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Microorganisms and Antibiotic Profile of Bacteria Associated With Three Stages of the Production of Flour from Wheat Grain (*Triticum aestivum*)

Aggrey Faith[1]*, Kugbenu, G. J[2] and Obire, O[3]
[1&3]Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria
[2]Department of Botany and Microbiology, University of Lagos, Akoka, Yaba, Lagos
***Corresponding Author:** *faithaggrey7@gmail.com*

ABSTRACT

Microbial contamination of wheat flour can result in food borne illness and disease outbreak from consumption of contaminated wheat flour and its derivative products. Hence this study is aimed to investigate the prevalence of microorganisms in different stages in the production of wheat flour in a Port Harcourt Flour Mill. Two sets of samples were obtained from three different stages of wheat flour production lines namely; UTF, production, and bagging on two different sampling periods. The samples were collected using sterile containers properly labeled according to standard microbiological procedures and then transported to the Laboratory for analyses using standard microbiological techniques. Antibiotic sensitivity profile of the isolated bacteria was carried out according to methods recommended by Clinical Laboratory Standard Institute. Results showed that mean total heterotrophic bacterial counts ranged from 6.3×10^5 to 2.25×10^6CFU/g; Total fungi counts ranged from 5.0×10^3 to 2.2×10^4CFU/g, *Salmonella/Shigella* counts ranged from 1×10^2 to 5.0×10^2CFU/g, and Total coliforms 0.0CFU/g. Three Gram positive bacteria belonging to the genera: *Bacillus*, *Clostridium*, and *Staphylococcus*, and three Gram negative bacterial species which includes *Escherichia coli*, *Serratia*, and *Salmonella* species were isolated and identified from samples of the three different stages in the production of wheat flour. The Most Probable Number results showed presence of coliform bacteria and was identified as *Escherichia coli* and *Serratia* spp. Results of Antibiotic sensitivity revealed that all the Gram positive isolates were susceptible to Gentamycin while all the gram negative bacterial isolates were susceptible to Ciproflox, Nalidixic Acid and Septrin. Findings of the study revealed the presence of potential pathogens in the flour samples investigated. Therefore, it is recommended that microbial test should be carried out on wheat grains and decontaminated before flour production, proper handling of wheat before and during production and addition of antimicrobial agent during tempering.

Keywords: Wheat (*Triticum aestivum*), flour, bacteria, fungi, food-borne illness, antibiotics resistance, public health

Introduction

Wheat (*Triticium* spp) is any of several species of cereal grasses of the genus *Triticum* (family Poaceae) and their edible grains. Wheat is widely cultivated for its seed, a cereal grain which is a worldwide staple food (FAOSTAT, 2014; Encyclopaedia Britannica, 2024). Wheat is one of the oldest and most important of the cereal crops. Botanically, the wheat kernel is a type of fruit called a caryopsis. Wheat grains, are composed of the starchy endosperm, or food-storage portion, constituting about 85 percent; several outer layers that make up the bran, constituting about 13 percent; and the oily germ, or embryo plant, approximately 2 percent. Of the thousands of *Triticum* varieties known, the most important and widely grown are common wheat (*Triticum aestivum*), used to make bread; durum wheat (*T. durum*), used in making pasta (alimentary pastes) such as spaghetti and macaroni; and club wheat (*T. compactum*), a softer type, used for cake, crackers, cookies, pastries, and flours (FAOSTAT, 2014; Encyclopaedia Britannica, 2024).

Wheat flour is a powder made from grinding wheat seeds or grains; making it usable for human consumption. There are different types of wheat flour, distinguished by the amount of gluten they contain, their color, the parts of the grain used, and the type of wheat. Wheat flour is an essential ingredient in bread, cakes, cookies, and most baked. White flour is made from only the endosperm. Brown flour includes the germ and bran. Whole grain flour includes all three parts.

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Once each part has been separated, it is ground into a powder. White flour has a naturally yellowish color but is often bleached or mixed with oxidizing chemicals to produce a white color (Lamsal and Faubion, 2009).

