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ISOLATION AND IDENTIFICATION OF FUNGAL ISOLATES FROM CANNED BABY FOOD SOLD AT SOME MAJOR MARKETS IN KANO METROPOLIS

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ABSTRACT

Food contamination by pathogens has become a global issue of health concern. The challenges involved fully grown adult and children. In food production, contamination during preparation processes, use of contaminated containers materials and storage. This causes a lot disease condition most especially in children e.g. bacterial, fungal and parasitic infections. In the study, twenty (20) of canned baby food samples were purchased from different markets, shops and supermarkets in Kano metropolis. One hundred microliters of each of the diluted samples used above will also be plated onto Patato Dextrose Agar. Petri plates were incubated at 25°C for 96 h and examined daily for fungal growth. Fungal colonies on inoculated samples were observed and sub cultured on SDA for identification. It is observed that total number of 20 fungal isolates was discovered in the study, five different markets, Sabon gari, Singer, Rimi, Tarauni and sheka respectively. Highest incidence was obtained from Aspergillus niger (40%), then followed by Penicillium spp (25%), Aspergillus flavus (20%) and lastly, Aspergillus fumigatus (15%). This indicated that measure of public health concerning has to be taken on the canned food sold for the children. Enforcement agency need strengthen their effort to make sure exiting guideline and laws are followed to make sure they are fully implemented.

Key words: Canned baby food, Contamination, Isolation, Identification and *Penicillium* spp

INTRODUCTION

Feeding infant with powdered formula and ready to eat infant food issued from birth through first two (2) years to feed millions of infants over the world. This kind of nutrition represents a rich source of nutrients and contains ingredients from various origins. A part of given nutritive food value, it is considered an excellent medium to support bacterial and other microbial growth and carrying potential risk of exposure to foodborne pathogens. Infants and babies are more susceptible to infection by such pathogens because of their less well developed immune system and lack of competing intestinal flora Havelaar, (2015). Low moisture content of dried infant foods and even ready to eat baby's acts as inhibitory factor with respect to bacterial and fungal spores or vegetative that have survived drying or processing. These microorganisms cannot grow and play any direct role in their spoilage. Their occurrence in the products is a great significance served as an index of hygienic standards maintained during production, processing and handling Abdullah and Jahan, (2010). But, reconstituted infant foods are considered to be a food class of high risk due to the infants susceptibility to enteric bacterial pathogens in order to mortality WHO, (2015). Epidemiological evidence found that the majority of outbreaks worldwide due to B. cereus have been associated with concentrations (105 cfu/g in implicated foods). Infants and babies are more susceptible to infection by such pathogens because of their less well-developed immune system and lack of competing intestinal flora Havelaar, (2015).

The microflora of dried milk powders depends on the number and type of fungal species in the raw milk or milk by-product, preheating temperature, operation conditions, evaporator and/or dryer and plant hygiene Amany, (2013). *Aspergillus flavus* contamination of infant milk formulas was well documented in previous studies (Amany, 2013; Khalil *et al*., 2017). *Aspergillus niger* was among the primary microbes associated with baby food contamination as reported by FAO/ WHO Expert. In spite of that *Aspergillus niger* was classified as category C or of low risk, its prevalence in infant food was sufficiently high to cause food borne infection outbreaks (Ben Salem *et al*., 2012 and Muhammad *et al*., 2016). The spores are resistant to many processes as low and high temperatures, desiccation, disinfectant agents, ionization; radiation and ultraviolet light (Kauffman, 1974 and Threlfall, 1990). *B. cereus* has been reported to produce aflatoxin and 1 emetic toxin. From them, heamolysine BL (HBL) and non-hemolytic enterotoxin (NHE) consists of 3 different exo-proteins while the other toxins, ENTFM, CYTK and BCE consists a single protein Newton, (2012). Therefore, this work aimed to investigate the incidence of *Bacillus* spp. and its enterotoxigenic virulence factors in infant formula and ready to use baby food collected from Cairo and Giza markets and pharmacies under Egyptian condition of production, storage and distribution.

Food-borne pathogens were recently the cause of disease outbreaks reported to result in high morbidity rates in Hail and Abha: 39 cases of *Staphylococcus aureus* and 26 cases of *Salmonella enteritidis,* respectively (Khalil *et al*., 2017). Although food-borne diseases do not always result in acute gastroenteritis, food represents an important vehicle for transmission of food-borne diseases. Diarrheal diseases are the commonest manifestation of food poisoning and in some cases, it is highly lethal too. The

aim of the study is to isolate and identify the fungal isolates that are associated with contamination of canned baby food commonly sold in some markets in Kano metropolis.

MATERIALS AND METHODS

Sample Collection

A total of twenty (20) of canned baby food samples were purchased from different markets, shops and supermarkets in Kano metropolis which includes: Sabon gari, Singer, Rimi, Tarauni and Sheka Markets. Samples were packed separately, kept and transported Laboratory for microbiological investigation at Sa'adatu Rimi College of Education Kumbotso, Kano.

