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Assessment of Microbial Colonization of Hands of Nursery School Pupils in Orlu, Imo State, Nigeria

Helen Ifeoma Udujih [a], Peace Ngozi Chuwuemeka [b], Chizaram Winners Ndubueze [b*] and Mitchelle Ifechi Udujih [c]

[a] Institute of Infectious Diseases, Biosafety and Biosecurity, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.
[b] Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
[c] Department of Medical Laboratory Science, Rhema University, Aba, Abia State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the microbial colonisation of the hands of nursery school pupils in Orlu, Nigeria. Ethical approval was obtained from the management of the schools, and thirty participants were recruited for the study. Samples were collected by swabbing the palms of the study participants before and after meals. This was followed by transporting the samples to the laboratory to isolate and identify bacterial and fungal species. The antibiotic susceptibility pattern of the bacterial isolates was also determined. The study's results were analysed to obtain the mean, standard deviation, and

**Corresponding author: E-mail: chizaramwinnersndubueze@gmail.com;*

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frequency distribution. In this study, the isolated bacterial organisms include *Staphylococcus aureus*, *Salmonella* sp, *Escherichia coli, Staphylococcus epidermidis,* and *Bacillus* sp. Furthermore, fungal organisms, including *Penicillium, Aspergillus*, and *Fusarium*, were also isolated. These findings suggest that bacterial and fungal pathogens colonise the hands of some nursery school pupils in the study locations. Therefore, parents, caregivers, and teachers of nursery school pupils must ensure that these should learn and adhere to the practice of good hand hygiene.

Keywords: Fungi; bacteria; hand hygiene; microbial load; school; children.

1. INTRODUCTION

It is common knowledge that microbes can proliferate in higher numbers and adapt to various environmental conditions, making them ubiquitous [1,2]. Their vast habitats, including soil surfaces, acidic hot springs, radioactive wastes, the earth's crust, organic materials, and the bodies of plants and animals, indicate their capacity to adapt and multiply [3]. Furthermore, the population and site of infections in humans vary. While some microbes are harmless commensals, others may turn on the host and cause diseases [4].

One organ in the human body that is prone to colonisation by microorganisms is the hand. This is because it is being used to perform various activities. True to this, school children engage in activities that require using their hands, predisposing them to different infectious agents. Furthermore, there seem to be cases of poor hand hygiene and loose adherence to other infection practices among some of the school children in developing countries [5]. It is, therefore, essential that good hand hygiene, particularly hand washing with soap and water, is promoted among these schoolchildren to mitigate the spread of infectious diseases [6-9].

Hand hygiene is regarded as a defining characteristic of infection control strategies, which, when followed correctly, efficiently eradicate transitory bacteria from the hands of humans [10]. Unfortunately, some variables highlight unhealthy habits and inadequate hygiene, particularly among school-aged children. These include having limited access to handwashing facilities and often coming in touch with pollutants [11]. In addition, the lack of adequate institutional policies and dedication to proper hygiene practices also pose significant obstacles [12]. Therefore, it is pertinent that adequate infection control strategies are implemented to ensure that the risk of diseases like cholera, dysentery, typhoid fever, diarrhoea, and measles is significantly lowered.

It has been reported that in developing countries, 4.9 million children per 1000 die annually from diarrheal infections in their first five years of life, with 1.8 million deaths caused by diarrhoea in children under the age of five [13]. This demonstrates that diarrhoea is a severe public health concern. It has also been noted that the palms, particularly those of nursery school pupils, might host various harmful microbes that can survive on the hands for prolonged periods, potentially causing gastrointestinal infections such as diarrhoea [14]. Concerned by these reports, this study was carried out to assess the microbial load of the hands of nursery school pupils in Orlu, Imo State, Nigeria. This was achieved by determining the microbial load of their hands before and after mealtime.

2. MATERIALS AND METHODS

2.1 Study Population and Sample Collection

Thirty nursery school pupils were recruited for the study, and their perception of hand hygiene was assessed by questionnaires devised following WHO's guidance for evaluating hand hygiene practices. In addition, a station was established for sample collection in each school. Each child's dominant hand was swabbed using the sterile, moistened swab. These swabs were randomly taken starting from the palm area, leading up to all five fingers (including the creases and nail beds), and ending in the dorsal part of the hands. The swabs were then transported to the laboratory within 2 hours. Six schools in Orlu were randomly sampled for this study.

