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RESEARCH ARTICLE OPEN ACCESS

Isolation, Identification and Antibiotic Resistance Profile of Public Health Threat Enteric Bacteria from Milk and Dairy Products Retail in Abakaliki, South-East, Nigeria

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Abstract

Milk and foods made from milk is manufactured into more stable dairy products of worldwide value, such as butter, cheese, ice cream, and yoghurt. Consumption of contaminated milk or dairy products by pathogens causes human gastrointestinal infection, which leads to diarrheal disease in human and hospitalization or death in severe cases especially among elderly and children. An assessment of milk and dairy products was designed to determine the microbiological quality of milk and dairy products consumed in Abakaliki, Nigeria. Culture techniques were usedfor isolation of enteric bacteria from retail dairy productsand disk diffusion method were used to determine the Antibiotic Resistance profile of isolates. Bacteria pathogens isolated were characterized and identified using morphological and biochemical techniques. SPSS and Chi-square test were used for the analysis of the study,P-value of 0.02 indicates a significant difference between the bacteria pathogens counts. A total of 161 pathogenic bacteria were isolated from 100 dairy products. Salmonellaspp heard (26.1%), *Escherichia coli* (44.1%) and *Shigella* spp. (29.8%). All identified isolates were found to be 100% susceptible to ciprofloxacin and gentamycin, with 66.7% for ofloxacin. Augmentin, ampicillin, chloramphenicol and spectinomycin was 100% resistant. Data obtained confirm that milk and dairy products retailed in Abakaliki pose a serious public health threat to consumers due to the presence of pathogenic bacteria. Standard and good storage conditions, as well as environmental and personnel hygiene should be practiced to prevent contamination of milk and dairy products for the safety of consumers.

Keywords: Isolation, Identification, Antibiotic Resistant Profile, Enteric Pathogens, Milk, Dairy Products

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INTRODUCTION

Foodborne diseases result from the consumption of food contaminated or infected by pathogens.[1] These diseases cause serious and continued challenges to human health globally.[2] The epidemiology of foodborne diseases has changed rapidly due to changes in the environment and the ability of pathogens to adapt to a new environment.[3,4] Foodborne pathogens cause illness and death especially in developing countries, leading to a loss of labour force that could have contributed to economic growth.[5,6] Poor environmental sanitation, changes in eating habits, and lack of hygienic practice during production, packaging, transportation, storage and retail of food are contributing factors that leads to contamination of dairy products.[7] Annually, millions of cases of foodborne illness and thousands of associated deaths occur especially in developing countries.[8,9] The primary materials for the production of dairy products such as ice cream, yoghurt, butter and cheese are water, condensed and dried milk. Contamination of the primary materials affects the quality of the dairy products. Pathogenic microorganisms contaminate dairy products through impure air in the processing environment or through poor personnel hygiene during processing, as well as the use of contaminated water during preparation and poor storage condition.[10] The nutrient composition of milk and dairy products ensures a favourable environment for different microorganisms to grow and multiple.[11,12] Drug-resistant microorganisms are widely in circulation in the environment with a significant rise in their negative effects over the past few years as a result of empirical antibiotic use and knowledge deficiency.[13-16] Public health threat pathogenic bacteria of the *Enterobacteriaceae* family are mostly implicated in foodborne infections globally.[17,18] *Salmonella* spp., *Shigella* spp., *Listeria* spp., *Escherichia coli, Enterobacter* spp., *Campylobacter* spp. and *Yersinia* spp. are among public health threat pathogenic microorganisms commonly implicated in foodborne diseases.[19-21] Thus, this study aimed to determine the microbiological quality of various dairy products consumed in Abakaliki, and the antibiogram of the pathogenic enteric bacteria isolates from milk and different dairy products.

