MICROBIOLOGICAL ASSESSMENT OF HOME-PACKED CHILDREN'S MEAL, ANTIBIOTIC SUSCEPTIBILITY AND PREVALENCE OF INTESTINAL PARASITES AMONG PUPILS IN SELECTED SCHOOLS IN BENIN CITY, NIGERIA

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Abstract

Outdoor play among other factors predispose schoolchildren to intestinal parasitic infections. Home-packed children's meal which they take to school could be contaminated with microorganisms. In this study, a total of fifteen (15) samples of schoolchildren's meal obtained from five (5) selected private primary schools in Benin City and faeces of the pupils were analyzed using standard microbiological and parasitological methods. Antibiotic susceptibility test of the bacterial isolates involved disc agar diffusion method while molecular methods applies to plasmid DNA isolation and profiling of the isolates. Bacteria isolated from the food samples and their percentage occurrence include *Escherichia coli* (30 %), *Staphylococcus aureus* (25 %), *Streptococcus* sp. (20 %), *Bacillus cereus* (15 %) and *Bacillus subtilis* (10 %) while the fungal isolates were *Penicillium notatum* (25 %), *Aspergillus niger* (25 %), *Saccharomyces cerevisiae* (17 %), *Fusarium* sp. (17 %), *Rhizopus stolonifer* (8 %) and *Aspergillus flavus* (8 %). Prevalence of intestinal parasites detected in the faeces include *Ascaris lumbricoides* (37.5 %), *Giardia intestinalis* (37.5 %), *Entamoeba coli* (12.5 %) and *Hymenolepis nana* (12.5 %). Gram positive bacterial isolates were sensitive to gentamicin and ofloxacin; same applies to Gram negative isolate against ofloxacin and ciprofloxacin. All the food samples met the International Commission on Microbiological Specification for Food (ICMSF) criteria for THBC. In order to prevent schoolchildren from intestinal parasites and foodborne pathogens through faeco-oral transmission route, good kitchen hygiene practices, personal and environmental hygiene and proper monitoring of children's activities are recommended.

Keywords: Foodborne pathogens**,** ready-to-eat foods, faeco-oral route, antibiotic resistance, hygienic practices, foodborne diseases

Introduction

Outbreak of food poisoning is likely to occur in elementary schools for obvious reasons which include cross contamination, storage of food at ambient temperature, long hours between the time food is prepared and served (Malm *et al*., 2015; Abdullah and Ismail, 2021). Children in elementary schools usually between the ages of 6 to 11 years spend not less than six hours per day in school. Within that period, they are likely to be very hungry because of classroom and outdoor activities. Since schoolchildren lack stamina to withstand hunger for a long period, providing lunch for them in school is necessary. At least one third of daily nutritional requirement of pupils should come from lunch. The ability of pupils to learn and pay more attention in class is achieved when huger is satiated during school hours. Going to school with home-packed children's meal is convenient and safer for schoolchildren than patronizing food vendors selling ready-to-eat (RTE) foods (Ugochukwu *et al*., 2014). It is a thing of concern that schoolchildren's meal could be contaminated with chemicals, heavy metals and pathogenic microorganisms which could affect their health (Meldrum *et al*., 2009; Andarwulan *et al*., 2010; Adolf and Azis, 2012). According to Odeyemi and Sani (2016), 700,000 children in a particular region in southeast Asia die annually due to consumption of contaminated foods. Outbreak of foodborne diseases in schools have been reported in different countries (Pascual, 2020). In the US, slightly higher than 600 outbreaks of foodborne diseases were reported between 1973-1999 (Oranusi *et al*., 2007). It is surprising that a similar information obtained from schools in Nigeria is lacking. Children are prone to manifest foodborne diseases than adults because their immune system is not well developed (Lund and O'Brien, 2011; Aziz and Dahan, 2013; Esena and Owusu, 2013).

Diverse groups of bacteria, fungi, parasites and viruses are associated with foodborne diseases in humans (Esena and Owusu, 2013). *Staphylococcus aureus, Salmonella* sp., *Bacillus cereus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus* spp., *Escherichia coli, Enterobacter aeruginosa, Klebsiella pneumoniae* and *Citrobacter freundii* were encountered in various RTE foods sold in elementary school premises. Few of these bacterial isolates were detected in various foods served to pupils under the school feeding programme (Santana *et al*., 2009; Okolie *et al*., 2012; Trindade *et al*., 2014). Also detected in the RTE foods sold within the elementary school premises are fungal isolates which include *Aspergillus niger, A. fumigatus, Rhizopus* spp., *Penicillium viridicatum, Mucor* spp., *Fusarium* spp., *Candida albicans, C. dubliniensis, Candida* sp., *Cryptococcus* sp. and *Citrobacter freundii* (Afolabi *et al*., 2012; Ngueugang *et al*., 2021).

