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MICROBIOLOGICAL ASSESSMENT OF SOME DAY CARE FLOORS WITHIN ILORIN METROPOLIS

Sule*[1] I. O. and Awe[2] O. A.

[1, 2] Department of Microbiology, University of Ilorin, PMB 1515, Ilorin, Kwara State

Corresponding Author's Email Address: suleism@gmail.com Phone: +2348056663764

ABSTRACT
There are increasing number of daycare centres in many cities where nursing mother keep their children during working hours. Many of these children come home with episode of diseases. Floor is one of the avenues for disease transmission. It is therefore necessary to carry out microbiological investigation of these floor surfaces. Ten day-care centres within Ilorin metropolis were involved in this study. Swab samples were collected from an area of 25cm by 25cm of the floor at each location. The samples were analyzed and tested for total viable bacterial, faecal coliform, and fungal count. The bacterial count ranged from 1.8 x 10^3 to 2.0 x 10^4cfu/ml while the faecal coliform count ranged from 0 to 100 cfu/ml. Similarly, the fungal count ranged from 4.0 x 10^2 to 4.0 x 10^3cfu/ml. A total of thirteen different bacterial isolates and six different fungal isolates were identified. The bacterial isolates were *Bacillus licheniformis, Aeromonas* sp*., Bacillus coagulans, Micrococcus sedentarius, Staphylococcus aureus, Corynebacterium kutscheri, Bacillus megaterium, Staphylococcus epidermidis, Acinetobacter* sp*., Bacillus polymyxa, Corynebacterium* sp*., Citrobacter* sp*.,* and *Proteus* sp*.* while the fungal isolates were *Aspergillus clavatus, Geotrichum candidum, Penicillium chrysogenum, Saccharomyces cerevisiae, Fusarium culmorum,* and *Aspergillus niger*. It was concluded that some pathogenic microorganisms were present on the floors of some day care centres. Hence, there is need for the operators of the day care centres to improve on the level of hygiene; and the local health authorities need to regularly visit these day care centres to see if they are operating within the minimum standards.

Keywords: Day care, Floor, Bacteria, Fungi, Hygiene

INTRODUCTION

Day care centre is a place where infants and children are kept when their parents have gone to work. The workers at the daycare centres take care of the children requirements in term of feeding, nurse caring, excretion and general comforts (Wallace & Ebrahim, 1981; Arif & Ebrahim, 1995; Olaitan & Adeleke, 2006). The children group comprises about 45% of the population in developing countries and the infection rate is highest in the 0 - 5years of age (Gamblin, 2006). Infants and young children are more prone to infectious diseases; and some of the diseases suffered are environmental in origin and can be contained and prevented by an intelligent operator who has acquired the relevant basic hygiene skill. Dirty environments may harbour infectious agent and predispose the children to skin infections (Benson & Abraham, 1995). A good day care is however able to keep its environment in good working order; free from all preventable epidemics.

The ease of spread of communicable diseases generally depends on the environment, the children themselves and the employees of these day care centres who take care of these children. Often, these children come home with episodes of diarrhea, gastroenteritis, respiratory and skin diseases.

Microorganisms are very ubiquitous; they are present on the floors, in the air, surfaces like shelves and so on. Alvarez *et al*. (1994) observed that gastrointestinal diseases contribute to a major health problem in day care centres due to the fact that most of the children are non- toilet trained.

At young ages children have immature and inexperienced immune systems, so they acquire virtually every infection causing agent they are exposed to. As a result, when an infection is introduced into the day care setting, it could spreads round the entire room, and affects all the children in the facility.

Another significant factor in day care infections is the close physical contact among the participants (both children and adults). For example, diseases such as impetigo, head lice, and scabies could spread by physical contact. Infections that spread by the oral route can easily pass from individual to individual as different babies teethe on the same toys or as toddlers suck their thumbs after touching contaminated surfaces like floors. In addition, some diseases are spread by droplets.

The objectives of this research were to determine the bacterial and fungal loads and species of some day care centres; determine the occurrence of the bacterial and fungal species on the day care floors; enumerate the population of *E. coli* on the floor surfaces; and determine the sanitary appraisal of these day care centres in order to know the possible sources of contaminations.

MATERIALS AND METHODS

Sampling sites

Ten different day care centres within Ilorin metropolis were sampled and they were coded A to J.

Collection of samples

Firstly, permission was taken from the authorities of the day care centres prior to sample collection. Samples were collected in the early hours of the day between 8 am and 10 am just after the hygiene procedures have been observed and with the presence of about 80% of the children. A measured portion of the floor 25cm x 25cm was swabbed with a sterile swab stick on each occasion and taken to the laboratory immediately for analysis.