MATERIALS AND METHODS

Study area and duration

The study was conducted in Abakaliki the capital of Ebonyi State, South-East Nigeria. Abakaliki is located at Latitude 6.32485 and Longitude 8.11368. It is part of West Africa and in the Northern hemisphere. The study was conducted from August to December 2022 at the Microbiology laboratory of Alex Ekwueme Federal University Ndufu Alike, Ebonyi State, Nigeria.

Study design and samples collection

A total of 100 samples (ice cream, yoghurt, butter, cheese, dried milk) of dairy products were randomly collected from dairy shops and stores in Abakaliki metropolis, Ebonyi State, Nigeria. Twenty of each of the samples were transmitted to the microbiology laboratory in an insulating icebox as soon as possible for examination.

Bacteriological examination

The study was conducted using cultural techniques for the detection of enteric bacteria following the international standard guideline from ISO, 20976-1:2019(E).

Biochemical tests

All isolates were gram stained to detect if isolates were gram-negative or positive. Suspected bacteria pathogens stored on nutrient agar were identified by inoculation unto tubes of different biochemical test mediums such as; Motility Indole Urea (MIU) agar, Simmon Citrate Agar (SCA), Triple sugar iron (TSI), Methyl Red Voges Proskauer (MR-VP) broth, incubated for 24hr at 37°C. *Escherichia coli* and *Salmonella* spp. isolates are motile, *Shigella* spp. was not. All isolates are urease and citrate negative. *Escherichia coli* was indole positive while *Salmonella* spp. and *Shigella* spp. are indole negative. TSI agar test showed yellow slop and yellow butt for *Escherichia coli* while it showed red slop and yellow butt for *Salmonella* spp. and pink for both slop and butt for *Shigella* spp. *Escherichia coli* produced gas while *Salmonella* spp. and *Shigella* spp. doesn't. *Salmonella* spp. produces Hydrogen Sulphide (H_2S) or (Blackened), while *Shigella* spp. and *Escherichia coli* don't.

Antimicrobial susceptibility test of enteric bacteria

The antimicrobial susceptibility profile for all enteric bacteria of concern (*Salmonella* spp., *Escherichia coli*, and *Shigella* spp.) was carried out using the disc diffusion method on Mueller Hinton agar. The following antibiotics was used; ciprofloxacin (5µg), ampicillin (10 µg), augmentin (30 µg), gentamycin (10µg), perfloxacin (5µg), ofloxacin (5µg), streptomycin (10 µg), trimethoprim-sulfamethoxazole (1.25 µg /23.75 µg), chloramphenicol (30 µg) and spectinomycin (10µg). Within 30 minutes of applying the discs, the plates were inverted and incubated aerobically at 35°C for 16–18 hrs. After incubation, the control and test plates were examined to ensure the growth is confluent or near confluent. The zone of inhibition was measured in millimetres and interpreted as described in Haile et al.[22]

Data analysis

Data was analyzed using the statistical software programme SPSS version 20.0. (SPSS 20.0 Command Syntax Reference, SPSS Inc. Chicago, 2013). The Chi-square test was utilized to assess significant differences between each pathogen from different dairy products.

