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Microbial Exposure Assessment of Fresh and Smoked Pork Meat within Ado-Ekiti Metropolis, Nigeria

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Authors' contributions

This research work was carried out with great collaboration among all authors. Author PIO laid a great designed for the research study, carried out literature review, wrote protocol and the necessary draft. Authors GOA and NOA were instrumental in laboratory works, including collection of data. Authors SOO and RA were pivotal in performing the statistical analysis and as well managed all analyses. All authors read and approved the final manuscript for publication.

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ABSTRACT

The breeding and production environments of pigs tend to be exposed to microbial contaminations and could portend a potential public health hazard if not well managed. This study investigated bacterial and fungal loads of commercial fresh and smoked pork in order to ascertain their wholesomeness. Total aerobic bacterial and fungal count were done on nutrient agar and potato dextrose agar respectively, while antimicrobial susceptibility test to selected commercial antibiotic discs carried out using Disk Diffusion Technique. All the twenty samples (Fresh and Smoked) cultured yielded bacterial growth with a range of 2.2 - 9.0 × 10^4 CFU/g (smoked) to 1.0 – 6.3 × 10^6 CFU/g (fresh). On the other hand, the fungal loads ranges from 1.0 – 6.0 × 10^2 CFU/g(smoked) to

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1.0 -5.0 × 10^4 CFU/g (fresh). The bacterial isolated and the ratios in fresh and smoked samples were*Staphylococcus aureus* (6: 11), *Escherichia coli* (5: 8), *Bacillus cereus* (4: 7), *Salmonella* spp. (2: 3), *Proteus* spp. (0: 4), *Enterobacter* spp. (1: 2), *Shigella* spp. (0:2) and *Pseudomonas aeruginosa* (1: 0), while their fungal counterpart included *Aspergillus niger* (4: 2), *Aspergillus terreus* (2; 2),*Fusarium oxysporum*(4: 0), *Penicillium* spp. (4: 2), *Rhizopus* spp. (3: 5), *Mucor spp.* (0: 4); *Geotricum candidum* (0: 2) and *Microsporium* spp. (0: 2). Most of the *S.aureus* (> 58.8%) and *P. aeruginosa* (100%), and *B. cereus* (100%) demonstrated remarkable resistance to the majority of the tested antibiotics. These findings are of public health concern because most of the bacterial and fungal isolates have been implicated in foodborne infections. Hence, there is a need for stricter sanitary measures during the rearing and production to reduce the level of microbial contaminations.

Keywords: Pork meat; antibiotics; Salmonella spp; foodborne infections; antibiotic resistance.

1. INTRODUCTION

Meat has been known for its nutritive composition which is why it is being consumed by many people worldwide (Bradeeba*et al*., 2013). The protein profile of meat consists of amino acids which have been described as excellent due to the presence of all essential ones required by the body (Bradeeba*et al*., 2013). A large proportion of the world's population relies on meat as a source of food. Enteric bacteria can cause infections in humans when undercooked meat products are consumed. [1]. It has also been proved that protein and vitamins (especially A and B12) in meat could not be substituted by plant sources, this is further justifying the nutritive importance of the former.Domestication of pigs started somewhere around 5000 years ago [2]. Pigs have great potential to fulfil the demand of meat for the increasing population of the world because of their high feed conversion ratio, high prolific rate, short gestation period and great adaptability with respect to food and climate [2].The word 'pork' was derived from the French 'porc' and Latin 'porcus' meaning "pig". Pork has been proved to be an important source of food worldwide contributing about 40% to the total meat production around the world. Pork is the most perishable of all important foods since it contains sufficient nutrients needed to support the growth of microorganisms.

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses. [3]. Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation [4]. Sources for contamination of the pork can be abattoir, storage at the retailer's stall or shop, heavily contaminated utensils and benches used for the handling of pork. Pork contamination occur by a variety of ways, including bowel rupture during evisceration, indirect contamination with tainted water and also handling and packaging of finished pork products. A great diversity of microbes inhabits fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature and transportation means of raw meat, [5]. Raw meat may harbour many important pathogenic microbes i.e. *Salmonella spp*., *Campylobacter jejuni, Yersinia enterocolitica*, *E. coli*, *S. aureus* and to some extent, *Listeria monocytogenes*, making the meat a risk for human health, as without the proper handling and control of these pathogens, food borne illnesses may occur, [6].