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Isolation and enumeration of bacteria and fungi
The swab stick from each sampling site was shaken thoroughly in 10ml of sterile distilled water and this gave 10^{-1} dilution. Dilution was done up to 10^{-2} and plating was done from 10^{-1} and 10^{-2} dilutions using pour plate technique and nutrient agar for the isolation of bacteria while spread plate technique and potato dextrose agar was used for the isolation of fungi. The bacterial and fungal plates were incubated at 37°C and 25°C for 48 and 72 hours respectively (Sonia *et al*., 2013).

Enumeration of faecal coliform
Eosin methylene blue agar medium was used for the isolation of faecal coliform (*Escherichia coli*) using spread plate technique. After incubation at 37°C for 24hours the numbers of typical colonies with greenish metallic sheen were taken as faecal coliforms (Fawole & Oso, 2007).

Isolation of pure culture and preservation of the isolates
The bacterial and fungal isolates were subcultured on nutrient agar and potato dextrose agar respectively in order to obtain pure culture of each isolate. Pure cultures obtained were stored in the refrigerator at temperature not less than 4°C (Willey *et al.,* 2008).

Characterization and identification of isolates
The bacterial isolates were characterized on the basis of their colonial morphology, cellular morphology and biochemical reactions. The biochemical tests performed included: catalase test, oxidase, starch hydrolysis, citrate utilization, urease, coagulase, indole production, triple sugar iron agar, carbohydrate fermentation, oxidative and fermentative utilization of sugars, nitrate reduction, haemolysis and growth in 6.5% sodium chloride (Fawole & Oso, 2007).

The bacterial isolates were then identified by making reference to standard texts (Patricia *et al.,* 1970; Buchanan & Gibbons, 1974). Similarly, the fungal isolates were characterized based on their macroscopic and microscopic characteristics in order to identify them (Onions *et al.,* 1981).

Sanitary appraisal of day care centres
Each of the day care centre was examined for the possible sources of contamination using parameters such as number of babies, nature of floor, source of water for mopping the floor, methods of cleaning the floor, frequency of floor cleaning, whether the windows and doors were screened with nets or not, and whether the children were allowed to wear foot wears or not.

Statistical analyses
Statistical analysis package SPSS 15.0 was used to determine the mean, range, standard deviation and then one way analysis of variance was used to determine the differences within the means (SPSS, 2010).

RESULTS

Microbiological analyses
The bacterial count of the floor ranged from 1.8×10^3 to 2.0×10^4cfu/ml/25cm by 25cm of the floor surface while the faecal coliform count ranged from 0 to 1.0×10^2cfu/ml//25cm by 25cm of the floor surface. All the sampling sites were free from *E. coli* except at sampling site G. Furthermore, the fungal count ranged from 4.0×10^2 to 4.0×10^3cfu/ml//25cm by 25cm of the floor surface (Table 1).

The bacterial species identified were *Bacillus licheniformis, Aeromonas* sp., *Bacillus coagulans, Micrococcus sedentarius, Staphylococcus aureus, Corynebacterium kutscheri, Bacillus megaterium, Staphylococcus epidermidis, Acinetobacter* sp., *Bacillus polymyxa, Corynebacterium* sp., *Citrobacter* sp., and *Proteus* sp. The occurrence of bacterial species in each of the day care floor was as presented in Table 2.

Similarly, the fungal species identified were *Aspergillus clavatus, Geotrichum candidum, Penicillium chrysogenum, Saccharomyces cerevisiae, Fusarium culmorum* and *Aspergillus niger* and their occurrence were as presented in Table 3. The sanitary appraisal of the day care centres were as presented in Table 4.

Table 1: Microbial counts on the floors of some day care centres within Ilorin metropolis

