Isolation and identification of potential high risk pathogens from blenders used in grinding some food stuffs in a local community market in rivers state: a public health concern

Abstract
Blenders used in cutting and grinding food substances are often times not cleaned up after use. This leads to the proliferation of microorganisms, as these food substances contain nutrients that encourage microbial growth. Samples were obtained randomly in the market by scraping blenders that had been used to blend Okazi (Gnetum africanum), Ogbono (Irvingia gabonensis), Egusi (Citrullus lanatus) and Crayfish (Procambrous clarkia) in a major local market in Elele Community in Ikwere Local Government Area of Rivers State. The samples collected were subjected to bacteriological analysis for the isolation and identification of pathogens in food substances. However, results from this study showed that samples from the blender used in blending Ogbono had the highest bacterial colony forming units (1.67x10^6) and the least bacterial colony forming units was from that of the crayfish (2.72x10^4). However, out of the ten bacterial isolates identified, Staphylococcus aureus had the highest frequency of 24% while Xanthomonas sp. and Pseudomonas sp. had the least frequency of 4% each respectively. The presence of these pathogenic bacteria poses a huge threat of toxins production and associated diseases and thus should provoke a massive public health concern among stakeholders. Nonetheless, cleaning of blenders before and after use should therefore be encouraged among the market local foodstuff traders, even as the importance of health education and awareness on personal hygiene and food safety should be strongly underpinned in our local communities, so as to reduce the increasing trend of possible food borne epidemic of unimaginable proportion among the weak and most vulnerable subjects in the hinterlands.

Keywords: blenders, Okazi, Ogbono, Egusi, Crayfish, pathogenic bacterial, hygiene, community market, health protection

Introduction
Markets are places where buying and selling of products takes place. In most markets found in developing countries, especially in the rural areas, it is probably believed that sanitation of the environment and the cleaning of the drainages are rarely carried out to discourage an outbreak of foodborne epidemics. The traders are probably concerned chiefly on how fast they could finish their products and maximize their profit than paying attention to environmental sanitation details. However, preliminary observatory investigation through weeks of daily monitoring of the level of hygiene outcome in the market by the researchers, revealed that cups used in the measurement of products such as rice, beans, melon and other seed-like products are not cleaned before and after use, thus this would likely promote the presence and proliferation of microorganisms in these cups and measuring buckets, and subsequent transfer same to the products being measured. When these seed products are taken to be ground, the person concerned with this also, does not take out time to clean his machine before and after use, thus providing another good environment for the growth and possible colonization of new microorganisms different from those found in the seeds. Nonetheless, blenders are thus, machines (either electrically or manually operated) used to cut or crush substances (especially food and fruits) into smaller pieces. After blending, there are usually residues of the substance that are left on the blender machine. In most rural communities and markets, after blending, the researchers observed that the blenders are not washed. But are used over and over again, which often provides conducive media for the growth of microorganisms, possibly pathogenic ones to thrive. Nonetheless, such an unprofessional practice remains a massive promoter of an outbreak of foodborne epidemic, such as gastroenteritis and other seeming stomach disorders among unsuspected subjects, especially in rural settings, where access to the functional health system and availability of drugs has been a massive problem begging for attention over the years.1

Nevertheless, Irvingia gabonensis (African bush mango seed) popularly called Ogbone in Nigeria is a fruit that has a fleshy part and a nut which has a hard shell and the seed or kernel. The seeds have a hull (outer brown testa) and two white cotyledons. In Nigeria, the seeds are sun-dried, ground into powder and used as soup thickener. It is the food gum component of these seeds that acts as a thickening agent mainly in hot water.2 It is the food gum component of these seeds that acts as a thickening agent mainly in hot water.3 In the Bwenba Community of Uganda, the white cotyledons are roasted and eaten; the roasted seeds give aroma and flavor to foods especially vegetables.4 Proximate analysis of its seeds has shown that they contain 14.1% carbohydrate, 8.65% protein, 2.1% moisture, 1.4% crude fibre, 16.8% ash and 38.9% dietary fibre.5 Solvent extraction of the seeds yielded a fat content of 68% to 75%.6 Citrullus lanatus (egusi melon) is the biological ancestor of watermelons but compared with the later whose flesh is red and sweet, egusi melon’s juicy flesh is green or pale yellow and is bitter.6 Egusi seeds contain vitamin B2 and C, fat, carbohydrate, riboflavin,
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No. of colonies  Viable count (cfu/g)
---  ---
15  2.80x10^4
162  1.62x10^2
162  3
12  2.80x10^0
11  1.62x10^2
272  3
3  2.80x10^1