Food habits of society have substantially changed due to rapid urbanization and hurried way of living, resulting in increased demand for ready to cook and ready to eat meat products. Consumers have become more selective conscious of quality, concerned about value for money, freshness and health aspects of meat food products. Meat is not only highly susceptible to spoilage, but also frequently implicated to the spread of food-borne illness, various biochemical changes and microorganisms are associated with meat, during the process of slaughter, processing and preservation [7]. Approximately 69% of gram negative bacteria are known to cause bacterial food borne disease. [8]. Several researchers have reported that the meats sample were contaminated with high level of *Klebsiella pneumoniae*, *Enterobacter sp,Pseudomonas aeruginosa*, *E. coli, Salmonella sp, Serratia marcescens* and *Proteus vulgaris*, *Staphylococcus aureus* and *Bacillus* sp. [1]. On the other hand, food-borne pathogens are able to disseminate from contaminated meat to the surfaces and can spread infections in the community.

A great diversity of microbes inhabits fresh meat, but different types may become dominant depending on pH, composition, textures, storage temperature, and means of transporting raw meat. The meat available at retail outlets comes through a long chain of slaughtering and handling, where each step may pose a risk of microbial contamination. This has resulted to a lot of diseases as well as infections when consumed by human beings. To control food-borne illnesses and to keep the microbial load of raw and processed meat in check, food safety requirements should be followed strictly in accordance with HACCP (Hazard analysis critical control point). One of the major sources of protein is meat and since the community consume meat it is important to perform microbial analysis on the meat been sold at the retail shops. This will go in long way toward identify the microbes associated with cooked and uncooked meat and proper measures as required will be put in place to curb the further contamination and avert danger associated with their consumption. This study is aimed at determine the extent of exposure of fresh and smoked pork meat purchased to pathogens within Ado-Ekiti, Ekiti State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Location

This research was carried out in a retail market called Oja Oba market in Ado Ekiti, Ekiti State, Nigeria which falls within Ado local government Area (Fig. 1).

2.2 Sample Collection

Meat samples for the study were collected from three different open meat markets of Ado Ekiti. A total of 20 samples of pork (approximately 20 g), including 10 raw (fresh) and 10 roasted (smoked) were randomly collected from different retailers in Oja Oba market into a sterile container and transported in ice-packed thermo-cool bag (4-8°C) to the laboratory for analysis.

2.3 Sample Preparation

Macroscopic examination included physical appearance of the meat samples to look for any gross pathological lesions, checking for any blood, fresh or clotted, on the sample surface and also to detect faecal contamination of the samples, if any. The samples were processed within 4-6 hours of collection. The Standard Plate Count (SPC) of pork samples was done by using pour plate method.

The sample containers were opened aseptically, and samples were cut using sterile forceps and knife into sterile containers. Serial dilution method for pour plate technique was adopted. Each raw and roasted pork sample was pounded using a mortar and pestle. One (1) grams of each sample was weighed out, and homogenized into 10ml of sterile distilled deionised water and vigorously shaken in a conical flask to dislodge adhered bacteria. Tenfold dilution of the homogenates was made using sterile pipettes and one (1) ml from the aliquot was transferred serially to other test tubes containing 9ml of distilled water up to 10^{9}.