Fungal analysis

One gram (1g) of each sample of canned baby food sample was put into nine militer (9mL) of sterilized distilled water, then four folded serial dilutions were carried for each sample and plated on the Patato Dextrose Agar. Petri plates were incubated at 25°C for 96 h and examined after every 24 h for fungal growth. Fungal colonies observed on the samples were sub cultured on Sabouraud Dextrose Agar (SDA) for identification. Morphological and cultural characteristics of the growing cultures were evaluated for preliminary identification. Then fungal colonies were subjected to microscopic identification as described by (Gilman, 2001).

A pure colony of each obtained and subsequently be maintained. The maintenance will be done by sub-culturing each of the different colonies on to the PDA plates and incubated at room temperature again for 9 h. In sub-culturing, baiting method was used which involves the use of a sterile wire loop to pick the culture growth and then transferred to the subculture media, after which the petri dishes were labeled accordingly.

Identification of Fungi

The technique of John, (2001) was adopted for the identification of unknown isolate using direct microscopy. The identification was achieved by placing a drop of normal saline on clean free grease slide with the aid of a wire loop, where a small portion of mycelium from fungal culture was removed and placed on the drop of normal saline. The mycelium was spread very well on the slide with the aid of a wire loop. A cover slip was gently applied with a little pressure to eliminate air bubbles and over flowing. The slide was mounted and observed with 10X and 40X objectives lenses respectively. The species encountered were identified in accordance with the method adopted by Cheesbrough, (2012). The main characteristics will be employed in their identification are as follows: 1.Hyphae: Septate or Non-septate 2. Mycelium: Colored or Non-colored 3. Spores: Types of asexual, nature of spores 3. Presence of special structures, such as stolon, rhizoids and foot cells.

RESULTS AND DISCUSSIONS

The number of fungal species isolated from canned baby food sold at some major markets in Kano as shown in the table 2. It was observed that total number of twenty (20) different fungal isolates were discovered in the study, from five different markets, Sabon gari, Singer, Rimi, Tarauni and sheka respectively. Highest incidence the fungal isolates was obtained from *Aspergillus niger* (40%), then followed by *Penicillium* spp (25%), *Aspergillus flavus* (20%) and lastly, *Aspergillus fumigatus* (15%). In the study *Aspergillus niger* had highest incidence of contamination of canned baby food, this similar with the findings of Oje *et al*., (2018) which found some level of fungal growth in the canned food with presence of aflatoxins. The high frequency of members of the genus Aspergillus might be due to two reasons. Firstly, during processing and storage conditions which the products may come in contact with the mycelium of the fungal isolates. In addition, *A. flavus* and *A. niger* were pre-dominant fungal isolates found in the peanut, producing molds in the market under warm condition (Horn, 2015).

It revealed that *A. niger* and *A. fumigatus* contaminating already packaged food mostly agricultural commodities such as flour of maize wheat and barley sold in the markets. The moderate incidence growth of *Penicillium* spp might be due to the presence of moisture as reported by Horn, (2015) that conditions resulting in the high incidence of *Penicillium* spp in already package food materials were attributed to moisture content. While other reasons are associated with good processing practice, that is Standard Operation Procedure (SOP), handling and storage conditions.

CONCLUSION

From the results obtained it shows that there was high incidence of fungal isolates of *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus flavus* and *Penicillium* spp from baby canned food sold in the major markets in the Kano metropolis. This indicated measures of public health concerning on the canned food sold for the children. Enforcement agency need to put more measure and guideline for the existing law to fully implemented.

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Table 1: Identification of Fungal isolate from canned baby food

S/N	Fungal Isolate	Characteristics features	Identity Sources
1.	*Aspergillus niger*	Dark greenish colony, septate multi-Nucleated hyphae with holy sprinkler like Conidios6pheres.	Burnet, (1976), John, (2001) Gillman, (2001), Bayman *et al*.,(2002)
2.	*Aspergillus fumigatus*	Blue green colony, unseriate columnar Conidial heads with phialides limited to The upper part of the vesicle.	Gillman, (2001)
3.	*Aspergillus flavus*	Yellow colony, septate and multinucleate Hyhae with sprinkler head (conidia) on the Conidiospheres	Burnet, (1976), John, (2021), Gillman, (2001), Raper and Fennel, (1965), Klinch, (2002)
4.	*Penicillium* spp	Greenish-blue colony, septate hyphae with Conidiophore bearing brush like conidia	Burnet, (1976), John, (2001), Gillman, (2001)

Table 2: Distribution of Fungal isolates identified from different Markets within Kano Metropolis

Market	*A. niger*	*A. fumigatus*	*A. flavus*	*Penicillium spp*	Total
Sabon Gari	3	1	2	1	7
Singer	1	1	1	ND	3
Rimi	1	ND	ND	1	2
Tarauni	2	1	1	2	6
Sheka	1	ND	ND	1	2
TOTAL	**8**	**3**	**4**	**5**	**20**
Incidence (%)	**40**	**15**	**20**	**25**	**100**

ND – Not Detected

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