RESEARCH ARTICLE

Antimicrobial Susceptibility Patterns of Agbo Herbal Remedies Sold in Enugu Metropolis

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ABSTRACT — Around 80% of people living in developing countries rely on herbal remedies for their primary healthcare needs. This study aims to determine the antimicrobial susceptibility patterns of bacteria isolates found in Agbo samples in Enugu. A cross-sectional study design was used. The research spanned from October 10th to November 25th, 2024. Twenty (20) Agbo samples were collected from five popular markets in Enugu. The pH values ranged from 2.77 to 6.71 (mean=4.32). The samples were cultured on Eosine Methylene Blue agar, Salmonella-Shigella agar, and Mannitol Salt agar, and incubated at 37 degrees Celsius for 24 hours. Bacterial isolates were identified based on their morphological characteristics and standard biochemical tests. Staphylococcus aureus, Escherichia coli, Salmonella spp, and Klebsiella spp were isolated. *Staphylococcus aureus* had the highest frequency (90%), followed by *Klebsiella* spp (55%), *Salmonella* spp (50%) and lastly *Escherichia coli* (20%). The bacterial isolates were sub-cultured onto new plates and incubated for 37 degrees Celsius for 24 hours. Colonies were picked from the pure sub-culture for susceptibility testing using the Kirby Bauer disk diffusion method to determine susceptibility patterns of the isolates to five antibiotics —ciprofloxacin µg, augmentin, gentamicin, ampicillin, and ceftriaxone. All the isolates were susceptible to ciprofloxacin except *Staphylococcus aureus.* All were resistant to ampicillin. *Staphylococcus aureus* was susceptible to augmentin and ceftriaxone. *Escherichia coli* was susceptible to gentamycin. *Salmonella* spp was susceptible to augmentin, gentamycin, and ceftriaxone. *Klebsiella* spp was susceptible to augmentin. The results of this study shows that herbal medicines sold in Enugu are contaminated with pathogenic bacteria. This underscores the need for the enforcement of standardization and strict hygienic requirements for herbal medicine practitioners. Ciprofloxacin is the ideal antibiotic for treating bacterial infections acquired from taking Agbo products sold within Enugu.

Keywords: Agbo, Herbal Remedies, Bacterial contamination, Antimicrobial susceptibility

I. INTRODUCTION

According to a 2010 report by the World Health Organization (WHO), about 80% of the population in developing countries, including Nigeria, depend on traditional medicine for their primary healthcare needs (WHO, 2010). Agbo, is a Yoruba name for different concoctions of herbs and roots sold across Nigeria that are used to treat various conditions such as malaria, hemorrhoids, body pains, and sexually transmitted infections.

Agbo is prepared by soaking plant parts like leaves, stems, roots and barks in either water or alcohol, the preparation is allowed to stand for days before it is dispensed into plastic bottles and then hawked on the streets by mostly Yoruba women (Ejukonemu and Isiosio, 2019).

Sometimes the preparation is boiled when water is used as the preferred solvent. The widespread acceptance of herbal remedies such as Agbo is driven by factors such as easy accessibility, cultural approval and affordability (Ogbole et al., 2023). Also, there's evidence that with the right constituent plants, Agbo can be an effective cure for diseases such as malaria (Erhunse et al., 2023). There's no doubt that traditional medicine plays and will continue to play a crucial role in Nigeria, particularly among low-income populations.

There are laid down regulations in Nigeria regarding the manufacture, distribution, and

marketing of herbal medicines (Osuide, 2002). However, these regulations are hardly followed by herbalists that prepare traditional medicines such as Agbo nor enforced by the appropriate government agency. The unregulated and unsupervised preparation of Agbo products poses public health risks as studies have documented high levels of bacterial and fungal contamination in Agbo samples sold in urban areas (Ejukonemu and Isiosio, 2019). Pathogenic bacteria can be introduced into Agbo at several points during the production and distribution process. It could be from the harvested herbs or unhygienic practices during the preparation and storage of the herbal medicine (Ola et al., 2013). These pathogenic bacteria pose a significant health risk, and are likely responsible for side effects such as stooling and vomiting reported by those who use herbal medicine (Amorha et al., 2018).