In the production of refined flour, the purpose of the milling process is to separate the endosperm from the other kernel portions. In the production of whole wheat flour, all parts of the kernel are used. In modern milling of refined flours the wheat kernels are cleaned and tempered by the addition or removal of moisture and then split open by a pair of rolls. The finest particles, called break flour, are sieved out and bagged (Berghofer *et al*., 2003; COFCO, 2018). Coarser particles of endosperm (called semolina) and pieces of bran with endosperm attached are then subjected to a series of rolls in which semolina of steadily reducing size is gradually ground to flour and the bran separated out. Wheat flour generally has an Aw level of 0.87 or lower depending on flour moisture and temperature. The concern with wheat flour is that bacteria and fungi can be carried or stored and emerge when the flour is being processed.

Wheat microbial contamination can be found along the crop production chain, including the stages of preharvest, harvest, transportation, storage, and processing (Los *et al*., 2018). The contaminating microbes can be carried by different elements such as animals, air, water, dust, and contaminated equipment (Laca *et al*., 2006; Los *et al*., 2018). Furthermore, different weather conditions, such as precipitation level and relative humidity level, as well as specific field microflora can influence the type and amount of microbial load on the kernels (Sabillón *et al*., 2019; Sabillón *et al*., 2020). The microorganisms found on wheat grains include enteric pathogens, such as the gram-negative bacteria *Escherichia coli* and *Salmonella* and the gram-positive bacteria *Bacillus cereus*, yeasts, and mycotoxin-producing fungi from the genera *Aspergillus*, *Penicillium*, and *Fusarium*, among others (Manthey *et al*., 2004; Laca *et al*., 2006).

Foodborne illness and outbreaks associated with contaminated wheat flour is as a result of contaminated wheat grains. The microbial load is typically found on the surface of wheat kernels. However, during the dry milling process, the microbial contamination is redistributed among the milled products (Berghofer *et al*., 2003), leading to inadequate microbiological quality of refined wheat flour.

Furthermore, with the increasing trend of consuming whole grain products, the risk of microbial contamination in whole wheat flour and its derived products is prevalent. The low water activity (aw < 0.60) of flour does not support microbial growth, nevertheless contaminant spores along with dormant microorganisms will remain viable for extended periods being a potential health hazard (Eglezos, 2010; Manthey *et al*., 2004).

In a study conducted in 21 American universities, Byrd-Bredbenner *et al*. (2008) reported that 53% of the participants incurred in the risky eating behavior of consuming raw homemade cookie dough. Eating unbaked products that should be cooked before consumption appears to be a widespread practice. For instance, in a study that linked ready-to-bake commercial prepackaged cookie dough with *E. coli* contamination (Neil *et al*., 2012), patients admitted buying dough for its raw consumption without having plans to bake it.

Furthermore, (Harris and Yada, 2019) have compiled cases of outbreaks of foodborne illness and recalls due to microbial contamination of wheat flour and wheat-based products. Some of the most recent listed cases related to the consumption of raw wheat flour products in the USA include a dough mix contaminated with *E. coli* O157:H7 in 2016 and a cake mix contaminated with *Salmonella* Agbeni in 2018 (Harris and Yada, 2019). From these cases, the outbreak investigations pointed to contaminated wheat flour as the main suspect of being the pathogen carrier.

In the milling process, microbial contamination may occur which could lead to several food borne illnesses (Laca *et al*., 2006; Los, *et al*., 2018). Therefore, even when consumers are warned about the risks of consuming unbaked wheat-based products, the industry should explore alternatives to decontaminate wheat and wheat flour to ensure consumers' safety despite their risky eating behaviors.

This study was therefore aimed at isolating the microorganisms (bacteria and fungi) and investigating the antibiotic profile of bacteria associated with three stages of the production of flour from wheat grain (*Triticum aestivum*) as to assess the implications of the consumption of the wheat flour product to public health.

