BA=Co-trimoxazole PR=Cephalexin TE=Tetracycline CF=Cefotaxime CP=Ciprofloxacin LE=Levofloxacin LZ=Linezolid CX=Cloxacillin RF=Roxithromycin LM=Lincomycin GM=Gentamicin

coli showed moderate resistance while *Klebsiella* spp was highly sensitive to the conventional antibiotic discs.

Table 3 Showed antibiotic susceptibility pattern of the isolated Gram-positive organisms. *Staphylococcus aureus* and *Streptococcus*spp were mostly resistant to the pool of antibiotics while *Lactobacillus* spp showed higher sensitivity.

Discussion

The result of this study demonstrates substantial bacterial contamination of baby diapers sold in markets within Enugu metropolis. Nine out of fifty diapers that were sampled showed bacterial growth

in different levels. The result agrees with the work done by Uko *et al.*¹ and Akin *et al.*⁷ who reported bacterial contamination from unused infant diapers. The findings from the present study reveal that *Escherichia coli* and *Staphylococcus aureus* had the highest growth of 3 each. Other isolated bacteria were *Streptococcus* spp, *Klebsiella* spp and *Lactobacillus* spp 1 each. These findings are consistent with the work by Akin *et al.*⁷ and Odio *et al.*⁸ who also reported the presence of similar bacteria in baby diapers. This present study also agrees with the findings of Uko *et al.*¹ who also isolated *Staphylococcus aureus* and *Escherichia coli* from unused diapers purchased at pharmaceutical stores and supermarkets/ superstores within Ikot

Ekpen metropolis. This study is in contrast to the study of Tuzun *et al.*¹⁰ who reported no bacteria isolates from baby diapers.

The presence of these microorganisms could pose a serious problem to infant care. *Staphylococcus aureus* can secrete toxins that can cause gastrointestinal disorders¹¹. 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. *Lactobacillus* spp commonly found to be present in milk and other dairy products and a vaginal flora, is a non-pathogenic bacterium that generally inhibits the pathogenic process of other pathogenic bacteria¹². These bacteria could be potentially hazardous to infants due to their poorly developed immune systems. In addition, the isolation of *Escherichia coli* is a good indicator of fecal contamination probably from the water used during manufacturing. *Klebsiella* spp is a member of the family *Enterobacteriaceae* that can colonize the upper respiratory tract¹³. Its presence in diapers could be due to contamination with sputum of manufacturers or other handlers possibly when they cough or sneeze, contaminating the environment with these organisms.

The hand is one of the major sources of contamination and could serve as a microbial vehicle. Some of these microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp are normal body flora that colonizes the skin, nasal cavity, pharynx and gastrointestinal tracts and these can contaminate the palms of diaper manufacturers handler and distributor, which can then be transferred to the diapers. *Escherichia coli* isolated from brand BM bought from Artisan showed a count of 1.3×10^3 CFU/ml, *Staphylococcus aureus* isolated from brand KD bought in Ogbete had the highest count at 2.9×10^3 CFU/ml. *Klebsiella* spp and *Lactobacillus* spp recorded a count of 1.6×10^3 CFU/ml and 2.3×10^3 CFU/ml respectively while *Streptococcus* spp recorded the least count with 1.0×10^3 CFU/ml. These high counts of organisms in diapers are of great concern because of the vulnerability of the population that makes use of these products. This agrees with the work by¹ who reported similar counts from bacteria isolated from baby diapers.