Microbiological analysis

One gram (1g) of each samples was added to 9ml of normal saline from which a 10-fold serial dilution was carried out and 0.1 ml from dilution (10^-1 to 10^-5) of each sample was plated on freshly prepared Nutrient agar plates in duplicates; these were incubated in a well regulated incubator for 24 hours at 37°C for the isolation of bacteria pathogens. Pure cultures were obtained by sub-culturing distinct colonies on freshly prepared culture plates. Identification of bacterial isolates was carried out using Gram’s stain reaction, motility, indole, spore formation, sugar fermentation tests, starch hydrolysis and catalase, MR-VP, coagulase and oxidase tests. Pure cultures that had been identified were stored on Nutrient Agar slants as described in Cheesbrough. Multiple antibiotic tests were carried out by preparing the cultures according to MacFarland’s standard. Using sterile swabs, the cultures were streaked on Mueller Hinton agar, a ring containing 8 antibiotics (Gentamicin, Ofloxacin, Augmentin, Cefazidime, Cefuroxime, Nitrofurantoin, Cefixime and Ciprofloxacin) was placed on each plate and the plates incubated at 37°C for 24 hours.

Results

Randomly selected blenders that had been used to ground egusi, ogbono, okazi and crayfish (local food stuffs) in a major local Elele market were scrapped and inoculated on Nutrient agar (bacteria medium) aseptically. The bacteriological analysis results revealed that the blender used to ground ogbono had the highest bacterial count of 1.67 x 10^5 compared with that of egusi (2.80x10^4), okazi (1.62x10^4) and crayfish (2.72x10^4) as shown in Table 1. Regardless of the high dilution factor (10^-7) used, the microbial load in the blenders used to ground okazi and ogbono was still high. Of the ten isolates, Staphylococcus aureus had the highest frequency with a percentage occurrence of 24 as shown in Table 2. Xanthomonas sp. and Pseudomonas sp. had the lowest frequency of 4% each. On all four blenders, there was the occurrence of Staphylococcus aureus while Escherichia coli and Klebsiella sp. occurred only in the blender used for egusi. Bacillus sp. occurred in the egusi, crayfish and ogbono blenders. Micrococcus sp. and Pedicoccus sp. occurred in the blenders used for okazi and egusi. Lactobacillus sp. occurred only in the blenders used for egusi and crayfish. Figure 3 shows a plate were multiple antibiotic sensitivity test for Klebsiella sp. was carried out. Figure 4 shows the percentage sensitivity of the isolates to the antibiotics with a good percentage exhibiting resistance to Augmentin and 100% being susceptible to Ofloxacin (Figures 1 & 2).

### Table 1: Bacterial load count of ground food samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>No. of colonies</th>
<th>Viable count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egusi</td>
<td>10^-4</td>
<td>282</td>
<td>2.80x10^4</td>
</tr>
<tr>
<td>Okazi</td>
<td>10^-2</td>
<td>162</td>
<td>1.62x10^4</td>
</tr>
<tr>
<td>Ogbono</td>
<td>10^-2</td>
<td>162</td>
<td>1.67x10^4</td>
</tr>
<tr>
<td>Crayfish</td>
<td>10^-4</td>
<td>272</td>
<td>2.72x10^4</td>
</tr>
</tbody>
</table>

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Table 2 Frequency of isolated bacteria according food stuffs examined

<table>
<thead>
<tr>
<th>Isolated organism</th>
<th>Frequency</th>
<th>Okazi</th>
<th>Egusi</th>
<th>Crayfish</th>
<th>Ogbono</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>12 (24%)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4 (8%)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>8 (16%)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>4 (8%)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>7 (14%)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>2 (4%)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>4 (8%)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xanthomonas sp.</td>
<td>2 (4%)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pedicoccus sp.</td>
<td>4 (8%)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3 (6%)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: -, absent; +, present

Figure 1: A picture of food stuff ground machine taking in the local market study environment.