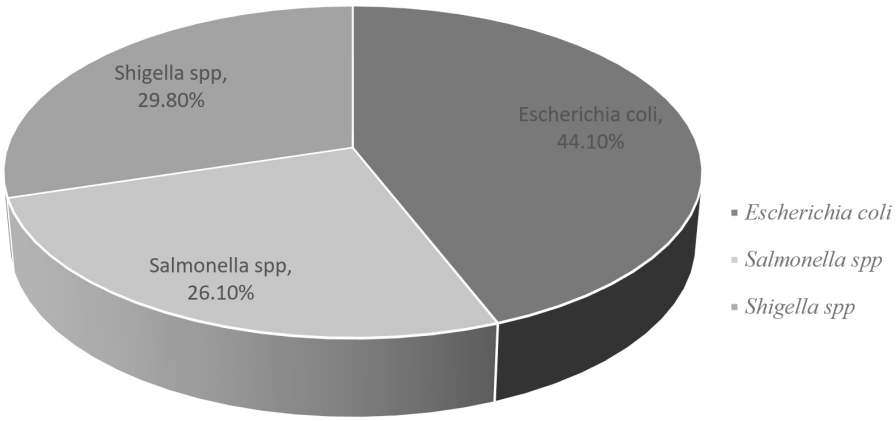

Figure 1. Percentage prevalence of bacteria in dairy products

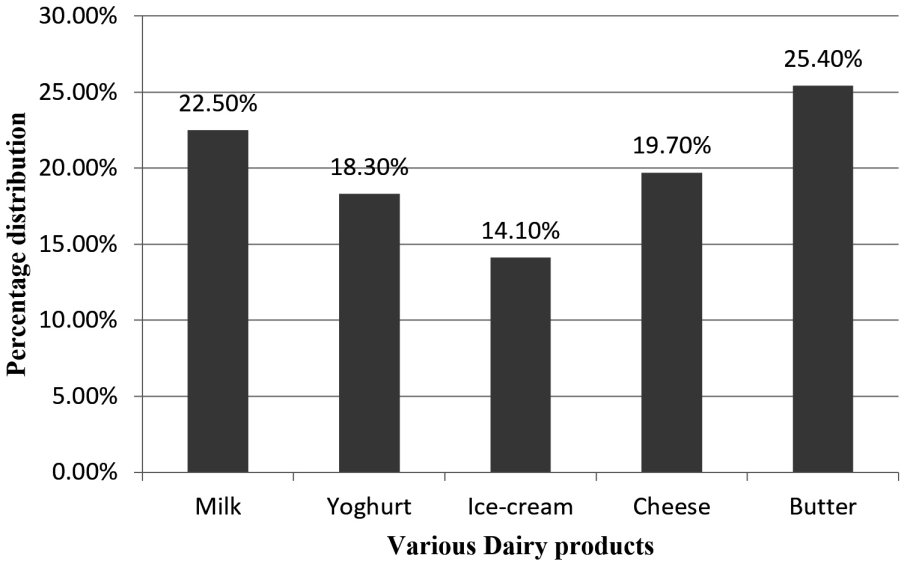

Figure 2. Percentage distribution of *Escherichia coli* in dairy products

RESULTS

Bacteriological examination of dairy products through culture techniques and identification of isolates through morphological and biochemical methods implicated *Escherichia coli* (44.10%), *Salmonella* spp. (26.10%) and *Shigella* spp. (29.80%) as the prevalent bacteria. *Escherichia coli* was most prevalent and *Salmonella* spp. least prevalent (Figure 1). Among the dairy products, butter had the highest *Escherichia coli*, while ice cream had the least (Figure 2). The percentage distribution of *Salmonella* spp. heard yoghurt with the highest percentage and butter with the least (Figure 3). The highest percentage of *Shigella* spp. was detected in dried milk, while ice cream had the least (Figure 4). The antibiogram of the isolates confirmed that ciprofloxacin and gentamycin were completely susceptible while ampicillin, augmentin, chloramphenicol and spectinomycin were completely resisted by the isolates (Figure 5). The percentage resistance and susceptibility confirmed that *Escherichia coli* was 50% resistance and 50% susceptibility. *Shigella* spp. and *Salmonella* spp. was 60% resistance and 30% susceptible, respectively, with 10%