The susceptibility profile of all the organisms isolated was done and the result showed

that *Escherichia coli* was sensitive to sparfloxacin, ciprofloxacin, perfloxacin and tarivid, and resistant to amoxicillin, augumentin, gentamycin, streptomycin and septrin. This agrees with result of Akin *et al.*⁷ which also recorded multi-drug-resistant specie of *Escherichia coli* from baby diapers. *Klebsiella* spp was sensitive to ciprofloxacin, perfloxacin, augumentin, gentamycin, tarivid and streptomycin, intermediate for sparfloxacin and amoxicillin and resistant to chloramphenicol. This disagrees with the findings of Fertans *et al.*¹⁴ where *Klebsiella*spp showed 90% resistance to similar antibiotics used in this present study. *Staphylococcus aureus* was only sensitive to linezolid and ciprofloxacin and resistance to ampicillin, lincomycin, cefotaxime, levofloxacin, cloxacillin, roxithromycin, Lincomycin, and gentamicin. This result agrees with the report of Gurung *et al.*¹⁵ where *Staphylococcus aureus* was also highly resistance to the same antibiotics. *Lactobacillus* spp showed resistance to Ampicillin, Trimoxazole, and cefotaxime and was sensitive to other antibiotics; this is in line with the findings of Sharma *et al.*¹⁶ where *Lactobacillus* spp was resistant to Ampicillin, Co-trimoxazole, and cefotaxime.

Although there was no statistically significant difference between bacterial contamination of diapers across different markets and different brands ($p > 0.05$), it still calls for more hygienic manufacturing and distribution processes and also stricter adherence to manufacturing standards set by the appropriate control agencies. The law demands that producers and retailers must ensure that the goods and services that they offer to customers are safe and of merchantable quality, failure of which could lead to a lawsuit for negligence.¹⁷ Moreover, Article 1 of the Child's Rights Act 2003 provides for the best interest of children to be of paramount consideration. There is no doubt that the production and sale of diapers is an action concerning children and the selling of contaminated diapers runs against the best interest of children and must be dealt with legally.

Strengths and limitations

The study provides an opportunity to analyse hygienic procedures used throughout the diapers' life cycle, including manufacture, storage, and distribution. This study's findings can provide

significant data for establishing or reinforcing regulatory requirements for manufacturers of diapers, importers, and merchants. These standards may specify specified thresholds for permissible amounts of bacterial contamination, resulting in increased industry compliance and safer products in the market. However, the study is confined to Enugu Metropolis, and the findings may not apply to other places with differing market dynamics, environmental circumstances, and hygiene standards. Also, the study covers a specific point in time, although the bacterial contamination levels and profiles may fluctuate over time due to factors such as changes in production methods, storage conditions, and handling practices. Finally, the study may not identify all bacterial species found in nappies, particularly non-culturable bacteria or those that require special growth conditions.

Recommendations

The government at all levels as a matter of urgency and in compliance with the provisions of the Child Rights Act 2003 should set standards using the appropriate government agencies to ensure clean and healthy diaper manufacturing, packaging, and distribution practices.

Moreover, diaper manufacturing companies should strictly adhere to the standards set for regulation to minimize the possibility of contamination. These standards include all directives by all Nigeria's health and monitoring agencies. Further, since most contaminations occur from water, it is recommended that water sources to diaper manufacturing industries should be properly purified as unclean water may also pose a threat of contamination. There is also a need for mothers and childcare providers to purchase diapers only from the brands certified by NAFDAC to have significant compliance with the standard of operations during their production process. Finally, the Laws in Nigeria should be amended to proffer strict liability to accentuate the standard of care for manufacturers and vendors of infant diapers.

Conclusion

This study demonstrated that baby diapers can be contaminated with pathogenic bacteria which can pose a health hazard to infant care. The numbers of contaminated diapers were generally low, indicating

non-severity of the microbial occurrence. However, presence of these bacteria on diapers should not be overlooked by the manufacturers, control agencies (NAFDAC) and distributors. *Staphylococcus aureus* and *Escherichia coli* had the highest frequency of isolation. *Klebsiella* spp, *Lactobacillus* spp and *Streptococcus* spp were isolated once each. Results from this study should thus serve as a means for public health education, also paving the way for more investigation to abolish the occurrence of microorganism in diapers and to suggest the best possible precautions to be taken during production and distribution of the products.

Conflict of interest

None

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None

Contribution of authors

Iniekong Philip Udohand Edith Nwosu conceived and designed the study.

Gerald E. Ibeh collected and analyzed the data

Edwin C. Arum and Ikenna C. Okoli prepared the manuscript.

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