Schoolchildren are predisposed to intestinal parasitic infections because they usually play with moist soil, defecate openly and walk on barefoot. Unhygienic practices, lack of access to potable water and toilet facilities in some elementary schools in developing countries is associated with high prevalence of gastrointestinal parasites in pupils (Oyeniran *et al*., 2014; Sisay and Lemma, 2019). Parasites could be ingested by schoolchildren as a result of salad and vegetables included in their diet. Amissah-Reynolds *et al.* (2019) isolated *Giardia lamblia, Entamoeba histolytica, Moniezia* spp., *Trichuris trichuria* and *Entamoeba coli* from

RTE salads. In a related study, Agbalaka *et al*. (2019) reported that fresh vegetables which include carrot, cucumber, garden egg, among others, were contaminated with parasites. The prevalence of intestinal parasites in schoolchildren in different locations have been reported by many researchers (Asemahagn, 2014; Odo *et al*., 2016). An estimated population of 250 million people living in Sub-Saharan Africa are infected with at least one or more species of intestinal nematode. Intestinal parasitic infections in school children is associated with poor educational performance, stunting of linear growth, physical weakness, loss of appetite and mental problems (Elameen *et al.*, 2019).

To avoid patronizing RTE foods sold by vendors with high risk of contamination, many parents insist on preparing food at home which their children take to school (Sachar and Sharma, 2019). The nutritional contents of lunch packs of primary school children in Nnewi, Nigeria was determined by Ugochukwu *et al*. (2014). The study did not evaluate the microbiological quality of the lunch packs nor determine the prevalence of intestinal parasites among the schoolchildren. Unhygienic cooking and storage practices involved in preparing schoolchildren's meal at home could lead to food contamination. Although many parents have the notion that home-packed meal is microbiologically safe for schoolchildren, there is dearth of information to support it. Therefore, this study is aimed at carrying out microbiological analysis of home-packed schoolchildren's meal, antibiotic sensitivity test of the bacterial isolates and determine the prevalence of intestinal parasites among pupils in selected private primary schools in Benin city, Nigeria.

Materials and Methods

Study location and sample collection

Five (5) private primary schools were selected randomly in Benin city for this study. Figure 1 is the map of Benin city, Edo state, Nigeria showing where the schools are located. A total of fifteen (15) samples which comprise of three (3) samples of home-packed schoolchildren's meal obtained from three (3) randomly selected pupils in each primary school were collected using sterile universal plastic containers with a lid. The schoolchildren's meal comprises of white rice, jollof rice, fried rice, noodles, fried yam, plantain and boiled egg. Immediately after sampling, they were deposited inside ice packs and transported within 2 hours to the Laboratory for analyses. From the pupils whose meals were collected, samples of their faeces were also collected using fifteen (15) sterile universal plastic bottles. All the samples were properly labeled and put inside a big sterile bag and transported to the Microbiology Laboratory, Wellspring University, Benin city, for analyses.

Ethical considerations

The study protocol was approved by Wellspring University, Benin city, before the study commenced. Also, approval was given by authorities in charge of the selected primary schools in Benin city before samples were collected from pupils for laboratory analyses. Informed consent of parents or guardians of the pupils selected randomly for the study was obtained. Participation in the study was voluntary. At any time, the pupils earlier selected for the study could withdraw without further obligation. The names of pupils who participated in the study and their respective primary schools remain anonymous.

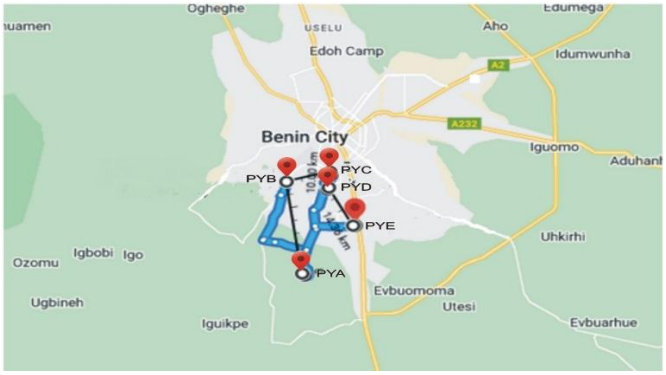

Figure 1: Map of Benin city, Nigeria showing the location of selected primary schools.; Source: Google map

Serial dilution

Twenty-five (25) grams of food sample from each pupil was homogenized and added to 225 ml of sterile normal saline (1:9) in a sterile conical flask to obtain 10^{-1} homogenate. From the inoculum, 1 ml was pipetted into 9 ml sterile normal saline. Ten-fold dilution was carried out by stepwise transfer of 1 ml dilution from the first test tube into subsequent tubes containing 9 ml sterile normal saline using sterile pipettes for each transfer. Exactly 1 ml dilution 10^{-3} was pipetted into separate sterile petri dishes in duplicates.

Microbiological analysis

Determination of total heterotrophic bacterial count

Under aseptic conditions, 1 ml dilution 10^{-3} was transferred into sterile petri dishes in duplicates and 15 ml of freshly prepared nutrient agar (L-S Biotech, India) was added to the petri dishes containing the diluted samples. The plates were rocked gently to achieve uniform distribution of the inoculum and the medium. The inoculated plates were incubated at 37 °C for 24 hours. The total number of colonies on the culture plates was counted after the incubation period using a hand tally counter. The result obtained was calculated using the formula below and expressed as colony forming units per gram (CFU/g).

$$\text{CFU/g} = \text{no. of colonies} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ plated}$$

Determination of total fungal count

The procedure used for determining the total heterotrophic bacterial count was applied in determining the total fungal count except that 10^{-3} dilution was plated out on potato dextrose agar (L-S Biotech, India) and incubated at room temperature (28±2 °C) for 7 days. The total number of colonies on the culture plates were counted after the incubation period using a hand tally counter. The result obtained was calculated using the formula below and expressed as colony forming units per gram (CFU/g).