In Enugu metropolis where Agbo herbal remedies are readily available and consumed, there is a paucity of information on its bacteriological profile, leaving a critical gap in public health knowledge. This study is necessary to identify bacterial contaminants in Agbo sold in Enugu and also understand their antimicrobial susceptibility patterns. The aim of this study is to evaluate the bacterial contamination of Agbo products being sold to the residents of Enugu Metropolis and to determine the sensitivity reactions of the identified bacterial isolates.

II. MATERIALS AND METHODS

A. Sample Collection

The samples were collected in sterile plain containers at the markets and transported to the laboratory immediately afterwards. Four samples were collected from each market, with different intended uses. Agbo Iba for malaria, Agbo Jedi-Jedi for hemorrhoids, Agbo Ara-riro for body pains, and Agbo Atosi for infections

B. pH Determination

The pH of the samples was determined using a digital pH meter. The pH meter was calibrated using buffer powders with pH values of 6.86, 4.01, and 9.18 dissolved in 250ml of distilled water at a temperature of 25°C. The water temperature was measured with a digital thermometer. The pH meter was rinsed and gently blotted dry with a paper towel in between each calibration and reading.

C. Microbiological Analysis

Each sample was inoculated on Eosine Methylene Blue agar, Mannitol Salt agar, and Salmonella-Shigella agar plates using an inoculating loop of 2µL. The plates were incubated at 37°C for 24 hours after which they were read. Suspected bacterial species were identified based on colony morphology and gram staining properties, and their presence was confirmed using biochemical tests: catalase, coagulase, oxidase, and indole tests. The presence of salmonella species was confirmed using H and O antisera slide agglutination test. The identified bacteria species were sub-cultured on new plates and incubated for 24 hours at 37°C.

D. Antimicrobial Susceptibility Testing

Pure bacterial isolates were picked from the sub-cultured plates and suspended in normal saline solution to obtain a density of 0.5 McFarland standard. A commercially purchased 0.5 McFarland standard prepared using latex particles was used as a reference. The bacterial suspension for each organism was inoculated on freshly prepared Muller-Hinton agar plates. Sterile forceps were used to place ciprofloxacin 5µg, augmentin 30µg, gentamycin 10µg, ampicillin 10µg, and ceftriaxone 30µg antibiotic discs on the plates. The plates were incubated at 37°C for 24 hours. The zones of inhibition were read with a transparent ruler in mm. The susceptibility of the bacteria to the antibiotics was determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables.

E. Data Analysis

Data from the research was analyzed and presented as frequencies, percentages, and tables to show pH readings, bacterial contaminants present in Agbo products sold in Enugu and their antimicrobial susceptibility patterns.

III. RESULTS

Sample source	Sample type	pH
Ibeagwa market	*Agbo Iba*	6.71
	Agbo Jedi-Jedi	3.19
	Agbo Ara-riro	2.77
	Agbo Atosi	4.53
Abakpa market	*Agbo Iba*	3.42
	Agbo Jedi-Jedi	4.58
	Agbo Ara-riro	4.60
	Agbo Atosi	5.57
Ogbete market	*Agbo Iba*	4.78
	Agbo Jedi-Jedi	3.02
	Agbo Ara-riro	5.41
	Agbo Atosi	5.93
Old Artisan market	*Agbo Iba*	6.30
	Agbo Jedi-Jedi	4.21
	Agbo Ara-riro	5.54
	Agbo Atosi	3.86
New market	*Agbo Iba*	4.08
	Agbo Jedi-Jedi	3.39
	Agbo Ara-riro	4.66
	Agbo Atosi	5.88

