

Microbial Assessment of Aprons Worn By Some Street Food Vendors In Awka South, Anambra State, Nigeria

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ABSTRACT: Many food vendors touch money and other contaminated items with their bare hands before serving meals to customers without cleaning them. This creates a pathway for microorganisms to spread from their hands to the aprons and then the food. A total of six swabs were aseptically collected randomly from different parts in the respective aprons of food vendors from six randomly selected street food vendor points within the premises of the study area. The isolates' cultural and morphological characteristics were identified. Four bacterial and six fungal isolates were found in the aprons. The bacterial isolates include *Staphylococcus aureus* (32.7%), *Bacillus* spp.(21.8%), *Klebsiella* spp.(11.1%) and *Escherichia coli* (34.01%) while the fungal isolates include *Mucor* spp. (12.1%), *Candida* spp. (17.1%), *Microsporum canis* (17.1%), *Penicillium* spp. (9.7%) and *Aspergillus* spp. (24.3%) for fungi were isolated. *Escherichia coli* and *Aspergillus species* were the most prevalent bacterial and fungal isolates respectively. It was observed that aprons of food-vendors who stay in close proximity to garbage dumps contained higher levels of pathogenic organisms. The results of this study showed that most food vendors fail to maintain proper food hygiene, which raises concerns for the public's health. Education of food vendors on personal, environmental, and food hygiene is crucial since it will help to reduce apron contamination and improve the safety of the food provided at vending locations.

KEYWORDS: Aprons; cross-contamination; food-vendors; microorganisms

1. Introduction

Food vending has a long tradition in many developing countries like Nigeria. The trade has evolved over time, from vendors carrying a single food item on their heads to pushing several foods in wheelbarrows and carts along the streets and markets. The contamination of surfaces and food served by vendors can pose public health risks to the consumers [1]. Street foods includes various food items sold in public places. The United Nations Food and Agricultural Organisation (FAO) estimated that over 2.5 billion people consume street foods daily [2]. According to [3], some street food vending sites were sanitary and significantly high number of food vendors had good food handling practices.

However, there is growing evidence that street food consumption is increasingly affecting people of all socioeconomic groups worldwide. Aprons, protective clothing worn over other types of casual clothing, are indispensable items in the wardrobe of both adults and children. Aprons are worn as needed for work that involves clothes getting dirty or damaged and protect against stains, grease, water, etc. [4]. The risk of serious foodborne illness outbreaks associated with street food remains a threat in many parts of the world, with street vendors' ignorance about the causes of foodborne illness being a major risk factor [5]. While many consumers value hygiene when choosing street food vendors, they are often unaware of the health risks associated with street food [6].

Numerous studies have shown that food handlers lack basic knowledge and formal training in food hygiene and safety in most parts of Africa. The common foodborne pathogens associated with street-vended foods include *Clostridium perfringens*, *Escherichia coli*, *Shigella* spp., *Campylobacter jejuni*, *Staphylococcus aureus*, *Salmonella* spp., and *Bacillus cereus* [7]. Reports by [4] shows that the level of knowledge of street food vendors about food safety practices in urban Nigeria is very high, but this knowledge has not been fully applied in practice. This situation is often aggravated by the lack of personal hygiene among street food vendors. Poor food suppliers and poor waste disposal methods increase the risk of food contamination with pathogenic microorganisms, leading to the risk of foodborne illness outbreaks. Studies on the microbiological quality of street food in Africa and other parts of the world have revealed that street food is heavily contaminated by various microorganisms. The lack of regulations on street food business by state management agencies is a big challenge in terms of food hygiene and safety. An earlier study of street food in developing countries found that the inspection of registered fast food restaurants was rudimentary to non-existent [8]. So it is not surprising that food suppliers are not regulated by the local government. The study aims to isolate and characterize the bacterial and fungal contaminants on the aprons worn by selected food vendors in Awka South Local Government Area of Anambra State, Nigeria.

2. Materials and Methods

2.1. Study area.

The study was conducted in Awka South Local Government Area, Anambra State, Nigeria, situated at Latitude: 6.2443° and Longitude: 7.1237°.

2.2. Sample collection.

A total of six swabs were aseptically collected randomly from various parts of the respective aprons of food vendors in six randomly selected canteens within the premises of the study area. These canteens were specifically chosen if they were located near dumpsites and contaminated environments. The swabs were labeled A, B, C, D, E, and F. Each sterile swab stick was dipped into normal saline and used aseptically to swab the chest and lower body region of the respective aprons of each food vendor. After collection, the swab sticks were placed in sterile containers and promptly transported to the laboratory for analysis. This procedure was carried out in accordance with the method used by [9].