$$\text{CFU/g} = \text{no. of colonies} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ plated}$$

Detection of coliforms

Under aseptic conditions, 1 ml dilution 10^{-3} was transferred into sterile petri dishes in triplicate and 15 ml of freshly prepared MacConkey agar (Oxoid) was added to the petri

dishes containing the diluted samples. The plates were incubated at 37 ºC for 24 hours after which purplish-red colonies surrounded by reddish zone of precipitated bile were counted as total coliforms using a hand tally counter. The result obtained was calculated using the formula below and expressed as colony forming units per gram (CFU/g).

$$\text{CFU/g} = \text{no. of colonies x } \frac{1}{dilution\ factor} \text{ x } \frac{1}{volume\ plated}$$

Obtaining pure isolates

Repeated subculturing of the isolates were carried out to obtain pure cultures. Discrete bacterial colonies on the petri dishes were picked using a sterile wire loop and plated on freshly prepared nutrient agar and incubated at 37 °C for 24 hours. Similarly, discrete fungal isolates were also plated on potato dextrose agar (PDA) and incubated at room temperature (28± 2 ºC) for 5 days. The pure isolates obtained were stored in slants and kept inside a refrigerator until further identification of the isolates were completed.

Identification of bacterial isolates

The colonial morphology of the bacterial isolates in the petri dishes were observed and noted. Gram staining of the bacterial isolates were carried out, followed by biochemical tests which include catalase, coagulase, citrate, oxidase, motility, urease, indole, methyl red, Voges-Proskauer and sugar fermentation tests (Shoaib *et al*., 2020).

Identification of fungal isolates

The colonial characteristics and morphology of fungal isolates in the petri dishes were observed and noted. Mycelia growth observed on the PDA culture plates were teased out and placed on two (2) drops of lactophenol on top of a clean glass slide. Gently, a coverslip was placed on top of the preparation and observed under the microscope using x10 and x40 objective lens. Identification of the fungal isolates based on morphological characteristics was achieved using a guide published by Watanabe (2010).

Detection of intestinal parasites in stool samples

The method described by Michael *et al*. (2017) with slight modifications was used to study stool samples obtained from schoolchildren. About 1 g of faeces inside universal sterile containers was emulsified with 4 ml of normal saline and mix properly. The mixture was gently placed on microscopic slides. The preparation was observed using a light microscope at 100x and 400x magnifications to ascertain whether cysts and ova of parasites are present. A similar procedure was carried out using iodine in place of normal saline. A standard chart showing ova and cyst of different intestinal parasites was used as a reference to identify the parasites seen in the stool samples.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on each bacterial isolate using the disc diffusion method also referred as Kirby-Bauer disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards (NCCL, 2003) guideline to evaluate the sensitivity of test organisms to various antibiotics. The test isolates were grown on nutrient agar and incubated at 37 ºC for 24 hours. Thereafter, the colonies were suspended in sterile normal saline and the inocula density were adjusted to 0.5 McFarland turbidity standards. A sterile swab stick was used to apply the broth medium containing each isolate onto the surface of a freshly prepared Mueller-Hinton agar plate. The antimicrobial discs used were ceftazidime (CAZ 30µg), gentamycin (GEN 30µg), ofloxacin (OFL 5µg), ciprofloxacin (CPR 5µg), erythromycin (ERY 10µg), cefuroxime (CRX 30µg), cefixime (CXM 5µg), and augmentin (AUG 30µg) (Oxoid), Basingstoke, United Kingdom. The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton agar plate and incubated at 37 °C for 24 hours. After incubation, diameters of zones of inhibition were measured using a transparent metre ruler. The result was interpreted as resistant (≤19 mm), sensitive (≥ 23 mm) and intermediate (20-22 mm) in accordance with standard specified by Clinical Laboratory Standard Institute (CLSI).

Multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance (MAR) index was determined for each bacterial isolate by dividing the number of antibiotics in the sensitivity disc the isolate was resistant to by the total number of antibiotics tested (Krumpenam, 1983).

$$MAR\ index = \frac{a}{b}$$

Where:

a = the number of antibiotics which the test isolate demonstrated resistance

b = the total number of antibiotics used in subjecting the isolates to susceptibility test

Molecular analysis

Plasmid analysis of the isolates that showed multidrug resistance was carried out to ascertain if multidrug resistance exhibited by the isolates were solely plasmid or chromosomally mediated. The multiple drug resistance (MDR) classification was based on bacteria that were resistant to three (3) or more different classes of antimicrobial agents (Magiorakos *et al*., 2012).

Plasmid DNA isolation and profiling

Zero point five millilitre (0.5 ml) of the overnight broth culture (each isolate) was centrifuged. The supernatant was discarded. Two hundred microlitres (200 µl) of P1 Buffer was added to the pelleted cells followed by two hundred microlitres (200 µl) of P2 Buffer and mixed. It was incubated at room temperature (28±2 °C) for 2 minutes. Four hundred microlitres (400 µl) of P3 Buffer was then added and mixed. It was centrifuged at 16,000× g for 2 minutes. The supernatant was loaded inside the Zymo-spin™ IIN column and was centrifuged for 30 seconds. The flow through was discarded. Two hundred microlitres (200 µl) of Endo-Wash Buffer was added to the column in a collection tube and centrifuged for 30 seconds. Four hundred microlitres (400 µl) of plasmid Wash Buffer was added and centrifuged for 1 minute. The spin Column was placed in a new microcentrifuge tube and thirty microlitres (30 µl) of DNA Elution Buffer was added and centrifuged for 30 seconds (Sambrok and Russell, 2001; Ranjbar *et al*., 2007).