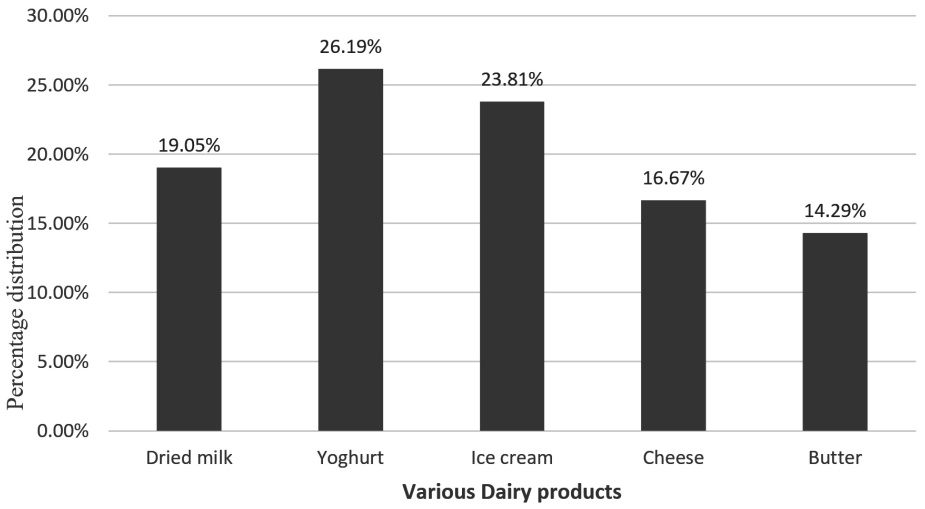

Figure 3. Percentage distribution of *Salmonella* spp. in dairy products

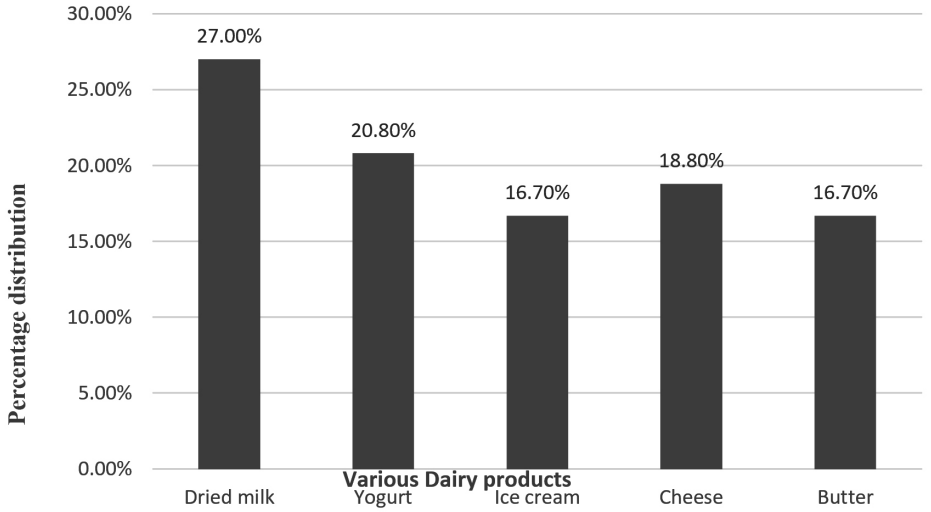

Figure 4. Percentage distribution of *Shigella* spp. in dairy products

intermediate (Figure 6). Out of 100 dairy products sampled, a total of 161 bacteria was isolated (Table 1). The first line of antibiotics for the treatment of dairy foodborne disease are ciprofloxacin and gentamycin (Table 2).

DISCUSSION

In the present study, out of 100 dairy products sampled, a total of 161 isolates were isolated from all the samples. Statistical analysis showed a significant difference between bacteria