Sampling locations	Bacterial count (cfu/ml) x 10^3	Faecal coliform count (cfu/ml) x 10^2	Fungal count (cfu/ml) x 10^2
A	$2.3^{a} \pm 0.1$	$0^{a} \pm 0.0$	$7^{abc} \pm 0.5$
B	$12^{c} \pm 2.0$	$0^{a} \pm 0.0$	$11^{cd} \pm 2.0$
C	$4.3^{a} \pm 0.2$	$0^{a} \pm 0.0$	$24^{e} \pm 4.0$
D	$20^{e} \pm 3.0$	$0^{a} \pm 0.0$	$32^{f} \pm 3.0$
E	$7.2^{b} \pm 0.2$	$0^{a} \pm 0.0$	$7^{abc} \pm 1.0$
F	$15^{d} \pm 2.0$	$0^{a} \pm 0.0$	$40^{g} \pm 5.0$
G	$1.8^{a} \pm 0.3$	$1^{a} \pm 0.2$	$12^{d} \pm 2.0$
H	$10^{c} \pm 2.0$	$0^{a} \pm 0.0$	$4^{a} \pm 1.0$
I	$2.2^{a} \pm 0.2$	$0^{a} \pm 0.0$	$10^{bcd} \pm 1.0$
J	$4.2^{a} \pm 0.2$	$0^{a} \pm 0.0$	$6^{ab} \pm 1.0$

Values are means of 3 determinations ± standard deviation.
Values in the same column with different superscripts are significantly different at $p<0.05$

Table 2: Occurrence of bacterial species on the floors of some day care centres within Ilorin metropolis

Bacterial Isolates	A	B	C	D	E	F	G	H	I	J
Bacillus licheniformis	+	-	-	+	+	+	+	-	-	-
Aeromonas sp.	+	+	+	+	+	+	-	-	-	-
Bacillus coagulans	+	-	-	-	-	-	+	-	-	-
Micrococcus sedentarius	+	-	+	+	+	+	-	+	+	-
Staphylococcus aureus	+	-	+	-	+	-	-	-	-	-
Corynebacterium kutscheri	-	+	+	-	-	-	+	+	+	-
Bacillus megaterium	-	+	+	-	-	-	-	+	-	+
Staphylococcus epidermidis	-	-	+	+	-	+	-	-	-	-
Acinetobacter sp.	-	+	+	+	-	+	-	-	-	-
Bacillus polymyxa	-	-	-	-	-	-	+	-	-	-
Corynebacterium sp.	-	-	-	-	-	-	-	-	-	+
Citrobacter sp.	-	-	-	-	-	-	+	-	-	-
Proteus sp.	-	-	-	-	-	-	+	-	-	-

Key: +, isolated; -, not isolated

Table 3: Occurrence of fungal species on the floors of some day care centres within Ilorin metropolis

Fungal isolates	A	B	C	D	E	F	G	H	I	J
Aspergillus clavatus	-	+	+	+	-	-	-	-	-	-
Geotrichum candidum	-	-	+	-	+	-	-	+	+	-
Penicillium chrysogenum	+	+	+	-	-	+	-	-	-	+
Saccharomyces cerevisiae	-	-	-	+	+	+	+	-	+	+
Fusarium culmorum	-	+	-	-	-	-	-	+	-	-
Aspergillus niger	+	+	-	+	-	-	-	-	-	+

Key: +, isolated; -, not isolated

Table 4: Sanitary appraisal of some day care centres within Ilorin metropolis

Sampling locations	Number of babies	Nature of floor	Mopping water	Toilet facility	Floor cleaning	Cleaning frequency	Wearing foot wear	Windows and doors
A	4	r	bh	+	sm	ev	ac	s
B	13	r	w	+	sm	ev	ac	s
C	19	r	w	+	sm	ev	ac	s
D	13	cf	p	+	sm	ev	ac	s
E	4	t	w	+	sm	ev	ac	s
F	15	cp	p	+	sm	int	ac	s
G	23	cp	bh	+	sm	ev	ac	s
H	4	cp	w	+	sm	ev	ac	s
I	9	t	p	+	m	int	ac	s
J	5	t	w	+	m	int	ac	s

Key: +, present; r, rug; cf, cemented floor; t, tiled; cp, carpet; bh, borehole; w, well; p, pipeborne; sm, sweeping and mopping; ev, everyday; int, Intermittently; ac, allowed by children only; s, screened with net

DISCUSSION

This present research demonstrated the presence of bacteria and fungi on the floors of some day care centres within Ilorin metropolis. The bacterial counts across the daycare centres ranged from 1.8 x 10^3 to 2.0 x 10^4 cfu/ml//25cm by 25cm of the floor surface. In a similar study, Olaitan & Adeleke (2006) obtained bacterial counts that ranged from 2.0 x 10^4 to 9.6 x 10^6 cfu/ml in daycare centres in Abeokuta, Ogun, Nigeria. *E. coli* was obtained only in a day care centre and this corresponds to the day care centre with the highest number of children. In another study, Sonia *et al.* (2013) obtained bacterial count in the range of 1 – 1100 cfu/24cm^2 on the surfaces of some hospital furniture. Indoor and outdoor microflora are inevitable in our immediate environment as a result of activities of man like raising of dust, wearing of shoes from outdoor into indoor. Some of the organisms isolated in this study are pathogenic and could be dangerous to human's health.