Preparation of 0.8 % agarose gel (for plasmid DNA detection)

0.8 % agarose gel was prepared by dissolving 0.8 g agarose in 100 ml Tris EDTA Buffer. The mixture was heated in a microwave for 5 minutes to dissolve completely. It was allowed to cool at 56 °C and six microlitres (6 µl) of ethidium bromide was added to it. The agarose gel was poured into the electrophoresis chambers with gel comb, and allowed to solidify (Bikandi *et al*., 2004).

Electrophoresis

Ten microlitres (10 µl) of the molecular markers was loaded into the first well. Two microlitres (2 µl) of the loading dye mixed with eight microlitres (8 µl) of the plasmid DNA

extract were loaded in the other wells. Electrophoresis was performed at 90 v for 60 minutes. After electrophoresis, the products were visualized by Wealtec Dolphin Doc UV trans-illuminator and photographed. Molecular weights were estimated using molecular weight standard of the maker.

Results and Discussion

The results obtained from this study revealed that majority of the samples of schoolchildren's meal were contaminated with pathogenic microorganisms with few exceptions. Consumption of contaminated foods is a threat to health of school children. The total heterotrophic bacterial count (THBC) and total coliform count (TCC) of home-packed schoolchildren's meal obtained from fifteen (15) pupils in five (5) different schools is presented in Table 1 and 2, respectively. The result shows that THBC and TCC of the food samples was within the range of 0 - 1.2 x 10^5 and 0 – 2.3 x 10^4 CFU/g, respectively. In a related study, Ngueugang *et al.* (2021) reported that total mesophilic bacterial count (TMBC) of ready-to-eat (RTE) food sold in urban primary schools was within the range of 3.0 x 10^4 – 2.0 x 10^5 CFU/ml. Adolf and Azis (2012) reported that total coliform count of various foods served in elementary schools was within the range of 2.7 x 10^2 - 1.6 x 10^5 CFU/g. Both reports are in agreement with the results obtained in this study. According to International Commission on Microbiological Specification for Food (ICMSF), total bacterial count (TBC) and coliforms in food should not exceed 1.0 x 10^6 and 1.0 x 10^4CFU/g, respectively (Odu *et al*., 2019). All the samples of schoolchildren's meal met the ICMSF criteria for TBC. However, 13 out of 15 samples met the ICMSF criteria for total coliforms.

Total fungal count (TFC) of home-packed schoolchildren's meal obtained from fifteen (15) pupils schooling in five (5) different primary schools is presented in Table 3. The values obtained are within the range of 1.2 x 10^3 - 4.4 x 10^4 CFU/g. According to Ngueugang *et al.* (2021), total fungal count of ready-to-eat (RTE) food sold within the primary school was in the range of 0 - 2.8 x 10^5 CFU/ml.

Table 1. Total heterotrophic bacterial count of home-packed schoolchildren's meal from different schools

Sample			CFU/g
School PYA	9.0 x 10^3	-	1.2 x 10^5
School PYB	5.0 x 10^3	-	4.0 x 10^3
School PYC	1.5 x 10^4	1.0 x 10^4	1.0 x 10^3
School PYD	-	4.3 x 10^3	3.9 x 10^4
School PYE	2.7 x 10^4	2.6 x 10^3	3.1 x 10^3

Table 2. Total coliform count of home-packed schoolchildren's meal from different schools

Sample			CFU/g
School PYA	-	-	-
School PYB	-	-	1.0 x 10^3
School PYC	7.1 x 10^3	1.5 x 10^4	2.3 x 10^4
School PYD	-	-	4.0 x 10^3
School PYE	1.0 x 10^3	-	-

Table 3. Total fungal count of home-packed schoolchildren's meal from different schools

Sample			CFU/g
School PYA	1.0 x 10^4	2.0 x 10^3	3.8 x 10^3
School PYB	5.0 x 10^3	1.4 x 10^4	1.2 x 10^3
School PYC	4.4 x 10^4	3.2 x 10^3	9.0 x 10^3
School PYD	2.6 x 10^3	9.0 x 10^3	1.1 x 10^4
School PYE	6.0 x 10^3	8.0 x 10^3	1.3 x 10^3

Table 4 shows the result of biochemical characterization of bacterial isolates from home-packed schoolchildren's meal. The bacteria identified include *Bacillus cereus*, *B. subtilis*, *Streptococcus* sp. *Staphylococcus aureus* and *Escherichia coli*. This result is substantially in agreement with the findings of Okolie *et al*. (2012) which reported the presence of *E. coli*, *S. aureus*, *Pseudomonas* spp. and *B. cereus* in some foods sold to children in private primary schools. The presence of *E. coli* in home-packed schoolchildren's meal is an indication of faecal contamination from food handlers, the use of untreated water and dirty utensils in preparing food (Igbinosa *et al*., 2020).