2.4 Microbiological Analysis

One (1) ml of the diluents (inoculum) of 10^{5},10^{6} and 10^{8} was aseptically dispensed into separate sterile Petri dishes and 15ml of the already prepared molten agars of Nutrient Agar (NA), MacConkey Agar (MAC), and *Salmonella-Shigella* agar and Sabouraud Dextrose Agar (SDA) were poured on those plates. These samples were then incubated at 37°C for 24h, observed and recorded.Total viable aerobic bacterial and fungal counts were performed on Nutrient Agar and Sabourad Dextrose Agar. MacConkey agar was used for coliform enumeration and the isolation of *Shigella* spp. and *Salmonella* spp.on Salmonella-Shigella Agar. All plates were incubated at 37ºC for 24 h in an incubator. Sabouraud Dextrose Agar (SDA) plates were kept for 1week at room temperature for isolation of fungi. The plates were observed and the colonies were counted using colony counter to obtain the total heterotrophic bacteria counts (THBC), total Enterobacteriaceae Count (TEC), total coliform count (TCC) and total mycological count (TMC). The number of colonies counted was multiplied by the reciprocal of the dilution factor to determine the microbial load in colony forming unit per gram (CFU/g). The colonies were subculture to obtain pure colonies. Pure isolates of bacterial colonies were Gram differentiated and biochemically characterized (using the following tests: citrate utilization test, indole test, Mobility test, MR, VP, TSI, Urease and catalase test) and identified using the Berjey's Manual of Systematics of archaea and bacteria.

2.5 Antibiotic Susceptibility Testing

Antimicrobial susceptibility pattern of each isolate was done using conventional disc diffusion method according to National Committee

Laboratory Standards (NCCLS) recommendation as described earlier (Ogu *et al*., 2019). This was carried out using commercial multiple antibiotic discs. The discs used included Gentamicin (10 µg), Ampicillin (3010 µg), Ofloxacin (5 µg), Chloramphenicol (25µg), Ciprofloxacin (5µg), Tetracycline (30 µg), Norfloxacin (30µg), Cefuroxime (30 µg), and Amoxicillin (30g) for Gram-negative and Gentamycin (10 g), Cephalexin (30 µg), Cloxacilin (5µg), Ceftriaxone (30µg), Amoxicillin-clavulanic acid (augumentin) (30µg), Cotrimoxazole (25µg), Erythromycin (10µg), Clindamycin (10g), and Ciprofloxacin (5ug) for Gram-positive bacteria. A turbid suspension of the isolates was made in distilled water using 0.5 McFarland standard, prepared as a comparator. A sterile swab was dipped into the bacteria suspension, pressed on the side of the bottles to allow excess drip-off, and then used to evenly streak the entire surface of the Mueller-Hinton agar. Sterile forceps were then used to place the multiple antibiotic discs in a circular pattern on the media. The process was carried out for all the identified isolates, and the plates incubated at 37°C for 24 h. After incubation, the zone of inhibition for each antibiotic was measured from the centre of the disc to the point where clearing stopped.

3. RESULTS

Table 1 shows the estimation of the total viable bacterial counts and total coliform counts in both smoked and fresh pork meat samples. The total bacteria count ranged from 2.2×10^4 – 9.0×10^4 and 1.0×10^6 – 6.0×10^6 for smoked and fresh pork respectively. The total coliform count ranged from 1.0×10^2 – 6.0×10^2 for smoked pork and 1.0×10^4 and 5.0×10^4 for fresh pork., A total of 37 bacterial isolates were obtained from the fresh pork samples (FPS) with the occurrence of *Staphylococcusaureus* as well as pathogenic bacteria species, *Escherichia coli,Salmonella* spp. and *Enterobacter* spp. and 19 bacterial isolates from smoked pork samples.

Table 2 shows the probable identities of organisms isolated from smoked and fresh pork meat. They included *Staphylococcus aureus, Escherichia coli, Bacillus cereusProteus mirabilus, Salmonella* spp. *Staphylococcus epidermidis, Klebsiella* spp., *Proteus vulgaris, Bacillus* spp., *Staphylococcus* spp., *Salmonella typhimurium, Enterobacter* spp., *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli and Proteus* spp.,are the bacterial isolates obtained from smoked pork meat. *Listeria monocytogenes, Aeromonas hydrophila, Klebsiella pneumoniae, Escherichia* spp., *Shigella* spp., *Bacillus* spp., *Listeria* spp., *Staphylococcus* spp.,are the bacterial isolates obtained from fresh pork meat after subjecting them to various biochemical tests (citrate utilization test, indole test, Mobility test, MR, VP, TSI, Urease and catalase test).