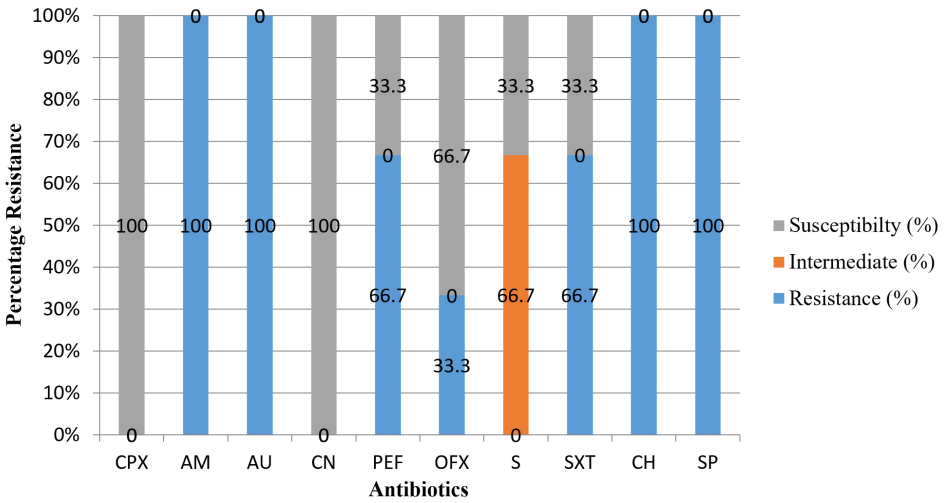

Figure 5. Percentage susceptibility profile of antibiotics among enteric bacteria from dairy products
KEY: CPX: Ciprofloxacin (5µg), AM: Ampicillin (10µg), AU: Augmentin (30µg), CN: Gentamycin (10µg), PEF: Perfloxacin (5µg), OFX: Ofloxacin (5µg), S: Streptomycin (10µg), SXT: Trimethoprim-Sulfamethaxazole (1.25/23.75), CH: Chloramphenicol (30µg), SP: Spectinomycin (10µg)

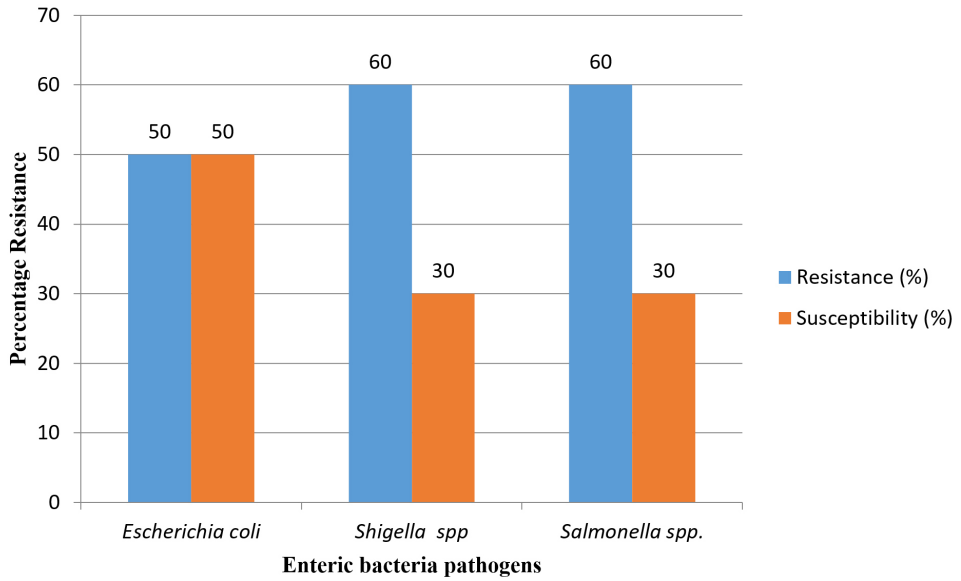

Figure 6. Percentage resistance and susceptibility of pathogenic bacteria from dairy food

Table 1. Distribution of pathogenic bacteria in dairy products

Dairy foods	No. of samples	*Escherichia coli*	*Salmonella* spp.	*Shigella* spp.	No growth
Milk	20	16	8	13	0
Yoghurt	20	13	11	10	0
Ice-cream	20	10	10	8	2
Cheese	20	14	7	9	1
Butter	20	18	6	8	2
Total	100	71	42	48	5

Table 2. Antibiotic susceptibility profile of bacteria isolates from dairy products