The presence of coliforms in the food samples is an indication of improper handling of food after cooking and poor kitchen hygienic practices. It is also a sign of faecal contamination due to careless handling of cooked food. Haileselassie *et al*. (2012) reported that food handlers are responsible for contaminating meals with enteric pathogens during preparation, handling, and post-processing. Poor hygienic practices of food handlers who are healthy carriers of *S. aureus* in their skin, nasal passage and throat could be responsible for contamination of schoolchildren's meal (Odu and Maduka, 2019). The presence of *Streptococcus* sp. in schoolchildren's meal is a threat to their health. *Bacillus* sp., found in the samples could be attributed to the use of untreated water to prepare food, contamination of foodstuff with soil, exposure of schoolchildren's meal to air and dust.

Table 5 shows the colonial characteristics and morphology of fungi isolated from home-packed schoolchildren's meal. The fungi isolated include *Penicillium notatum*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Fusarium* sp. In a related study, Afolabi *et al*. (2012) reported the presence of *A. niger*, *A. fumigatus*, *Rhizopus* spp, *Penicillium viridicatum*, *Mucor* spp and *Fusarium* spp in RTE foods sold in primary schools in South-West, Nigeria. The report is substantially in agreement with the findings from this study. The sources of fungi found in the schoolchildren's meal could be from soil. Poor handling of foodstuff could be a source of contamination of schoolchildren's meal with *Rhizopus* sp. which is ubiquitous in the soil, excreta from animals and rotting vegetable (Ahaotu *et al*. 2022).

Table 4: Biochemical characterization of bacterial isolates from home-packed schoolchildren's meal

Samples		Catalase	Coagulase	Oxidase	Citrate	Urease	Indole	Voges-Proskaeur	Motility	Methyl red	Glucose	Fructose	Lactose	Suspected isolates
School	a	+	-	-	+	+	-	+	+	-	+	+	-	*Bacillus cereus*
PYA	a	+	-	+	+	+	-	+	+	-	+	+	-	*Bacillus subtilis*
	b	-	-	-	-	-	-	-	-	-	-	-	-	No growth
	c	-	-	+	-	-	-	-	+	+	+	+	+	*Streptococcus* spp.
	c	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
School	a	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
PYB	b	-	-	-	-	-	--	-	-	-	-	-	-	No growth
	c	-	-	+	-	-	-	-	+	+	+	+	+	*Streptococcus* spp.
	c	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
	c	-	-	+	-	-	-	-	+	+	+	+	+	*Streptococcus* spp.
School	a	-	-	+	-	-	-		+	+	+	+	+	*Streptococcus* spp.
PYC	a	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
	b	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
	b	+	-	-	+	+	-	+	+	-	+	+	-	*Bacillus cereus*
	c	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
	c	+	-	-	+	+	-	+	+	-	+	+	-	*Bacillus cereus*
School	a	-	-	-	-	-	-	-	--	-	-	-	-	No growth
PYD	b	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
	b	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
	c	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
	c	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
School	a	-	-	-	-	-	--	-	-	-	-	--	-	No growth
PYE	a	+	-	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
	b	+	-	+	+	+	-	+	+	-	+	+	-	*Bacillus subtilis*
	c	+	+	+	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*

Key: + positive; - negative; a, b, c represent isolate codes

Table 5: Colonial characteristics and morphology of fungi isolated from home-packed schoolchildren's meal

Samples	Colonial characteristics	Morphology and cellular structure	Suspected isolates
School PYA and PYE	Colonies were velvety, yellow and green.	Conidiophores were smooth, and relatively short. Penicillia mycelia were arranged, very irregular and asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them; conidia were smooth and ellipsoidal.	*Penicillium notatum*
	Colonies were light grey, growing rapidly and filling the petri dish with dense cottony mycelia producing mass of sporangia.	A bundle of sporangiophore was formed. Sporangiophore was smooth-walled, aseptate, light brown, simple (arising in groups of 3-5 from stolons opposite rhizoids). Sporangia globose or sub-globose with some flattened base, contained many spores (white at first; then turned black).	*Rhizopus stolonifer*
	Colonies were light green-yellow. At maturity, conidia was straw-like and yellow-green.	Conidiophores grew from substrate, long hyphae with thickened finely denticulate wall. Conidia were typically radial. Vesicle was elongated. Phialides were in two layers: primary and secondary.	*Aspergillus flavus*
	Colonies with loose, white to yellow mycelium, rapidly becoming dark brown to black on the development of conidia.	Vesicles were light yellow brown. Phialides grew radially along the whole periphery of phialides. Primary phialides and secondary vesicles; both are brown.	*Aspergillus niger*
	White moist spherical surface with smooth shape.	The cells are prolate spheroids.	*Saccharomyces cerevisiae*
School PYB and PYD	Mycelium was grey-white with sparse floccose.	Oval microconidia. Microconidia were produced on richly branched conidiophores.	*Fusarium* spp.
	Colonies with loose white to yellow mycelium rapidly became dark brown to black as the conidia developed.	Vesicles were light yellow brown. Phialides grew radially along the whole periphery of phialides. Primary phialides and secondary vesicles; both are brown.	*Aspergillus niger*
	Colonies were velvety yellow and green.	Conidiophores were smooth and relatively short. Penicillia mycelia were arranged, very irregular and asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them; conidia were smooth and ellipsoidal.	*Penicillium notatum*
School PYC	Colonies were velvety, yellow and green.	Conidiophores were smooth and relatively short. Penicillia mycelia were arranged, very irregular and asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them; conidia were smooth and ellipsoidal.	*Penicillium notatum*
	White moist spherical surface with smooth shape.	The cells were prolate spheroids.	*Saccharomyces cerevisiae*
	Colonies with loose white to yellow mycelium rapidly became dark brown to black on the development of conidia.	Vesicles were light yellow brown. Phialides grew radially along the whole periphery of phialides. Primary phialides and secondary vesicles; both are brown.	*Aspergillus niger*
	Mycelium was grey-white with sparse floccose.	Oval microconidia. Microconidia were produced on richly branched conidiophores.	*Fusarium* spp.