Table 1: pH values of each sample

Bacteria isolates	Frequency (%)
Staphylococcus aureus	90%
Escherichia coli	20%
Salmonella spp	50%
Klebsiella spp	55%

Table 2: Frequency of bacterial isolates from samples

Sample source	Sample type	Media	Bacteria isolates
Ibeagwa market	*Agbo Iba*	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp, *Escherichia coli*
		SSA	*Salmonella* spp
	Agbo Jedi-Jedi	MSA	NG
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
	Agbo Ara-riro	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	NG
	Agbo Atosi	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG
Abakpa market	*Agbo Iba*	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	NG
	Agbo Jedi-Jedi	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
	Agbo Ara-riro	MSA	*Staphylococcus aureus*
		EMB	Klebsiella spp
		SSA	*Salmonella* spp
	Agbo Atosi	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG
Ogbete market	*Agbo Iba*	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG
	Agbo Jedi-Jedi	MSA	NG
		EMB	*Klebsiella* spp, *Escherichia coli*
		SSA	*Salmonella* spp
	Agbo Ara-riro	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	*Salmonella* spp
	Agbo Atosi	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
Old Artisan market	*Agbo Iba*	MSA	*Staphylococcus aureus*
		EMB	*Escherichia coli*
		SSA	NG
	Agbo Jedi-Jedi	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
	Agbo Ara-riro	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG

	Agbo Atosi	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
New market	*Agbo Iba*	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG
	Agbo Jedi-Jedi	MSA	*Staphylococcus aureus*
		EMB	*Klebsiella* spp
		SSA	*Salmonella* spp
	Agbo Ara-riro	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG
	Agbo Atosi	MSA	*Staphylococcus aureus*
		EMB	NG
		SSA	NG

Table 3: Agbo sources, samples and bacteria isolates present

MSA = Mannitol Salt Agar, EMB = Eosin Methylene Blue Agar, SSA = Salmonella-Shigella Agar
NG = No bacterial growth

Bacterial Isolates	**CPX 5µg**	**AU 30µg**	**GN 10µg**	**AMP 10µg**	**CTX 30µg**
S. aureus	23 mm (I)	35 mm (S)	13 mm (R)	19 mm (R)	25 mm (S)
E. coli	25 mm (S)	18 mm (R)	22 mm (S)	14 mm (R)	15 mm (R)
Klebsiella spp	27 mm (S)	20 mm (S)	12 mm (R)	13 mm (R)	21 mm (R)
Salmonella spp	25 mm (S)	19 mm (S)	21 mm (S)	11 mm (R)	25 mm (S)

Table 4: Antibiotics susceptibility of bacterial isolates

CPX = Ciprofloxacin AU = Augmentin GN = Gentamycin AMP = Ampicillin
CTX = Ceftriaxone

S = Susceptible, I = Intermediate, R = Resistant
Note: The susceptibility of each organism was determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables.

IV. DISCUSSION

Table 1 shows the pH values of the samples collected which ranged from 2.77 to 6.71, with a mean of 4.32. All the values fall within the acidic region of the pH scale. This results are consistent with results from existing body of research. The often extremely low pH values of Agbo can have serious health implications for consumers, including but not limited to bone problems and damage to the lining of the digestive tract (Korir, 2017).

Table 2 shows the frequency of bacteria isolates found in the samples. *Staphylococcus aureus* was extremely common with a frequency rate of 90%. *Staphylococcus aureus* is commonly found on the skin and inside the nose. The presence of this bacteria in samples at such a high rate is an indicator of poor hygienic standards observed by the herbal practitioners during the preparation process. *Staphylococcus aureus* produces a wide variety of enterotoxins which can cause food poisoning and symptoms like nausea, and vomiting with or without diarrhea (Argudin et al., 2010.