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Materials and Methods

Description of Study Area

Port Harcourt is located in the Niger Delta region; Southern Nigeria. The city is situated between latitudes $3^0$37' and $3^0$56'N and longitude $11^0$10' and $11^0$45'E, approximately 50km from the Atlantic Coast. Precipitation averages 3,030mm annual and a temperature average of 23℃. Port Harcourt is located in the Niger Delta region and in Southern Nigeria.

Wheat Flour Sample Collection

Wheat flour samples were aseptically collected from different stages of wheat flour production in a flour mill in Port Harcourt, Rivers State, Nigeria. A total number of six (6) samples from the UTF line, Production line, and Bagging stages were obtained. These were designated or simply coded as UTF, PD and BAG respectively. The samples were collected using a sterile container properly labeled and transported immediately in a cool box to the microbiology Laboratory of the Rivers State University, Port Harcourt for analyses.

Sterilization of Glassware and Media

All the glassware used during the study were thoroughly washed with detergent and rinsed with distilled water, dried and sterilized in the hot air oven at 160°C for 1½ hours. Sterilization of all media as well normal saline used for serial dilution was carried out in the autoclave at 121°C at 15psi for 15 minutes. The entire working area was also disinfected with 70% ethyl alcohol to reduce microbial contamination.

Preparation of Culture Media and Diluent

The following media used for the study were prepared following manufacturer's instruction and specifications; Nutrient agar (NA), Sabourand Dextrose Agar (SDA), MacConkey Agar (MCA), *Salmonella-Shigella* Agar (SSA), MacConkey Broth (MB), Mueller Hilton Agar (MHA), Nutrient Broth (NB), Preparation of Normal Saline (Diluent).

Microbial Analysis of Wheat Flour

Serial Dilution

One gram (1.0g) of the wheat sample was weighed and transferred into test tube containing 9ml of normal saline and shaken vigorously for a homologous suspension to form a stock solution. One milliliter (1ml) of the suspension was aseptically transferred to another test tube to give 10^{-1} dilution and serially diluted till a 10^{-5} dilution was obtained.

Cultivation, Enumeration and Isolation of Total Heterotrophic Bacteria and Total Fungi

Spread plates technique was used to determine total bacterial counts of the samples. An aliquot (0.1ml) of homogenized wheat flour sample from 10^{-4} was plated (inoculated) in duplicates into dried sterile nutrient agar plates and eventually spread with a sterile bent L shaped glass rod. The inoculated plates were incubated in inverted position at 37°C for 24 hours. All inoculations were done aseptically. The bacterial colonies observed after the incubation period, were counted and expressed as colony forming unit per gram (CFU/g) of the sample. With regards to fungi, the total fungi count (TFC) was determined by spreading plate technique 0.1ml aliquot from 10^{-2} and 10^{-3} were plated in duplicates into dried sterile SDA agar plate and eventually spread with a bent L shaped glass rod. The inoculated plates were incubated aerobically at ambient room temperature (28-30°C) for 3-5 days. The developed fungal colonies observed after the incubation period, were counted and expressed as colony forming unit per gram (CFU/g) of the sample.

Enumeration of Total Coliform and *Salmonella-Shigella*

Total coliform counts of the wheat flour samples 0.1ml aliquot of 10^{-3} and 10^{-4} of each sample were plated in duplicates in already prepared MacConkey agar, and *Salmonella-Shigella* agar for enumeration of total coliform of enteric bacteria (*E coli* and *Salmonella* respectively). The plates were incubated inverted at 37°C for 24-48hours for total coliform count. After the incubation period, the colonies were counted, and expressed as colony forming unit per gram (CFU/g) of the sample.

Sub-Culturing, Maintenance and Preservation of Bacterial and Fungal Microbial Isolates

The streak plate method was used to obtain pure cultures by repeated subculturing of each of the distinct colonies formed on freshly prepared nutrient agar plates and Sabourand dextrose agar plates for bacteria and fungi respectively. A sterile inoculating loop was used to make streaks of each of the isolates on the medium and incubation was done at 37°C for 24 hours for bacteria and at room temperature for 2-5 days for fungi.