2.2 Direct Examination Normal Saline

A drop of normal saline was added to each swab and allowed to settle. The sediment was placed on a grease-free slide and covered gently with a coverslip. The slide was then examined under a light microscope with 10x and 40x objective lenses to check for parasites.

2.3 Wet Preparation using Lugol's Iodine Solution

A tiny amount of the sediment was placed on a clean, grease-free microscope slide. This was followed by adding a drop of Lugol's iodine to the sediment. The preparation was then covered with a clean, grease-free coverslip and examined under the microscope with 10x and 40x objective lenses.

2.4 Isolation and Identification of the Test Organisms

Serial ten-fold dilutions of the samples were done using normal saline. Each sample had five tubes. However, only the first, third, and fifth test tubes were cultured. After incubation, the isolated test organisms were identified at the species level by Gram staining and various biochemical tests, including catalase, coagulase, urease, and oxidase.

2.5 Antibiotic Susceptibility Testing

Antibiotic Susceptibility testing of the identified bacterial isolates was done using the Kirby-Bauer disc diffusion method. Standardised bacterial inoculums equivalent to 0.5 McFarland were swabbed onto Mueller Hinton Agar plates. Single antibiotic discs (Himediu, India) of ampicillin (10ug), gentamicin (30ug), tetracycline (30ug) and chloramphenicol (30ug) were aseptically placed into the swabbed plates. The plates were incubated overnight at 37ºC, and the diameters of the zones of inhibition around the antibiotic discs were measured.

2.6 Statistical Analysis

Descriptive statistics, including the mean, standard deviation, and frequency distribution, were calculated using Microsoft Excel Version 16.83.

3. RESULTS

3.1 Socio-Demographic Characteristics of the Nursery School Pupil

Table 1 shows the socio-demographic characteristics of nursery school pupils. Out of the thirty pupils selected for this study, 12 (40%) were aged 2-4 years, while 18 (60%) were aged 5-6 years. Gender analysis showed that 16 (53.33%) were males while 14 (46.67%) were females. Furthermore, 10 (33.33%) of the pupils wash their hands before meals, while 20 (66.67%) do not. Also, 5 (16.67%) of the pupils wash their hands with soap after using the toilet, while 25 (83.33%) do not. Among the thirty pupils, 17 (56.67%) knew that failure to wash hands led to disease transmission, while 13 (43.33%) did not have this knowledge. Similarly, 17 (56.67%) had soaps for handwashing, while 13 (43.33%) did not have. On the other hand, 20 (66.67%) of the pupils wash their hands after meals, while 10 (33.33%) do not. Furthermore, 14 (46.67%) of the pupils wash their hands at school, while 16 (53.33%) do not. It was also observed that 12 (40%) of the pupils wash their hands at home, while 18 (60%) do not.

3.2 Identification of the Test Organisms

The test organisms were identified, as shown in Table 2 below, by their morphology, gram reaction, and biochemical tests.

3.3 Prevalence of Bacteria Isolate among Nursery School Pupils in Orlu

The table shows the prevalence of bacteria species Isolated from the hands of Nursery school pupils. Out of the thirty participants, nine bacterial organisms were isolated, giving a cumulative prevalence of 30% of bacterial colonisation of their hands. Eight of the thirty pupils had their hands colonised by *Bacillus* sp, six by *Salmonella* sp, six by *S. epidermidis*, five by *Salmonella* sp, and another five by *Staphylococcus aureus*.

3.4 Prevalence of Fungi Isolate among Nursery School pupils in Orlu

Table 4 shows the prevalence of Fungi isolates in the hands of Nursery school pupils. *Aspergillus* was observed in 50% of the respondents, *Penicillium* was observed in 33.33%, and *Fussaruim* was observed in 16.67%.

3.5 Bacterial Count of the Pupils' Hands Before Meal

Table 5 shows the bacterial count of the hands of the nursery school pupils from the six schools before the meal. The bacterial count of *Staphylococcus aureus* ranged from $20x10^1$ to $41x10^3$ CFU/g. That of *E. coli* ranged from $11x10^1$ to $111x10^3$ CFU/g. Furthermore, that of *Salmonella* sp ranged from 0 to $19x10^1$. On the

other hand, *Bacillus* sp was only isolated from school D (23x10^{3}CFU/g), while *S. epidermidis* was isolated from only school F (25x10^{3}CFU/g).