The *Escherichia coli* in the samples could have been as a result of using contaminated water to prepare the Agbo remedies or not properly washing the herbs used. Most *Escherichia coli* strains are harmless and live in the intestines of humans and animals. However, virulent strains of the bacteria can cause a wide range of intestinal and extraintestinal diseases, including urinary tract infections (Pakbin et al., 2021).

Klebsiella spp can be found in a variety of places such as in the environment and gut of humans and other mammals. It is often spread through contact with contaminated water, soil, or hands of health personnel in nosocomial settings. In this case, the contamination is likely from the soil during harvesting of the herbs or the use of contaminated water in preparing Agbo. When ingested *Klebsiella pneumoniae*, the most medically important member of the *Klebsiella* species, can cause infections like pneumonia, urinary tract infections, septicemias, and soft tissue infections (Podschun & Ullmann, 1998). This occurs through translocation i.e. the bacteria finding its way into the other parts of the body from the gut.

Salmonella species can find their way into water used for domestic purposes when the water source becomes contaminated with infected human or animal stool. *Salmonella* species infection is one of the leading causes of acute diarrheal diseases (Popa & Papa, 2021). The clinical presentation of salmonellosis varies from common gastroenteritis to life-threatening enteric fevers. Therefore, the presence of this bacteria in products intended for public consumption is unacceptable.

Table 3 gives a detailed breakdown of the market sources, types of Agbo, and the bacterial isolates found on culture plates. All the markets had nine (9) bacteria isolates except New Market which had six (6).

Table 4 shows the antibiotics susceptibility of the isolates. Ciprofloxacin emerged the most effective against the bacteria isolates. *Staphylococcus aureus* was the only organism not susceptible to the drug. The same applies to augmentin; *Escherichia coli* was the only bacteria not susceptible to augmentin. In contrast, all the bacteria isolates resisted ampicillin. This could be because ampicillin is an older antibiotic that is easily accessible in Nigeria and resistant strains of bacteria have developed over the years due to antibiotic abuse. Therefore, ciprofloxacin and augmentin are ideal antibiotics for treating bacterial infections acquired from consuming Agbo products sold in Enugu state.

V. CONCLUSION

The results of this study are consistent with existing research on bacterial contaminants found in Agbo products being sold for public consumption. This raises legitimate questions about the hygienic standards that local herbalists follow when preparing these products. The presence of these bacteria in Agbo sold in Enugu is a serious public health issue. Unsuspecting consumers may ingest pathogenic and virulent strains of bacteria in a bid to cure existing health problems.

The pH of the samples was all in the acidic range. This can also be detrimental to the health of consumers whom may develop acid-alkaline imbalance which can lead to problems with the bones and joints. People who regularly consume Agbo herbal remedies are at high risk of developing these problems.

Another pressing concern is the increasing rate of antibiotics resistance of bacteria. This can complicate the management of diseases caused by resistant strains of these organisms in humans. In addition to antibiotic abuse, the use of Agbo products could be a source of introduction of multiple antibiotic resistant microorganisms in consumers.

The quality of Agbo products being sold in Enugu state and other parts of the country leaves a lot to be desired. Enugu state government must partner with and encourage relevant federal agencies like the National Agency for Food and Drug Administration and Control (NAFDAC) and their state branches to sanitize the herbal medicine market within the state. Strict quality control measures must be implemented to ensure that Agbo products are free of harmful bacteria and other undesirable contaminants. Herbal practitioners should be educated on hygienic preparation and storage methods. Preparation and dosing regimens should be standardized to ensure the efficacy and quality of Agbo products. This can only be achieved through collaboration of government agencies with herbal medicine practitioners. NAFDAC should certify and issue NAFDAC numbers to Agbo products that meet new stringent efficacy and safety requirements. Public awareness is also necessary to inform consumers of the risks of purchasing Agbo products that have not been certified by NAFDAC. Regular monitoring and follow-ups are equally important to ensure that stipulated standards are maintained even after a product has obtained NAFDAC certification.

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