2.3. Isolation of bacterial and fungal isolates from the aprons.

Each swab stick was inoculated aseptically onto freshly prepared Nutrient agar (NA) and Sabaraud Dextrose Agar (SDA) in petridishes for growth of bacterial and fungal isolates respectively. The NA plates were incubated at 37°C for 24 hours while the SDA plates were incubated at 37°C for 48-72 hours for SDA plates. This procedure was as used by [9].

2.4. Biochemical identification of bacterial isolates

The morphological features of the isolates were microscopically determined and biochemical tests were carried out to identify the isolates. Microscopic and biochemical tests done using standard methods includes Gram staining, motility, catalase, coagulase, urease, citrate, sugar tests, indole, methyl red tests and voges proskauer tests.

2.4.1. Gram staining.

This was done using previous methods as described by [10].

2.4.2. Catalase test.

This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H₂O₂). It was used to distinguish bacteria that produce catalase enzymes, such as *staphylococci*, from bacteria that do not produce catalase, such as streptococci. Usually, 3% H₂O₂ is used for conventional culture while 15% H₂O₂ is used to detect catalase in anaerobic bacteria [10].

2.4.3. Motility test.

This was done using the methods described previously in [10]. Bacterial motility is indicated by the diffuse growth zone extending from the inoculum line. Some organisms grow throughout the medium, while others have small areas or nodules that develop from the inoculum. Non-motile bacteria will grow only in the soft agar tube and only in the area where they are inoculated.

2.4.4. Coagulase test.

This was done using the previous methods described by [11]. Place one drop of physiological saline solution on a clean slide and produce a smear that isolates the test organism 24 hours ago. One drop of human plasma is added to obtain a suspension. Aggregation shows a positive result and it implies the ability of the tested organism to produce coagulase, an enzyme that coagulates plasma whereas a negative result does not observe agglutination.

2.4.5. Citrate test.

This was done according to the description of [12] and [11]. This detects the organisms' ability to use citrate as its sole carbon source. Simon's citrate is prepared by weighing 2.5 g sodium citrate, 1.5 g ammonium phosphate, 0.2 g magnesium sulfate, 1 g potassium dehydrogen phosphate and 0.1 g bromothymol blue and dissolving it in 1 liter of distilled water. homogeneous and distributed in the test. tube and then plug it back in with cotton. One seed of each isolate was inoculated on Kosers citrate medium and incubated at 37°C for 72 h. Positive citrate is confirmed by the formation of a blue color while green color initially indicates a negative result.

2.4.6. *Indole test.*

This test was performed as described by [11]. The peptone water weighing 1.5 g is placed in a 250 ml conical flask. 100ml distilled water is added gradually and shaken well. It was then enriched with 1 g of tryptophan and heated on an electric stove to homogenize and finally dispensed into test tubes and capped for sterilization in an autoclave at 121°C for 15 min. One seed of each isolate was inoculated in 5 ml of sterile peptone water with 1% tryptophan added in the test and incubated at 37°C for 48 h of culture, 0.5 ml of Kovacs indole reagent and gently shaken. In the case of a positive test, indole (present in the culture medium) dissolves in the reagent, which then turns pink or red and forms a layer on the surface of the medium. A yellow layer on the surface of the medium is observed.

2.4.7. *Urease test.*

This was done according to the description of [12] and [11]. Christenses urea agar was prepared by weighing 20 g plain agar, 1 g peptone, 1 g glucose, 0.1 g phenol red, 1.2 g disodium hydrogen orthophosphate and 5 g sodium chloride dissolved in 100 ml distilled water, Heat to dissolve completely. The pH was adjusted to 6.8 using an electrode pH meter to produce a yellow color, dispensed into multipurpose flasks, and sterilized in an autoclave at 121°C for 15 min. 5 ml of a sterile 40% membrane urea solution was placed aseptically into a universal bottle and allowed to solidify in an inclined position. One seed of each isolate was inoculated on Christenses urea agar and incubated at 37°C for 24 h. The red release indicates a positive urease test while the initial yellow indicates a negative test. Check the control has been read and saved.