Figure 2: A picture of ground machine used in blending local food stuffs in the study local market.

Figure 3: Plate showing multiple antibiotic sensitivity test for Klebsiella sp.

Figure 4: Key: S, sensitive; I, Intermediate; R, Resistant; GEN, Gentamicin (10µg); OFL, Ofloxacin (5µg); AUG, Augmentin (30µg); CAZ, Ceftazidime (30µg); CRX, Cefuroxime (30µg); NIT, Nitrofurantoin (300µg); CXM, Cefixime (5µg) and CPR, Ciprofloxacin (5µg).

Discussion

Blenders used in cutting food substances into smaller pieces are important, but the occurrence of microorganisms in the ones that have been used and re-used before being cleaned (if at all they are cleaned) is of public health importance. In the market, probably due to the large number of persons coming to blend food stuffs, the persons doing the blending end up re-using the blenders without proper cleaning not minding the source of the food stuff. Sometimes, the food stuff may be already or almost spoilt (as in the case of some fresh pepper and tomato brought for blending), thus depositing some food spoilage microorganisms on the blender. Without proper cleaning, these organisms may replicate sporadically and be transferred to the new food stuff brought for blending also.

Bacterial isolates were obtained from the samples. The origin of these microorganisms may have been from the food samples that were blended or better still, from the water used in cleaning the blender before use, or even from the environment such as the dust from the soil. Since the market is a busy one, particulate matter carrying microorganisms may have been deposited on the blenders. On the other hand, sometimes water used in cleaning the blenders may have been stored for a long period of time, and probably some pathogenic microorganisms may have proliferated in the water. Also, sometimes, food brought for blending may be spoilt already and so the bacteria from these decayed food samples may be deposited on the blender during the process. These scenario tend to put the unsuspecting public at massive potential risk with strong public health concern of food poisoning outcome, if not checked in good time to nip it at the board.

However, the isolation of Bacillus sp. from the blender that had been used to ground ogbono was in agreement with the study by Ekundayo et al., who isolated these organisms and some other pathogens from fermented ogbono seeds. The presence of Staphylococcus aureus and Bacillus sp. is a potential risk as these organisms are able to produce toxins which are harmful to humans when ingested. Bacillus sp. is normally found in the soil and may have been of vegetable origin. The consumption of these organisms in large numbers, probably above 100 cfu /ml could probably lead to gastrointestinal illness, though the degree and critical nature of the infection in an individual may differ, based on individual specific immune response capacity and integrity. It is strongly believed that those who are immune compromised, based on individual specific immune response capacity and integrity, may have a higher risk of an infection due to the degree and critical nature of the infection in an individual may differ, based on individual specific immune response capacity and integrity. Nonetheless, others who might be at risk also are the infants and the aged with weak immune competence. However, toxins of Staphylococcus aureus on the other hand when found in food samples and consumed can cause diarrhea, vomiting and stomach cramps. These are pathogenic cum food poisoning microorganisms, as such, should not be found in our food stuffs or instruments used in preparing and processing food, if the health of general public must be protected at all time.

The occurrence of S. aureus which are Gram positive cocci, catalase-positive, coagulase-positive, oxidase-negative and facultative anaerobes in all samples strongly suggests a high level of personal hygiene compromise in association with the usage of these blenders. Although S. aureus is often associated with the skin and mucous glands (especially in the nose of healthy persons) as commensals, it is also pathogenic at some point. It has been implicated as one of the main causes of community and hospital-acquired infections which may lead to serious public health consequences. Hospital-acquired infections also known as nosocomial infections resulting from S. aureus affects the lower respiratory tracts, bloodstream, skin and soft tissues as it can cause osteomyelitis and endocarditis among other infection. Toxins produced by S. aureus have been associated with toxic shock syndrome (TSS), staphylococcal foodborne diseases (SFBD) and scalded skin syndrome. Nevertheless, Bacillus species is also a Gram-positive facultative anaerobe; it is catalase-positive, motile and endospore-forming microorganism. They are able to form heat-resistant spores in the soil as their natural habitat; hence the contamination of food, with sand or dust blown up by air current may probably be one of the ways of introducing pathogens on the food stuffs. Nonetheless, the researchers also firmly believed that, their ability to form these spores is a major reason for their huge virulence and persistent pathogenicity in an infected host.