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The various isolates were differentiated by their colonial morphology on the respective media after the incubation period following the subculture.
Discrete and distinct microbial isolates (bacteria and fungi) were preserved and maintained on the nutrient agar slants and Sabourand dextrose agar slants respectively in McCartney bottles or bijou bottles and stored in the refrigerator at 4°C for further use. This is known as stock culture.

Characterization and Identification of Bacteria and Fungi from Wheat Flour Samples

The pure bacteria isolates were analyzed based on morphological characteristics and biochemical tests carried out on the pure isolates. Cultural and morphological characteristics observed for each bacteria colony after 24h of growth included colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation and Gram's stain reaction. The following biochemical tests were performed using the pure cultures of bacterial isolates and adopting the procedures of (Cheesbrough, 2006); Catalase Test, Methyl Red/Vogues Proskaur (MR-VP), Oxidase Test, Motility Test, Indole Test, and Citrate. Sugar Fermentation Test was conducted to detect Acid and/or gas production during fermentative growth with sugars (glucose, lactose, sucrose, etc.). Results of the morphological and biochemical characteristics of the isolates were compared with those of known taxa using Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

Pure cultures of fungi were obtained by sub culturing discrete colonies onto freshly prepared Sabouraud dextrose agar plates and incubated at 28ºC for 5 to 7 days. The colonies which developed were further subcultured onto agar slopes or slants and incubated at 28ºC for 5 to 7 days. The following standard characterization tests were performed in duplicate; macroscopic examination of fungal growth was carried out by observing the colony morphology-diameter, colour (pigmentation), texture and surface appearance. Microscopic examination was done by needle mount or wet mount method and observing sexual and asexual reproductive structures. A wet mount was carried out using pure cultures of the fungi isolated. Two drops of cotton-blue-in-lactophenol were placed on grease free clean glass slide and a small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain. Using two sterile needles, the mycelium was gently teased into the medium as to obtain an even spread of the fungal hyphae.

Each prepared slide was gently covered with a cover slip to avoid air bubble. The slides were observed under low and high power objective, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1998).

Estimation of Coliforms in Wheat Flour Samples

Estimation of the coliform bacteria was done using the most probable number technique (MPN technique). Reaction to MPN technique and thermotolerant coliform bacteria MPN index 100ml of each wheat flour sample was done using double strength MacConkey broth for 10ml of sample and single strength MacConkey broth for 0.1ml and 1ml of the sample. The test for the estimation of coliforms involves the following steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al*. (1999).

Antimicrobial Susceptibility Profile

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique using Mueller-Hinton agar medium and 0.5 MacFarland's (1.5×10^8 CFU/ml) was employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017) and Cheesbrough (2006).

Peptone water (0.1%) diluent was prepared. Five discrete colonies of the different identified isolates were inoculated into 5 ml of the broths and incubated at 35°C for 4 – 6 h. The inoculum for sensitivity testing was prepared from a broth that has been incubated for 4 – 6 h. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard.

The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method. The antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37ºC for 24 h. Interpretation of results was done using the diameter zones of inhibition in millimeters (mm) (CLSI, 2017).

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Results

The results of the microbial counts of the wheat flour samples obtained from different stages of production is as shown in Table 1. The Total Heterotrophic Bacteria (THB) count ranged from 6.3×10^5 to 2.25×10^6 CFU/g, Total heterotrophic fungi ranged from 5.0×10^3 to 2.2×10^4 CFU/g, *Salmonella Shigella* count ranged from 1.0×10^2 to 5×10^2 CFU/g, and Total coliforms was 0.0 CFU/g.