ISOLATES	CPX	AM	AU	CN	PEF	OFX	S	SXT	CH	SP
Escherichia coli	S	R	R	S	R	S	S	S	R	R
Shigella spp.	S	R	R	S	S	R	I	R	R	R
Salmonella spp.	S	R	R	S	R	S	I	R	R	R

Key: CPX: Ciprofloxacin (5µg), AM: Ampicillin (10µg), AU: Augmentin (30µg), CN: Gentamycin (10µg), PEF: Perfloxacin (5µg), OFX: Ofloxacin (5µg), S: Streptomycin (10µg), SXT: Trimethoprim-Sulfamethaxazole (1.25/23.75), CH: Chloramphenicol (30µg), SP: Spectinomycin (10µg)

species isolated across the sampling period (P<0.05). *Escherichia coli* had the highest distribution of bacteria isolates of 71 (44.10%), while *Salmonella* spp. had the least number of 42 (26.10%). The isolation rate of *Escherichia coli* agreed with a report by Tadesse et al.[23] which reported 44.57%. The results also agreed with Dadi et al.[24] that *Escherichia coli* was the most prevalent bacteria in dairy products while *Salmonella* spp. was the least. In this study, the highest distribution of *Escherichia coli* was discovered in butter (25.4%) and dried milk (22.50%), while the least was in ice cream (14.10%). *Salmonella* spp. was found to have the highest distribution in yoghurt (26.25%) and least in butter (14.30%). *Shigella* spp. was found to have the highest distribution in milk (27.10%), this agreed with a report by Abunna et al.[25] Above all, the water used for cleaning milking equipment and the air in the environment are the likely sources of contamination by enteric bacteria. However, the high percentage distribution of *Escherichia coli* may be due to poor hygiene practices in dairy products firms. The antibiotic susceptibility testing for *Escherichia coli* had an overall susceptibility of 50% and resistance of 50%, this slightly agreed with Fredrick et al.[26] who reported overall susceptibility of 64.35%. All isolates were 100% resistant to ampicillin, augmentin, chloramphenicol, and spectinomycin, the findings agreed with Federick et al.[26] The highest resistance (60%) and least susceptibility (30%) were reported for *Shigella* spp. and *Salmonella* spp. in the present study, this agreed with Demirci et al.[27]; Qamar et al.[28] Furthermore, *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. had 100% susceptible to ciprofloxacin and gentamycin, 66.7% for ofloxacin, 33.3% for pefloxacin. These findings agreed with Qamar et al.[28] who reported ciprofloxacin (100%). The present study suggestsciprofloxacin as the first drug of choice in the treatment of food-borne illnesses, followed by gentamycin. Moreover, hygiene practices should be encouraged among dairy food handlers to avoid contamination.

CONCLUSION

Pathogenic microorganisms are highly resistant to so many conventional antibiotics/ drugs, therefore regular antibiotic surveillance is necessary from time to time to determine antibiotics that are susceptible to a particular pathogen. Ciprofloxacin and gentamycin antibiotics were confirmed as the antibiotics/drugs for the treatment of illness caused by foodborne

pathogens from milk and dairy products. The present research work is of unique significance since no existing literature has any work on the microbiological quality of dairy products retail and consume in Abakaliki, Nigeria. The research work informs society of the poor hygiene standard during storage and retail of milk and dairy products, leading to cross-contamination of the dairy products and milk by pathogenic microorganism of public health threat (*Salmonella*, *Escherichia coli* and *Shigella*); therefore, close monitoring of milk and dairy products is important to avoid outbreak of foodborne disease caused by milk and dairy. There is therefore the need to improve the quality of materials (water and milk) used for production of dairy, as well as storage conditions, and environmental and personnel hygiene to meet food safety standards.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

OSC conceptualized the study. CVU, IY, KEA, and OJO performed culture and identification. ACK, MME, CCN, OCA, and DCI performed antibiogram of isolates. DOO and CSU performed isolation and characterization of the isolates. BU performed data analysis. OSC supervised the study and wrote the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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