A total of ten fungi comprising five genera were isolated from the fresh and smoked pork samples. They were identified based on their cultural, morphological and microscopic characteristics. The isolated fungi are revealed on Table 3. The fungi isolates were; *Microsporum* spp., *Geotrichum candidum, Cryptococcus* spp., *Aspergillus* spp., *Fusarium oxysporum.* The fungal isolates were matched with a colour chart to identify the specific colours of the fungi for their identification.

Fig. 1. Google Map of Ado-Ekiti State showing the retail market (Oja Oba market)

Table 1. Total viable bacterial counts in roasted and fresh pork meat

Samples	TBC (CFU/g)		TCC (CFU/g)	
	Smoked (× 10^4)	Fresh (×106)	Smoked (× 10^2)	Fresh (× 104)
1	2.2	6.3	1.5	1.00
2	2.5	1.00	4.00	2.1
3	7.00	3.00	6.00	5.00
4	3.00	2.00	1.6	5.00
5	8.00	1.1	2.00	3.2
6	3.5	2.7	3.2	1.8
7	3.00	6.00	1.00	1.2
8	9.00	2.3	1.5	2.3
9	2.7	2.3	2.00	1.2
10	4.8	2.00	2.3	2.8

TBC- Total viable bacterial counts; TCC- Total coliform counts

Table 2. Occurrence of Bacterial isolates from Smoked and Fresh pork meat

Isolates	Smoked pork meat	Fresh pork meat
Staphylococcus aureus (n=17)	6	11
Escherichia coli (n=13)	5	8
Bacillus cereus (n=11)	4	7
Salmonella spp. (n=5)	2	3
Proteus spp. (n=4)	0	4
Enterobacter spp. (n=3)	1	2
Shigella spp.	0	2
Pseudomonas aeruginosa (n=1)	1	0
Total	19	37

Table 3. Occurrence of f isolates Fungal from Smoked and Fresh pork meat

Isolates	Smoked pork meat	Fresh pork meat
Aspergillus niger (n=6)	**4**	2
Aspergillus terreus (n=7)	2	2
Fusarium oxysporum (n=4)	4	0
Penicillium spp. (n=6)	4	2
Rhizopus spp. (n=8)	3	5
Mucor spp. (n=4)	0	4
Geotricum candidum (n=2)	0	2
Microsporium spp.	0	2
Total	17	19

Table (5) shows antibiotics used in the experiment, all isolates except SP_3^3 that was susceptible to Amoxacilin, were resistant to Amoxacilin and Ampiclox, however, 99% of isolates were susceptible to Ciprofloxacin. Isolate SP_1^3 showed susceptibilty to Ciprofloxacin and Pefloxacin but was resistant to Gentamycin, Ampiclox, Zinnacef, Amoxacilin, Rocephin, Streptomycin, Septrin, Erythromycin, Sparfloxacin, chloranphenicol, Tarivid and Augmentin. In addition, isolates SP_1^2, SP_1^1, FP_2^3 and SP_1^2 were resistant to Gentamycin.

4. DISCUSSION

Pork contains nutrients such as protein, lipid, fiber, carbohydrate, as well as moisture. These constituents make the meat product susceptible to microbial growth. According to Jay, (2005) most organisms utilize protein, a carbohydrate in the presence of moisture to multiply and thrive very well. All pork samples analysed contained pathogenic microbial contaminants and were *Escherichia coli, Salmonella* spp.*,S aureus*, *Enterobacter* spp.*, Fusarium oxysporum*, and *Aspergillus* spp. The presence of *Salmonella* in the pork screened in their work. The wide spread distribution of the meat product makes the consequence of contamination with food poisoning microorganisms more serious. The isolation of these organisms from roasted pork is public health importance because they are pathogenic organisms and is worrisome on the

Table 4. Antibiotic susceptibility patterns of Gram-positive bacter ial isolates from fresh and smoked pork meat

Isolate	CP	FX	CX	AMX	CD	GN	E	CO	AM	AP
S. aureus(n=17)	S (11/17)	S(11/17)	R(13/17)	R (13/17)	R (13/17)	R (10/17)	R (12/17)	R (13/17)	R (17/17)	R(13/17)
B. cereus(n=11)	S (9/11)	S (9/11)	S (9/11)	R(11/11)	R(11/11)	R(11/11)	S(9/11)	S(9/11)	R(11/11)	R(11/11)