Table 1: Microbial Counts of Wheat Flour Samples in Different Stages of Production

Wheat Flour Sample	Microbial counts (CFU/g)			
	Total heterotrophic bacteria	Total heterotrophic fungi	Salmonella/Shigella	Total coliform bacteria
UTF	8.2×10^5	5.0×10^3	5.0×10^2	0.0
Production	6.3×10^5	2.2×10^4	1.0×10^2	0.0
Bagging	2.25×10^6	5.0×10^3	5.0×10^2	0.0

The Colonial and morphological characteristics and probable identity of fungi isolated from the wheat flour samples is presented in Table 2. While the morphological and biochemical characteristics and probable identity of the bacterial isolates of wheat flour samples obtained from three different stages of production, isolated and identified in this study is presented in Table 3. The results showed that three Gram's positive bacteria (belonging to the genera: *Staphylococcus* spp. *Bacillus* spp and *clostridium* spp, and three Gram's negative bacterial species (includes *Escherichia coli*, *Salmonella* and *Serratia*).

On the other hand, the distribution of bacteria and fungi isolated from the three samples of wheat flour are presented in Table 4 and Table 5 respectively. While Figure 1 and Figure 2 shows the prevalence (%) for bacterial species and fungi species respective. The organisms isolated and their prevalence (%) were as follows; *Staphylococcus* spp (16%), *Bacillus* spp (26%), *Clostridium* spp (21%), *Escherichia coli* (10%), *Serratia* spp (11%), *Salmonella* spp (16%), *Aspergillus* spp (4%), *Candida* spp (21%), *Fusarium* spp (2%), *Mucor* spp (8%), *Penicillium* spp (6%).

Table 2: Colonial and Morphological Characteristics and Identity of Fungi Isolated from Wheat Flour

Colonial characteristics	Morphological characteristics	Fungal Isolates
Cream coloured, smooth round glistening colonies. Convex and opaque with a yeasty odour.	Oval shaped cells, budding spherical to elongated cells forming pseudo mycelium.	*Candida* spp
Fast-growing colonies in blue- green velvety growth with white periphery with reverse white colour.	Septate hyphae, with Branched condiophores bearing phialides condida and arranged in chains on the phialides.	*Penicillium* spp
Initially white but changes after a few days producing conidial spores. Conidial leads were dark brown to black. Reverse side of the colony was colourless to light yellow.	Conidial heads are large, globose and dark-brown hyaline hyphae and septate.	*Aspergillus* spp
White fluffy and wooly soft cottony, with pink/ yellow colour on reverse slide.	Septate hyphae with sporangium filled spores sickle shaped macroconida with spores that is yellow in colour. Hyphae are hyaline, plailades and cylindrical conidiophores.	*Fusarium* spp
White fluffy growth, with reverse white color large white colonies/wooly whitish colonies which turns black later.	Non-sepate hyphae, absence of rhizoid, long Branched sporangiophore with sporangia. Columella is present.	*Mucor* spp

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Table 3: Cultural, Morphological, Biochemical Characteristics and Probable Identity of Bacteria Isolated from Wheat Flour during Production