Depicted in Figure 2 is the percentage occurrence of bacteria isolated from home-packed schoolchildren's meal of fifteen (15) pupils. Among the bacterial isolates encountered in the samples, *E. coli* (30 %) and *Bacillus subtilis* (10 %) had the highest and lowest percentage occurrence, respectively. The dominance of *E. coli* could be attributed to high adaptability of the coliform to a wide range of ecological conditions including animal host and their surrounding environment (Igbinosa *et al*., 2020).

The percentage occurrence of fungi isolated from home-packed schoolchildren's meal of fifteen (15) pupils is depicted in Figure 3. Among the fungal isolates, *P. notatum* and *Aspergillus niger* each had the highest percentage occurrence (25 %) whereas the least involved *Rhizopus stolonifer* (8 %) and *A. flavus* (8 %). The dominance of *A. niger* could be attributed to exposure of schoolchildren's meal to air in the environment. The spores of *A. niger* are capable of surviving in the air for a long time (Ahaotu *et al*., 2022).

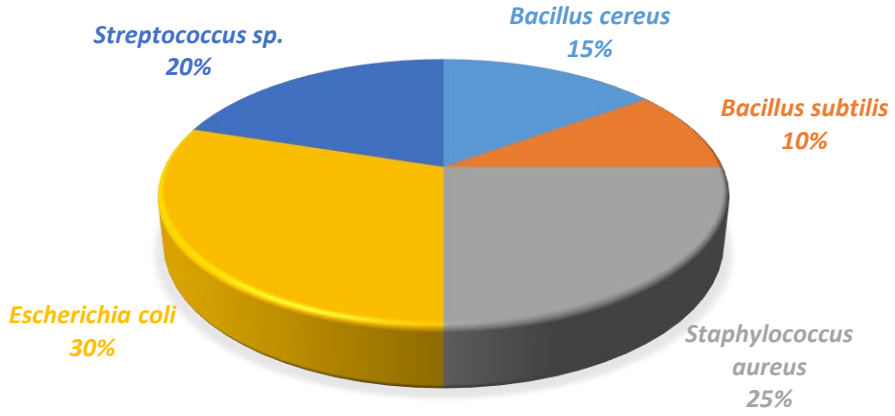

Figure 2. Percentage occurrence of bacteria isolated from home-packed schoolchildren's meal

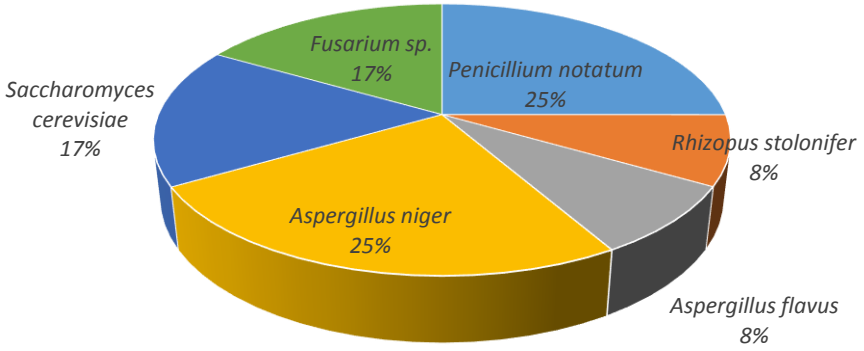

Figure 3. Percentage occurrence of fungi isolated from home-packed schoolchildren's meal

Presented in Table 6 is the percentage occurrence of intestinal parasites detected in the stool samples of fifteen (15) schoolchildren. *Ascaris lumbricoides* (37.5 %) and *Giardia intestinalis* (37.5 %) had the highest prevalence among the parasites detected in the stool samples. The parasites that recorded the lowest prevalence were *Entamoeba coli* (12.5 %) and *Hymenolepis nana* (12.5 %). In a related study, Oyeniran *et al*. (2014) reported that *Ascaris lumbricoides* (68.9 %) had the highest prevalence among the parasites detected in primary school pupils in Oshogbo, Nigeria. Our report is in agreement with their findings. The parasites detected in the faeces could be as a result of unhygienic practices among the schoolchildren, nibbling and sucking of their fingers. The habit of eating fruits by many schoolchildren without washing it could promote parasitic infection and reinfection. The presence of intestinal parasites in the schoolchildren could also be attributed to dirty toilets in the school premises,

insufficient water and soap to wash their hands after defecating.

Table 6. Intestinal parasites detected in stool samples of schoolchildren

Parasite	Frequency (%)
Ascaris lumbricoides	3 (37.5)
Giardia intestinalis	3 (37.5)
Entamoeba coli	1 (12.5)
Hymenolepis nana	1 (12.5)

Table 7 shows the physical characteristics and microscopic examination result of stool samples obtained from fifteen (15) schoolchildren. Eighty percent (80 %) and twenty percent (20 %) of the stool samples were semi-formed and solid/formed, respectively. Macroscopic observation further revealed that the stool samples were blackish (7 %), dark brownish (7 %), dark yellowish (13 %), brownish (27 %) and yellowish (46 %).