The bacteria isolated in this study were *Bacillus licheniformis, Aeromonas* sp*., Bacillus coagulans, Micrococcus sedentarius, Staphylococcus aureus, Corynebacterium kutscheri, Bacillus megaterium, Staphylococcus epidermidis, Acinetobacter* sp*., Bacillus polymyxa, Corynebacterium* sp*., Citrobacter* sp*.,* and *Proteus* sp. In a related study, Olaitan & Adeleke (2006) isolated *S. aureus, Bacillus* spp., *Klebsiella* sp., *Proteus* sp., *S. faecalis*, *P. aeruginosa*, *E. coli*, *S. dysenteriae, E. aerogenes* and *Micrococcus* spp. from the floor of daycare centres in Abeokuta, Ogun, Nigeria. *Bacillus licheniformis* and *Bacillus coagulans* are ubiquitous organisms that are likely to enter the human digestive system often many times a day (Topley & Wilson, 2008). *Staphylococcus epidermidis* is a part of the normal flora of man. It is a true opportunistic pathogen, as it requires a major breach in the host's innate defenses. Healthy individuals can possess up to 24 strains of the species, some of which can survive on a dry surface for long periods. Septicemia and endocarditis are diseases associated with *S. epidermidis* (Nilsson *et al.,* 1998; Villari *et al.,* 2000).

Staphylococcus aureus is ubiquitous and may be a part of human flora. Persistent carriage of this organism is more common in children than in adults. Some individuals are regarded as nasal carriers and they may be divided into persistent carriers with high risk of infection and intermittent or non-carriers with low risk of infection (Belkum *et al.,* 2009). This organism also elaborates toxins that can cause specific diseases or syndromes (Verkaik *et al.,* 2010).

Proteus spp. are most commonly found in the human intestinal tract as part of normal human intestinal flora. Inoculum size is important and it has a positive correlation with the risk of infection (Luzzaro *et al.,* 2009).
The primary modes of dissemination of *Corynebacterium* are by airborne respiratory droplets, direct contact with droplets, or infected skin lesions (Coyle & Lipsky, 1990). They are common inhabitants of soils.

Citrobacter spp. infrequently cause serious infections in compromised hosts. Invasive *Citrobacter* infections are associated with a high mortality rate, with 33 to 48% of patients succumbing to *Citrobacter* bacteremia (Shih *et al.,* 1996).
Aeromonas spp. are emerging human pathogens suspected to cause gastroenteritis ranging from mild enteritis to cholera-like diarrhoea. It has also been reported to cause infections such as septicaemia, endocarditis, osteomyelitis, myonecrosis, haemolytic uraemic syndrome, meningitis, peritonitis, respiratory tract disease, and ocular infections.

Acinetobacter spp. have low virulence but are capable of causing infections. *Acinetobacter* infections are uncommon but, when they occur, usually involve organ systems that have a high fluid content e.g. respiratory tract, cerebrospinal fluid, peritoneal fluid, urinary tract (Krol *et al.,* 2009).

The fungal species isolated could also have implications on the health of the children in the day care centres. *Geotrichum candidum* frequently causes pulmonary infection but has also been reported to cause bronchial, oral, vaginal, cutaneous and alimentary infections. Many species of *Aspergillus* can produce Aspergillosis (Varga *et al.,* 2000)**.**

The sanitary appraisal of the floors revealed the possible sources of contaminations of the floors as children's foot wears, water used for mopping the floor, population and health status of the children and so on. Kaltenthaler *et al.* (1995) observed that gastrointestinal diseases continue to be a major health problem in primary schools in the United Kingdom due to surface contamination of carpets.

Conclusion

The floor of the day care centres contained different species of microorganisms some of which could be a threat to the health of the children. Hence, the following recommendations were made. The floors should be mopped regularly with disinfectant; activities such as sweeping that generate dust should be avoided; only children who have been vaccinated against childhood diseases should be admitted into the day cares; wearing of outdoor foot wears into the day cares should be avoided; sick children should not be allowed in the day cares; parents should choose the smallest day care group to minimize the number of children from whom their children could acquire infections; local health authorities should visit the day cares regularly in order to determine their level of hygiene; and good hygiene habits such as the regular cleaning and disinfection of the hands of the children and their handlers after handling of faeces or visiting the toilet should be adopted.

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