	Cultural Morphology					Microscopy		Biochemical									Sugar fermentation				Probable organism
Isolate code	**Colour**	**Elevation**	**Opacity**	**Size**	**Texture**	**Gram rxn**	**Shape**	**Catalase**	**Indole**	**Coagulase**	**Motility**	**Oxidase**	**MR**	**V.P**	**Starch**	**Citrate**	**Maltose**	**Glucose**	**Lactose**	**Sucrose**	
UTF1	Yellow	Round	Translucent	Small	Smooth	+ve	Cocci	+	-	+	+	+	+	+	+	+	AG	AG	AG	AG	*Staphylococcus sp*
UTF2	White	Round	Opaque	Small	Mucoid	+ve	Rod	+	-	-	+	-	-	+	+		A	AG	-	A	*Bacillus sp*
UTF3	Pale	Round	Opaque	Small	Smooth	-ve	Rod	+	-	-	+	+	+	-	-	-	-	-	-	-	*Salmonella sp*
UTF4	Yellow	Round	Translucet	Small	Smooth	+ve	Rod	-	+	-	+	-	+	-	-	+	-	AG	AG	AG	*Clostridium sp*
PD1	Yellow	Round	Translucent	Small	Smooth	+ve	Cocci	+	-	+	+	+	+	+	+	+	AG	AG	AG	AG	*Staphylococcus sp*
PD2	Creamy	Flat	Opaque	Small	Smooth	-ve	Rod	+	+	-	+	-	+	-	+	+	AG	AG	AG	AG	*Escherichia coli*
PD3	Red	Round	Opaque	Small	Smooth	-ve	Rod	+	-	-	+	-	-	+	-	+	-	AG	AG	AG	*Serratia sp.*
PD4	Yellow	Round	Translucent	Small	Smooth	+ve	Rod	-	+	-	+	-	+	-	-	+	-	AG	AG	AG	*Clostridium sp.*
BAG1	Clear	Round	Translucent	Small	Smooth	-ve	Rod	+	+	-	+	-	+	-	+	+	AG	AG	AG	AG	*Escherichia coli*
BAG2	Creamy	Flat	Opaque	Large	Mucoid	-ve	Rod	+	-	-	+	-	-	+	-	+	-	AG	AG	AG	*Serratia sp.*
BAG3	White	Round	Opaque	Small	Mucoid	+ve	Rod	+	-	-	+	-	-	+	+		A	AG	-	A	*Bacillus sp.*
BAG4	Pale	Round	Opaque	Small	Smooth	-ve	Rod	+	-	-	+	+	+	-	-	-	-	-	-	-	*Salmonella sp.*

Key: PD = Production; BAG = Bagging

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Table 4: Bacteria Isolates that Occurred in Wheat Flour in the Three Different Stages of Production

Bacterial isolate	Stages of production of wheat flour		
	UTF	Production	Bagging
Bacillus spp	+	+	+
Clostridium spp	+	+	+
Escherichia coli	-	+	+
Salmonella spp	+	-	-
Serratia spp	-	-	+
Staphylococcus spp	+	+	+

Key: + = isolated; = not isolated

Table 5: Fungi Isolates that Occurred in the Three Different Stages of production

Fungal isolate	Stages of production of wheat flour		
	UTF	Production	Bagging
Aspergillus spp	-	+	+
Candida spp	-	+	-
Fusarium spp	-	+	+
Mucor spp	+	-	+
Penicillium spp	-	+	+

Key: + = isolated; - = not isolated

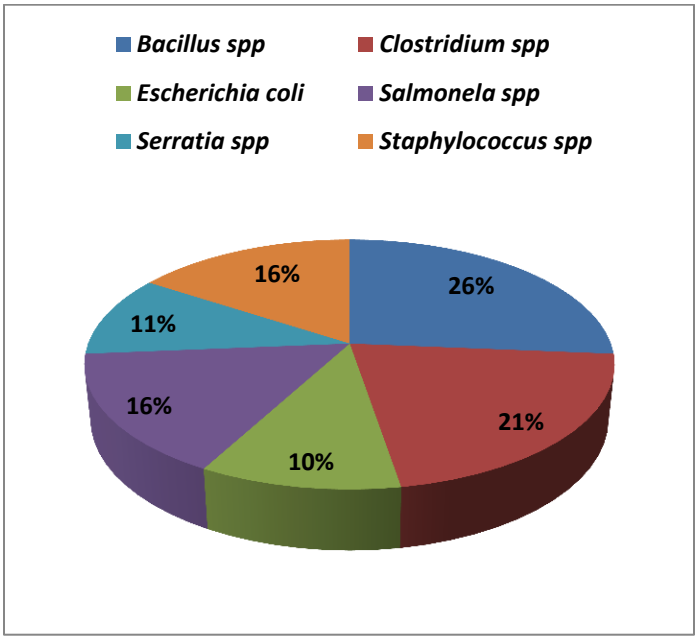

Fig. 1: Prevalence (%) of Bacteria Isolated from Different Stages of Wheat Flour Production