Table 7. Stool physical characteristics and microscopic examination

Samples		Consistency	Colour	Macroscopic examination	Microscopic examination (Normal saline)	Microscopic examination (Iodine)
School PYA	1	Solid/formed	Yellow	-	No egg was seen	-
	2	Solid/formed	Yellow	Undigested particles present	Eggs were visible	Presence of tape worm
	3	Semi-formed	Dark Brown	Presence of mucus	Eggs were visible	Presence of worm
School PYB	4	Solid/formed	Brown	Presence of mucus	-	-
	5	Semi-formed	Blackish	Presence of mucus	-	-
	6	Semi-formed	Brown	Presence of mucus	-	-
School PYC	7	Semi-formed	Dark yellow	Traces of blood	-	*Ascaris lumbricoides* decorticated eggs
	8	Semi-formed	Yellow	Presence of mucus	Presence of tape-like structure	*Hymenolepis nana*
	9	Semi-formed	Dark yellow	Traces of blood	*Giardia intestinalis* observed	*Ascaris lumbricoidees* decorticated eggs
School PYD	10	Semi-formed	Yellow	Presence of mucus	*Entamoeba coli* parasite observed	*Ascaris lumbricoides* decorticated eggs present
	11	Semi-formed	Yellow	Presence of mucus	*Giardia intestinalis* observed	
	12	Semi-formed	Yellow	Presence of mucus	*Giardia intestinalis* observed	
School PYE	13	Semi-formed	Brown	Presence of mucus	-	-
	14	Semi-formed	Yellow	Presence of mucus	-	-
	15	Semi-formed	Brown	Presence of mucus	-	-

The result of antibiotic susceptibility tests carried out on Gram-positive bacteria (*Bacillus cereus, B. subtilis* and *Streptococcus* spp.) and Gram negative bacteria (*E. coli*) isolated from schoolchildren's meal is presented in Table 8 and 9, respectively. The result obtained showed that Gram positive bacterial isolates were resistant to ceftazidime, ceftriaxone, cloxacillin and amoxicillin/clavulanic acid. However, the isolates were sensitive to ofloxacin and

gentamicin. *E. coli* was resistant to ceftazidime, cefruroxime, cefixime, augmentin and nitrofurantoin, but sensitive to ciprofloxacin and ofloxacin. In a related study, Ngueugang *et al.* (2021) reported that bacterial isolates from RTE foods sold in primary school premises displayed varying degree of susceptibility to some commonly used antibiotics. The study reported that *S. aureus* and *E. coli* were highly susceptible to ceftriaxone and oxacillin, but the isolates were less susceptible to penicillin G and amoxicillin. Mubarakat *et al*. (2018) reported that *B. cereus* isolated from food sold inside the premises of some elementary primary schools in Ilorin, Nigeria were resistant and sensitive to eleven (11) and five (5) antibiotics, respectively. In contrast, *Streptococcus lactis* also isolated from the food samples were resistant and sensitive to five (5) and eleven (11) antibiotics, respectively.

Table 8. Antibiotic susceptibility of Gram positive bacterial isolates from schoolchildren's meal

	Zones of inhibition (mm) in diameter/status							
Isolates/Antibiotics	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Bacillus cereus	12 (R)	11(R)	24 (S)	13 (R)	14 (R)	11 (R)	24 (S)	13 (R)
Bacillus subtilis	16 (R)	14 (R)	26 (S)	15 (R)	25 (S)	13 (R)	26 (S)	14 (R)
Streptococcus spp.	14 (R)	24 (S)	23 (S)	13 (R)	23 (S)	12 (R)	25 (S)	12 (R)
Staphylococcus aureus	15 (R)	13 (R)	25 (S)	14 (R)	12 (R)	13 (R)	24 (S)	26 (S)

Key: CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamicin, CTR= Ceftriaxone, ERY= Erythromycin, CXC= Cloxacillin, OFL= Ofloxacin, AUG=Amoxicillin/Clavulanic acid
S - Sensitive; **R** – Resistance

Table 9. Antibiotic susceptibility of Gram-negative bacterial isolate from schoolchildren's meal

	Zones of inhibition (mm) in diameter/status							
Isolates/Antibiotics	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR
Esherichia coli	15 (R)	17 (R)	16 (R)	14 (R)	26 (S)	13 (R)	14 (R)	25 (S)

Key: CAZ= Ceftazidime, CRX= Cefuroxime, GEN= Gentamicin, CXM= Cefixime, OFL= Ofloxacin, AUG=Amoxicillin/Clavulanic acid; NIT= Nitrofurantoin, CPR= Ciprofloxacin. **S -** Sensitive; **R** - Resistanc

Presented in Table 10 is the multiple antibiotic resistance (MAR) index of bacterial isolates from home-packed schoolchildren's meal for fifteen (15) pupils. The MAR of the isolates was within the range of 0.50 - 0.75. Among the bacterial isolates that demonstrated multidrug resistance, *E. coli* and *B. cereus* had the highest MAR index (0.75) each, whereas the lowest MAR index (0.50) involved *Streptococcus* sp. MAR index higher than 0.2 reported after testing any bacterial isolate is an indication that the organism originated from high risk sources of contamination where antibiotics are often used or misused (Krumpenam, 1983; Olisaka *et al.*, 2021). Since the MAR index of all the bacterial isolates encountered in the schoolchildren's meal exceeded 0.2, it is most likely that the isolates were exposed to the antibiotics indiscriminately and inappropriately.