S=Sensitive, R=Resistant, CX = Cephalexin, GN = Gentamicin, AP = Cloxacillin, AM = Ampicillin, CD = Clindamycin, CP = Ciprofloxacin, AMX = Amoxicillin-clavulanic acid, FX = Ceftriaxone, CO = Cotrimoxazole, E = Erythromycin. Zone of Inhibition: 0 -13 mm = Resistant; 14 -17 mm = Intermediate/sensitive;

Table 5. Antibiotic susceptibility patterns of Gram-negative bacterial isolates from fresh and smoked pork meat

Isolates	CP	CF	NF	OF	GN	TE	C	AM	AX
Salmonella spp. (n=5)	S(5/5)	S(5/5)	S(5/5)	S(4/5)	S(2/5)	S(5/5)	S(2/5)	R(5/5)	R(5/5)
Enterobacter spp. (n=3)	S (3/3)	S (3/3)	R (3/3)	R (3/3)	S(3/3)	S(3/3)	S(3/3)	R(3/3)	R(3/3)
Shigella spp.(n=2)	S (2/2)	S (2/2)	S (2/2)	S (2/2)	S(2/2)	S(2/2)	R(2/2)	R(2/2)	R(2/2)
Proteus spp.(n=4)	S(3/4)	S(3/4)	S(2/4)	S(2/4)	S(2/4)	S(1/4)	S(1/4)	S(1/4)	S(1/4)
E. coli (n=13)	S (9/13)	S (9/13)	S (9/13)	S (9/13)	R(6/13)	S(9/13)	R(11/13)	R(11/13)	R(11/13)
P. aeruginosa (n=1)	S (1/1)	S (1/1)	R (1/1)	S(1/1)	R(1/1)	R(1/1)	R(1/1)	R(1/1)	R(1/1)

**S = Intermediate sensitivity, S = Sensitive, R = Resistant, AM = Ampicillin, OF = Ofloxacin, C = Chloramphenicol, CF = Cefuroxime, TE = Tetracycline, AX = Amoxicillin, NF = Norfloxacin, CP = Ciprofloxacin, GN = Gentamicin. Zone of Inhibition: 0-13 mm = resistance; 14 -17 mm = Intermediate sensitivity; 18 mm and above = Sensitivity, NA=Not applicable*

fact that in the study area, many people like to consume this food product. *Salmonella* species are important food – borne pathogens. They are known to cause typhoid and non-typhoid illnesses and tends to be more severe with people in immunocompromised condition [9,10]. *Salmonella* causes an acute life - threatening illness and is mainly transmitted through urine or faeces of infected people or a chronic carrier. Some serotypes of *Salmonella* species are known cause non-typhoid salmonellosis of which results in gastroenteritis in humans. The symptoms include acute watery diarrhoea accompanied by nausea, cramps and fever. Blood in the stool may occur.

Animals are the main reservoir and transmission occurs by ingestion of contaminated food products from animals. *Staphylococcus aureus is* a normal flora of some body parts of man. According to Tauxe [11], it can be transmitted from person to product through unhygienic practices. Therefore, presence of *Staphylococcus aureus* in the roasted pork studied is an indication of possible contamination from human sources to the meat from the skin, mouth or nose of the handler which can be introduces directly into the food by contact or other aerial-droplet mechanisms such as coughing or sneezing. However, enterotoxin producing strains of *S. aureus* is a leading cause of food intoxication as it can produce extremely potent gastrointestinal toxin, *Escherichia coli* and *Enterobacter* species isolated in the study are enteric organisms. Their presence in the pork is an indication generally traceable to faecal contamination either direct or indirect means. They are normal flora of the intestine in human and animals and are widely distributed in the environment contaminating food and water. Moreover, their presences in foods are usually as a result of excessive human handling and possible contamination of pork itself during sales [12]. The pork that has been processed and kept for some days to be sold stand a chance to been contaminated especially when exposing such meat for consumers to see. *Escherichia coli* and *Enterobacter* species have been implicated in the ability to initiate the pathogenic cascade of sepsis leading to septic shock [13]. Notably is the fact that *Enterobacter* species are bacteria commonly known to further cause gastroenteritis, meningitis, and infection in the bladder. More so, an enterotoxigenic strain of *E. coli* is the most common cause of traveler's diarrhea and some strains of this pathogen can cause a wide variety of infections such as other forms of diarrhea and other gastrointestinal problems especially in a community setting. Pork or other food products that contain *E. coli* in its infective dose can be a continuous source of infections leading to complications and death especially among children and immunocompromised individuals. The fungi isolated from this study were mainly *Aspergillus niger* and *Fusarium oxysporum*. They have been known to produce mycotoxin which causes food intoxication to consumers. The *Aspergillus* spp is of medical significance because of the production of their aflatoxin. Their presence in food could be due to poor handling of the meat, unhygienic environment, improper storage facility and condition as well as lack of proper personal hygiene and even the prolonged exposure to the surroundings.