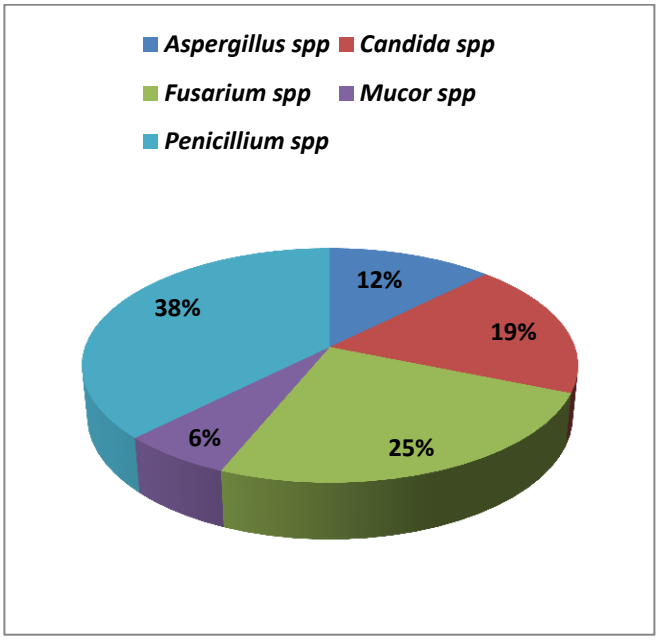

Fig. 2: Prevalence (%) of Fungi Isolated from Different Stages of Wheat Flour production

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The Antibiotics sensitivity profile of the Gram's negative bacteria isolated from wheat flour are as shown in Table 6.

While the antibiotics sensitivity profile of the Gram's positive bacteria isolated from wheat flour are as shown in Table 7.

Table 6: Antibiotics Sensitivity Profile of the Gram Negative Bacteria Isolated from Wheat Flour

Antibiotic	***E. coli* species N=24**			***Salmonella* species N=22**			**Overall sensitivity report for the Gram negative bacteria N=44**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amplicin (PN)	0	13	0	0	14	0	0	27	0
Augmentin (AUG)	0	0	0	22	0	0	0	0	0
Ceporex (CEP)	20	0	0	22	0	0	42	0	0
Ciproflox (CPX)	0	0	5	18	0	0	18	0	6
Gentamicin (GEN)	17	0	0	0	0	0	0	0	0
Nalidixicacid (NA)	24	0	0	20	0	0	44	0	0
Tarivid (OFX)	0	15	0	0	0	0	0	15	0
Septrin (SXT)	20	0	0	19	0	0	39	0	0
Streptomycin (S)	0	12	0	16	0	0	16	12	0

Key: Susceptibility (S), Intermediate (I), Resistant (R) (CLSI, 2017)

Table 7: Antibiotics Sensitivity Profile of the Gram Positive Bacteria Isolated from Wheat Flour

Antibiotic	***Bacillus* species (N=20)**			***Clostridium* species (N=30)**			***Staphylococcus* species (N=22)**			**Overall sensitivity report for the Gram positive bacteria (N=70)**		
	S	I	R	S	I	R	S	I	R	S	I	R
Amoxil (AML)	0	0	0	0	0	0	0	0	0	0	0	0
Ampiclox (AUG)	0	0	0	0	0	0	0	0	0	0	0	0
Chloramphemphenicol (CH)	0	0	5	0	0	0	0	0	0	0	0	5
Ciproflox (CPX)	0	12	0	0	10	0	0	0	0	0	22	0
Erythromycin (ERY)	0	0	0	0	14	0	0	15	0	0	29	0
Gentamicin (GEN)	18	0	0	30	0	0	22	0	0	70	0	0
Levofloxacin (LEV)	20	0	0	0	0	0	0	0	0	20	0	0
Rifampicin (RD)	0	0	0	0	0	0	0	15	0	0	15	0
Streptomycin (S)	0	14	0	0	0	7	18	0	0	18	14	7

Key: Susceptibility (S), Intermediate (I), Resistant (R) (CLSI, 2017)

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Most Probable Number (MPN)

MPN value per 100ml of wheat samples and 95% confidence limits for various combinations is shown in Table 8. The test confirmed the presence of coliform bacteria in the samples which were identified as *Escherichia coli* and *Serratia* spp.