The presence of Klebsiella species and Pseudomonas species in these samples suggest that, if the blender is used to blend another food item and it is either not cooked or not properly cooked before consumption, gastroenteritis may result as these microorganisms are often associated with gastrointestinal infections. It is therefore, very imperative that these microorganisms be eliminated from food samples, and material used in food preparation to prevent bacterial infections. Although antibiotics have been widely used to treat infections that are associated with these microorganisms, in the rural areas where there is little or no access to quality health care delivery, if one comes down with an illness, resulting from infection by these microorganisms, the result will either be that antibiotics will be abused leading to antibiotics drug resistance or the patient morbidity and mortality rate may increase geometrically. Therefore, it is of utmost importance that the food sample must not be a decayed one and the spread of dust within the environment where the blending is done, should be reduced as much as possible, so as to reduce contamination of the items. The resistance of the bacterial isolates to Augmentin is of public health concern, even as it is often used as a drug of choice in the treatment of bacterial infections. However, in place of it, Ofloxacin may be used for the treatment of such infections, but what is of massive worry for now is the scenario where the use of Augmentin which is a broad spectrum antibiotic has been abused in the local communities with a resistance feedback, then what next, in a changing world of antibiotic and multiple drug resistance history, even when living in the villages may not be financially strong to go for other alternative antibiotics with strong and broad spectrum capacity.

However, the importance of the provision of potable water in all the nook and cranny of our local markets by government and her agencies cannot be over emphasized. It is probably believed that non-provision of potable sources of water, which should be accessible regularly for washing and keeping the environment clean may likely promote these public health issues in our local markets, thus the public health implication of such practice is very massive, as cases of gastroenteritis and diarrhoea will be highly prominent in the area. This is a great public health concern and potential health risk that calls for urgent attention. Nevertheless, it is strongly believed that if these sceneries are not managed in good time, many subjects would be victims of food borne infections, even as the cases of antibiotic resistance saga in our local communities would continue to be on the increasing trend since the infected local populace always seek for medical attention through the practice of self-medication approach or the use of herbal mixtures that lacks accurate measurement of an active ingredients.

Conclusion and recommendation

Some Gram positive and Gram negative pathogenic microorganisms were isolated from blenders that had been used to blend egusi, ogbono, Azuonwu O, Azuonwu TC, Ndah MA. Isolation and identification of potential high risk pathogens from blenders used in grinding some food stuffs in a local community market in rivers state: a public health concern. J Microbiol Exp. 2019;7(4):183–187. DOI: 10.15406/jmen.2019.07.00258
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Irvingia gabonensis, suggesting a public health risk as the blenders are often used and re-used without sanitization. Staphylococcus aureus, Bacillus sp., Xanthomonas sp., Escherichia coli, Pediococcus sp., Pseudomonas sp., Klebsiella sp., Lactobacillus sp. and Micrococcus sp. were among the isolated bacterial species. Although they did not all occur in all the four samples, Staphylococcus aureus had the highest percentage occurrence. An unidentified organism was also isolated and had a percentage occurrence of 6%. The viable counts showed that ogbono had the highest microbial count. Nevertheless, it is strongly suggested that proper cleaning and disinfection of these blenders should be carried out before and after use to ensure that they are free from potential microbial pathogens, which are harmful to humans and possibly animals. Water used in cleaning these blenders must be potable water and should be accessible. The environment where the blenders are kept should be cleaned properly as there is a probability that dirt-carrying microorganisms may be deposited on the blenders or food samples to be ground. Awareness campaigns by public health workers on the dangers of using and re-using blenders without proper cleaning should be carried out from house to house, and even in the markets; not just in the urban areas but in the rural areas too, to encourage the proper cleaning of blenders and ground machines before and after use. Where possible, each household should be encouraged to have its own personal blender to avoid going to the market to blend, where you are not sure of the safety of the blending or ground machine being used for commercial purpose.

Studies on the microbial load of household blenders used in blending different food samples should be carried out so as to ascertain, if the isolated microorganisms were from the food samples or from the environment where the blenders are kept.

Acknowledgments

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Conflicts of interest

None reported among authors.

References