Other pre-disposing factors of contamination of the meat that could warrant the presence of these organisms could also be processing points, handling and selling. According to Tauxe, [11], the health status of animals prior to slaughtering, and prevailing circumstances in the slaughter contributes to the quality of meat from such animals. It was also noticed that in the study area, there is none of the station that cover this meat product but rather, they are placed on the net for passer-by to see and patronize. Hence, there is every tendency for atmospheric organisms to settle on these products thereby contaminating them. The customers' effect of touching and selecting the ones to buy, talking and interacting with the sellers before the net where the products are kept, even coughing, and sneezing at the sell points can bring organisms to settle on the products. Moreover, the condition of handlers packing the left-over that has not been sold into the containers to be exposed the next day, and the method of preservation of the meat equally is the source of microbial contamination. Other predisposing factors could account for the growth of these organisms in pork could be the feeding habit of the pig. Pig mostly feed on corn and soybean with a mixture of vitamins, and minerals added to the diet, the feed could serve as medium for the growth of these organisms. Moreover, the isolation of these organisms in roasted pork indicates a state of poor hygiene and environmental sanitation in some places where the meat is being processed to where it is being sold. The roasting, exposure as well as handling could also affect the meat quality.

Antibiotic-resistant is a major concern because of the limited therapeutic options for treating

infections [14]. The reduced susceptibility pattern of antibiotics showed by all isolates could be responsible for treatment failures in some clinical situations. Streptomycin as one of the antibiotics used in this experiment as shown in (Table 5) is not regularly used for treatment; but it is commonly used as a growth promoter in animals. Due to this reason, streptomycin could serve as a marker for resistant isolates moving through the food-chain. Among the multiple factors that confer the emergence of antibiotic resistant bacteria, the extensive and overuse of antibiotics in agriculture is believed as the most pivotal [15].

5. CONCLUSION

Roasted pork sold in Oja Oba market harbour microorganisms probably due to its environment and the utensils used during killing and selling period. It is very necessary that pork should be in good quality, and this comes as a result of good rearing condition, handling during slaughter, preparation method and transportation. Therefore, pork processors, handlers, and sellers should observe strict hygiene measures so that they may not serve as a source of inoculation of the microorganisms into the meat product. Meat handlers should be educated on the adverse effect of lack of proper personal, and environmental hygiene, and sanitation. Veterinary doctors should inspect the animal before it is slaughtered to establish the fitness of the meat for consumption. Government should set up local regulatory bodies to monitor and regulate the sale of pork. Emphasizing the need of clean environments and placing of the pork in well covered show-case. Consumers should insist on adequate reheating of the pork to destroy vegetative cells.

6. RECOMMENDATIONS

Public health programme is of good necessity to enlighten and educate the general public on the health implications of consuming contaminated meat products, highlighting the fact that the presence of these pathogenic microbial contaminants with high counts in the pork consumed could lead to an outbreak of disease in the study area and beyond. For improved hygienic meat handling the following recommendation are being made:

- Meat handlers and sellers should be educated on the adverse effects of the lack of proper personal and environmental hygiene and sanitation
- Good manufacturing practices should be strictly adhered to by butchers and those selling the meat. The equipment must be washed properly before use
- Adequate cooking of the fresh and/or smoked meat is required in order to kill all pathogens.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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