2.4.8. *Methyl red test (MR test).*

This is done according to the description of [13] and [14]. Phosphate-buffered glucose peptone medium is prepared by weighing 0.5 g peptone, 0.5 g glucose and 0.5 g dipotassium hydrogen phosphate (K₂HPO₄) in 10 ml of distilled water and heating on a plate, heating to dissolve completely and dispense into test tubes, cover with foil and cotton and sterilize at 121°C for 15 min. One seed of each isolate was inoculated at 37°C for 48 h. A few drops of methyl red were added to the culture medium. A positive MR test is indicated by a red formation while no change indicates a negative result.

2.4.9. *Voges Proskauer.*

The test organism was inoculated in glucose-phosphate peptone water and incubated at 270°C for 48 h. Then add 1 ml of 40% potassium hydroxide solution and 3 ml of 5% alpha-naphthol solution (VP reagent) in absolute ethanol. A positive reaction is indicated by the appearance of a pink color within 2 to 5 minutes [13].

2.4.10. *Sugar fermentation test.*

This test aims to determine if bacteria can ferment a particular carbohydrate. Weigh 1g (1g) of each sugar and place in separate beakers, dissolve 0.75g of peptone water in 500ml of distilled water and add a moderate amount of bromothymol blue to the mixture. 100 g of each mixture was added to each cone. bottle containing different sugars and mix well. Each type of 10ml sugar is put into test tubes containing Durham tubes upside down, plugged with

cotton wool and wrapped in aluminum foil, then autoclaved at 115 degrees Celsius for 15 minutes. After cooling, the test cultures were then added separately and the media were incubated at 35 °C for 24 h. The tubes were tested for acid-forming capacity, indicated by a yellow discoloration, and gas formation indicated by bubbles or voids in the upper part of the Durham tube. The organisms ferment carbohydrates and produce organic acids, thereby lowering the environmental pH and gas production [10].

2.5. Isolation, identification and confirmation of isolated fungal strains.

Place one drop of lactophenol cotton blue reagent onto a clean, dry glass slide. The culture ring is carefully used to inoculate the fungus onto a thin preparation plate placed on top of the thin preparation. The dye is allowed to act for 5 minutes. It was observed under a microscope with a low-power 40x objective for low-intensity screening. The identification is based on the shape and arrangement of the spores (conidial ontogeny) [10].

3. Results and Discussion

A total of four bacterial strains and six fungal strains were isolated from the aprons, including *Staphylococcus aureus* (32.7%), *Bacillus* sp. (21.8%), *Klebsiella* spp. (11%), and *Escherichia coli* (34.01%) for bacteria. The fungal strains comprised *Mucor* sp. (12.1%), *Candida* spp. (17.1%), *Microsporium canis* (17.1%), *Penicillium* spp. (9.7%), and *Aspergillus* sp. (43.8%). *Escherichia coli* was the predominant bacteria, accounting for 34.01%, while *Aspergillus* species constituted the majority of fungi, totaling 43.8%. Table 1 details the morphological characteristics of bacterial strains isolated from different types of aprons worn by grocers, while Table 2 presents the prevalence of bacterial strains isolated from the collected apron swab samples. *Escherichia coli* had the highest rate (34.01%), and *Klebsiella* spp. had the lowest rate (11.6%). Table 3 illustrates the macro- and microscopic appearance and characteristics of fungal strains isolated from different types of aprons worn by grocers, and Table 4 shows the prevalence rates of isolates, with *Aspergillus* species having the highest rate (43.5%) and *Penicillium* spp. the lowest (9.7%).

Table 1. Morphological properties of the bacterial isolates from the aprons.

Isolates	Margin	Elevation	Texture	Pigmentation	Form	Opacity
A	Entire	Convex	Smooth	Cream	Circle	Opaque
B	Undulate	Flat	Rough	Cream	Circle	Opaque
C	Undulate	Convex	Rough	Cream	Circle	Opaque
D	Undulate	Flat	Rough	Cream	Circle	Opaque
E	Flat	Convex	Mucoid	Pink	Circle	Opaque
F	Undulate	Flat	Rough	Cream	Circle	Opaque

The bacteria identified are of the following species: *Bacillus cereus*, *Escherichia coli*, *Klebsiella*, *Staphylococcus Aureus*, while fungal isolates included *Aspergillus niger*, *Microsporium canis*, *Mucor* spp., *Penicillium* spp., *Candida* spp. and *Aspergillus flavus*. *Escherichia coli* was the most common bacterium in the study as shown in Table 2. This is consistent with previous studies that reported *Escherichia coli* as one of the major food contaminant, alongside other microorganisms [15]. The presence of these bacteria in aprons indicates that food sold with an apron may have been contaminated during processing. However, other factors such as unhygienic practices can also cause aprons to become

contaminated after normal daily sales. These findings are similar to those reported by [16] who worked on lab coats of medical students.