Table 8: Most Probable Number Value for Various Combinations of Positive Tube Results

Wheat flour sample	Number of Tubes Giving Positive Reaction			MPN/100ml	95% Confidence limit	
	5 of 10ml	5 of 1ml	5 of 0.1ml		Lower	Upper
UTF	5	5	5	> 1600	-	-
Production	5	5	5	> 1600	-	-
Bagging	5	5	5	> 1600	-	-

Discussion

The study revealed the percentage of microbial contamination in the three stages of wheat flour production. The microbial counts of samples obtained from three stages in wheat flour production showed variations. The microorganisms present are as a result of cleaning methods, which includes magnets and the numerous steps of grinding and sifting to eliminate physical hazards in the finished flour, but causes microbial contamination, as well as the use of contaminated wheat grain (Sabillón *et al*., 2019; Sabillón *et al*., 2020).

In this study, six bacterial isolates and five fungal isolates belonging to their genera and percentage of occurrence in parenthesis were recorded/obtain within the sampling period as; *Staphylococcus* spp (16%), *Bacillus* spp (26%), *Clostridium* spp (21%), *Escherichia coli* (10%), *Serratia* spp (11%), *Salmonella* spp (16%), *Aspergillus* spp (4%), *Candida* spp (21%), *Fusarium* spp (2%), *Mucor* spp (8%), *Penicillium* spp (6%). These microbial isolates are known potential pathogens. These fungi are also known mycotoxin producers (Manthey *et al*., 2004).

MPN was used to confirm the presence of coliform bacteria in the samples as there was no growth on the agar plate, the MPN result showed the presence of *E. coli and Serratia* spp. Even though most of the may not be pathogenic, their presence in wheat flour in high amounts can still course food-borne diseases.

Results of Antibiotic sensitivity revealed that all the Gram positive isolates were susceptible to Gentamycin while all the gram negative bacterial isolates were susceptible to Ciproflox, Nalidixic Acid and Septrin. *E. coli* is a subgroup of fecal coliforms used as an indicator of fecal contamination. Although vast majority of *E. coli* are completely harmless, some strains of the bacteria have acquired genetic capabilities which enable them to encode virulence factors (Agata *et al*., 1995; Lim *et al.*, 2010). Obire *et al*. (2009) had also reported the susceptibility of *E. coli* to antimicrobial agents. Pathogenic *E. coli* strains cause diverse forms of bacterial induced illnesses with symptoms ranging from mild diarrhoea to severe complication and even death (Vidal *et al*., 2019).

The study revealed that the microbial load of at the bagging stages was higher than that of the UTF and production stage; this could be as a result of contamination at the packing scale and from individuals which handle the milling operation and final processing to form the final product, whereas the production stage had the least microbial load. Manthey *et al*. (2004) also reported the microbial loads, mycotoxins, and quality of durum wheat. While Laca *et al*. (2006) and Los *et al*. (2018) stated that, in the milling process, microbial contamination may occur which could lead to several food borne illnesses.

In conclusion, this study has revealed that, the microbial contamination in the three stages of wheat flour production from a wheat flour production company in Port Harcourt is as a result of contamination of wheat grain and poor manufacturing practices. The microbial load is typically found on the surface of wheat kernels. However, during the dry milling process, the microbial contamination is redistributed among the milled products, leading to inadequate microbiological quality of refined wheat flour.

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The microbial counts recorded in this study was not above the standard that total heterotrophic bacterial and fungal counts, for wheat flour must not be above 300 CFU/g while total and faecal coliform was 0 respectively. However, *Escherichia coli* was later isolated and identified from MPN positive test tubes of the samples. From the findings of this study, it is recommended that there should be public awareness of the microorganisms found on the wheat grains in other avoid contamination of the wheat flour during production. The wheat grains should also be tested for microorganisms such as coliforms and fungi and decontaminated as also stated by Hu *et al*. (2016) before wheat flour production and Good manufacturing practices should be maintained to prevent microbial contamination of wheat flour.

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