Table 10. Multiple antibiotic resistance (MAR) index of the bacterial isolates

Samples	Isolate	MAR index
School PYA	*Bacillus cereus*	0.75
	Bacillus subtilis	0.63
	Streptococcus spp.	0.50
	Staphylococcus aureus	0.63
School PYB	*Staphylococcus aureus*	0.63
	Streptococcus spp.	0.50
	Escherichia coli	0.75
School PYC	*Streptococcus* spp.	0.50
	Escherichia coli	0.75
	Bacillus cereus	0.75
School PYD	*Staphylococcus aureus*	0.63
	Escherichia coli	0.75
School PYE	*Escherichia coli*	0.75
	Bacillus subtilis	0.63
	Staphylococcus aureus	0.63

Plate 1 shows the agarose gel electrophoresis analysis of the plasmids DNA extracted from the multiple antibiotic resistant bacterial isolates. The absence of plasmid genes in *Bacillus cereus*, *Bacillus subtilis*, *Streptococcus* sp., *Escherichia coli*, and *Staphylococcus aureus* isolated from home-packed schoolchildren's meal is an indication that antibiotic resistance genes were not present in their plasmids.

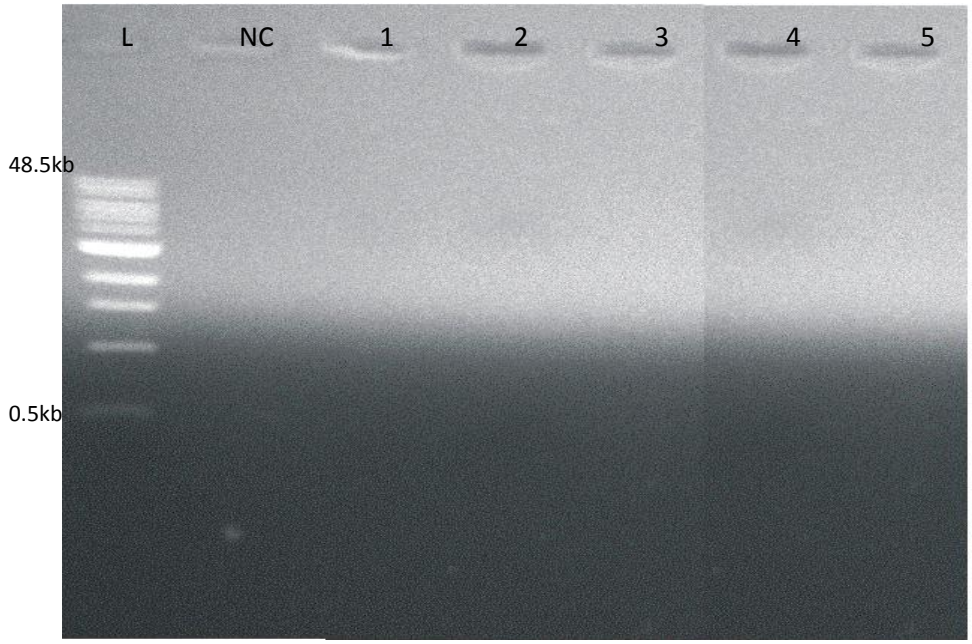

Plate 1: Plasmid profile of bacterial isolates analyzed with 0.8 % agarose gel electrophoresis stained with ethidium bromide.
L is 0.5kb-48.5kb DNA ladder (molecular marker). Isolates 1, 2, 3, 4 and 5 are negative for plasmid genes. NC is a no plasmid DNA template control.
Key: 1= *Bacillus cereus;* 2 = *Bacillus subtilis;* 3= *Streptococcus* sp.; 4= *Escherichia coli;* 5= *Staphylococcus aureus*

Conclusion

Home-packed schoolchildren's meal obtained from five (5) private primary schools in Benin city were contaminated with foodborne pathogens. The bacterial isolates were resistant to most of the antibiotics used in the study. Multiple antibiotic resistance (MAR) index reported in this study is an indication that bacterial species isolated from schoolchildren's meal have been unduly exposed to antibiotics. The plasmids of multidrug resistant bacterial isolates did not harbour antibiotic resistance genes. Among the intestinal parasites detected in the faeces of primary school pupils, *Giardia intestinalis* and *Ascaris lumbricoides* had the highest prevalence.

Recommendations

In a bid to prevent schoolchildren from intestinal parasitic infections and consumption of foods contaminated with foodborne pathogens which could result to frequent illnesses and hospitalization, thereby negatively affecting their education, it is recommended that:

(i) parents and guardians should observe personal hygiene, implement good kitchen hygiene practices and environmental sanitation
(ii) parents and guardians should desist from patronizing food vendors and insist on home-packed meal for the children to eat in the school
(iii) potable water should be made available to schoolchildren, both at home and in the school
(iv) school teachers should intensify their efforts in monitoring children activities within the school premises and always teach them good hygiene practices
(v) frequent deworming of schoolchildren should be carried out
(vi) strict regulations in the use of antibiotics
(vii) public health education and enforcement of food hygiene regulations should be sustained.

Conflict of Interests

The authors have declared that no conflict of interests exist.

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