Table 2. Prevalence of bacterial isolates from the swab samples collected.

Isolates	Frequency	Prevalence percentage
<i>Escherichia coli</i>	50	34.01%
<i>Staphylococcus aureus</i>	48	32.7%
<i>Bacillus cereus</i>	32	21.8%
<i>Klebsiella</i> spp.	17	11.6%

Table 3. Macroscopic and microscopic properties of fungal isolates from the aprons.

Isolates	Colony Description	Microscopic Features	Suspected Organism
A	Compact white turned black with aging	Septate, hyaline hyphae. Conidiophores are long with spherical vesicles at the apex.	<i>Aspergillus niger</i>
B	Cottony and white Reverse: deep yellow	They have septate hyphae that produce numerous macroconidia. They are truncated, thick-walled and spindle shaped with snout.	<i>Microsporium canis</i>
C	Cottony/woolly and white, turned greyish-brown with aging	They have non-septate hyphae called the sporangiophores.	<i>Mucor</i> spp.
D	Reverse: pale white	The entire structure, the conidiophores and extending conidia resemble a “brush”.	<i>Penicillium</i> spp.
E	Creamy/glabrous and white Reverse: white	Shows spherical to sub-spherical budding blastoconidia. Some were germ tube test positive detecting <i>Candida albicans</i>	<i>Candida</i> spp.
F	Cottony and powdery, turned yellow-green during Maturation. Reverse: Pale yellow	Septate, hyaline hyphae. Conidiophores are long with spherical/elongate vesicles at the apex.	<i>Aspergillus flavus</i>

Table 4. Prevalence of fungal isolates from the swab samples collected.

Isolates	Frequency	Prevalence percentage
<i>Aspergillus niger</i>	10	24.3%
<i>Microsporium canis</i>	7	17.1%
<i>Mucor</i> spp.	5	12.1%
<i>Penicillium</i> spp.	4	9.7%
<i>Candida</i> spp.	7	9.7%
<i>Aspergillus flavus</i>	8	19.5%

The pathogens isolated in this study were similar to those reported by [1][17]. Presence of *Bacillus cereus*, *Escherichia coli*, *Klebsiella*, *Staphylococcus* sp., *Aspergillus niger*, *Microsporium canis*, *Mucor* spp., *Penicillium* spp., *Candida* spp. and *Aspergillus flavus* reported in this study is also consistent with the findings of [2]. In addition, the results of this work are also consistent with those of [17] who reported the presence of *S. aureus*, *Bacillus* spp., *Escherichia coli*, *Pseudomonas species*, *Sacchromyces* spp., *Rhizopus* spp. and *Aspergillus* spp. in the hands of food vendors in Abeokuta Metropolis, Ogun State, Nigeria. Biological contaminants such as bacteria, viruses, fungi, protozoa, and helminths are the leading cause of foodborne illnesses of varying severity, ranging from mild illness to chronic illness or disease, life-threatening, or both [18] and [19]. In developing countries, these contaminants are responsible for foodborne illnesses such as cholera, campylobacteriosis, gastroenteritis, salmonellosis, shigella, brucellosis and amoebiasis [17].

4. Conclusion and Recommendation

The isolation of bacterial and fungal pathogens from dress vendors' aprons indicates that cross-contamination of food by street vendors is a primary cause of illness among consumers. Food outlets located near landfills have been observed to harbor more pathogens, potentially facilitated by flies. Despite the positive contributions of caterers to society, they also pose adverse effects on public health. Therefore, this study emphasizes caution in patronizing food suppliers. The identification of bacterial and fungal pathogens from caterers' aprons reflects poor hygiene standards and underscores the need for regular testing by regulatory agencies. It is crucial that food suppliers receive training in food handling practices and adhere to general good hygiene practices (GHP) to mitigate the risk of foodborne illnesses and ensure the safety of consumers.

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Competing Interests

The Authors hereby declare that competing interests does not exist regarding the publication of this research.

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