3.6 Bacterial Count of the Pupils' Hands After Meal

Table 6 shows the bacterial count of the hands of Nursery school pupils after meals. The bacterial count of *Staphylococcus aureus* ranged from 22x10^1 to 41x10^3 CFU/g. That of *E. coli* ranged from 0 to 113x10^3 CFU/g. Furthermore, that of *Salmonella* sp ranged from 0 to 86x10^3. On the other hand, *Bacillus* sp was only isolated from school D (25x10^{3}CFU/g), while *S. epidermidis* was isolated from only school F (26x10^{3}CFU/g).

3.7 Antibiotic Resistance Pattern of Bacteria Isolated from the Hands of Nursery School Pupil

All the isolated organisms were sensitive to Cephalexin and Ampiclox but resistant to Drovid. Furthermore, *Bacillus* sp and *S. epidermidis* were sensitive to Gentamycin, while *Escherichia coli* was sensitive to Clindamycin.

Table 1. Socio-demographic characteristics of the nursery school pupil

Variable	Frequency	Percentage (%)
Age		
2-4	12	40
5-6	18	60
Sex		
Male	16	53.33
Female	14	46.67
Washing of hands before meal		
Yes	10	33.33
No	20	66.67
Washing of hands with soap after using the toilet		
Yes	5	16.67
No	25	83.33
Does failure to wash hand transmit infection disease		
Yes	17	56.67
No	13	43.33
Presence of Soap water for handwashing		
Yes	17	56.67
No	13	43.33
Hand washing after meal		
Yes	20	66.67
No	10	33.33
Handwashing at school		
Yes	14	46.67
No	16	53.33
Hand washing at home		
Yes	12	40
No	18	60

Table 2. The identities of the organisms after isolation

Morphology	Gram Reaction	Urease	Catalase	Coagulase	Organism
+ Cocci	+	+	+	+	*S. aureus*
Rods	-	-	+	+	*E. coli*
-Rods	-	-	+	-	*Salmonella* sp
+Rods	+	-	+	-	*Baccillus*
+ Cocci	+	-	+	-	*S. epidermidis*

Key: + = Positive - = Negative

Table 3. Prevalence of bacteria Isolate among nursery school pupils in Orlu

Organisms	No. of Samples	Frequency (Percentage of Positive cases)
Staphylococcus aureus	5	1
Escherichia coli	6	2
Salmonella sp	5	1
Bacillus sp	8	3
S. epidermidis	6	2
Total	30	9 (30%)

Table 4. Prevalence of fungi isolate among nursery school pupils in Orlu

Variable	Frequency	Percentage (%)
Aspergillus	15	50
Penicillium	10	33.33
Fusarium	5	16.67
Total	30	100

Table 5. Bacterial count of the hand of nursery school pupil before meal

Sample	A	B	C	D	E	F
S. aureus	$101x10^1$	$20x10^1$	$86x10^1$	$38x10^1$	$85x10^1$	$41x10^3$
Escherichia coli	$111x10^3$	$11x10^1$	$8x10^3$	NG	$82x10^1$	$83x10^3$
Salmonella sp.	NG	$2x10^1$	$28x10^1$	$19x10^1$	NG	NG
Baccillus sp.	NG	NG	NG	$23x10^3$	NG	NG
S. epidermidis	NG	NG	NG	NG	NG	$25x10^3$

Key: A, B, C, D, E, and F = Codes for the different schools; NG = No Growth

Table 6. Bacterial count of the hands of Nursery School pupils after meal

Sample	A	B	C	D	E	F
S. aureus	$102x10^1$	$22x10^1$	$88x10^1$	$38x10^1$	$87x10^1$	$41x10^3$
E. coli	$113x10^3$	$13x10^1$	$10x10^3$	NG	NG	NG
Salmonella sp	NG	$14x10^1$	$30x10^1$	$21x10^1$	$82x10^1$	$86x10^3$
Bacillus sp	NG	NG	NG	$25x10^3$	NG	NG
S. epidermidis	NG	NG	NG	NG	NG	$26x10^3$
Mean Values	1.71	0.81	1.96	0.98	2.82	0.02

Key: A, B, C, D, E, and F = Codes for the different schools; NG = No Growth

Table 7. Antibiotic resistance pattern of bacteria Isolated from the hands of Nursery School Pupil

Bacteria Isolated	D	CX	GN	AC	CD
S. aureus	-	+	-	+	-
E. coli	-	+	-	+	+
Salmonella sp	-	+	-	+	-
Bacillus sp	-	+	+	+	-
S. epidermidis	-	+	+	+	-

KEY: D = Drovid; CX = Cephalexin; GN = Gentamycin; AC = Ampiclox; CD = Clindamycin; (+) = sensitive; (-) = Resistant

4. DISCUSSION

This study assessed the microbial colonisation of the hands of nursery school pupils in Orlu, Imo State, Nigeria. It was found that the prevalence of bacterial colonisation of the hands of the pupils was 30%. A comparison of the findings from this study to previous research shows that the value recorded in this study was lower than the value (81%) reported by Vivas et al. [15] and the 88% reported by Ogba et al. [16]. This suggests a reduction in the spread of bacterial

infection in schools. The promotion of intervention programmes such as hand washing, a clean environment, and healthy hygiene among pupils and their guardians might have played a key role in the reduction in the prevalence rate of bacterial infections among the pupils.

Although the overall prevalence rate of bacterial infection in the hands of primary school pupils was low, it could be that the small sample size and other factors might have resulted in this lower prevalence rate. This is probably true as the feedback from the completed questionnaires shows that most pupils do not wash their hands before meals. Therefore, further research should be carried out to better understand why the prevalence rate of bacterial colonisation among the pupils was low despite not adhering to handwashing programmes. The research should include a larger number of participants and examine other possible factors, including pupils' contact with animate and inanimate objects.

Although the 30% prevalence rate recorded in this study is lower than that of previous studies, it is still a cause for alarm and necessitates rapid and sustainable interventions. Awareness and sensitisation programmes on the importance of hand hygiene among children and their guardians should be intensified. Furthermore, water and soap should be constantly made available in designated areas on the school premises to ensure they are used effectively.

Another factor to consider is the age of the children. Most of the participants of this study were between 5 and 6 years of age, which is the age at which children play a lot. Moreover, some children cannot care for themselves at this age, meaning their parents and teachers need to be more involved in ensuring that these children maintain proper hand hygiene. Furthermore, the bacterial counts of the pupils' hands before and after mealtime were high. This could be attributed to the fact that most respondents did not wash their hands properly before and after meals, suggesting an urgent need to teach the children how to wash their hands properly.

In this study, both the fungal (*Aspergillus*, *Penicillium*, and *Fusarium*) and bacterial (*S. aureus, S. epidermidis, E. coli, Bacillus* sp., and *Salmonella* sp.) organisms isolated from the hands of the pupils are potential pathogens in humans, suggesting a threat to public health. Among these bacterial pathogens, the one with the highest prevalence was *Bacillus* sp*.* This finding disagrees with the reports by Chen et al. [17] and Ogba et al. [16], in which *S. aureus* had the highest prevalence*.* Furthermore, the isolation of *Escherichia coli* from these children's hands suggests the possibility of faecal contamination. Detecting Staphylococcus aureus is also important for public health since it can cause various diseases, particularly food poisoning.

The antibiotic susceptibility test showed that the isolates resisted some available conventional antibiotics. This is a cause for alarm as the manifestation of the diseases caused by these pathogens might be difficult to treat. It is also very important to perform molecular typing of pathogens and detect plasmid-resistant genes in future research. The findings will go a long way in helping epidemiologists and public health experts to develop more efficient interventions. Furthermore, it is imperative that a study on the microbial colonisation of the hands of the parents, teachers, guardians, and nannies of the children be done. This is because there is a high likelihood that these children might have picked up the pathogens from them or they are at high risk of being infected by the children. The findings from the study would also help advance public health interventions.

5. CONCLUSION

In conclusion, it was observed in this study that there were high bacterial and fungal counts on the samples collected from the hands of the nursery school pupils. This could be attributed to the fact that the pupil did not wash their hands properly before and after meals and before and after going to the toilet. This can cause a widespread of food-borne diseases, which can lead to the death of nursery school pupils. Therefore, parents and caregivers of nursery school pupils should be enlightened through public health education on the importance of washing hands before and after meals. Moreover, future research on this is encouraged in other locations and, if possible, includes molecular identification or sequencing of the isolated pathogens.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of manuscripts.

ETHICAL APPROVAL

Letters of introduction detailing the nature of the research were submitted to the selected schools in Orlu, Nigeria, and approval for sample collection was obtained from the schools' management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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