World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Isolation and antibiotic susceptibility analysis of retailed powdered milk from Orie Uga Market in Aguata, Anambra State, Nigeria

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World Journal of Advanced Research and Reviews, 2023, 18(02), 682–689

Publication history: Received on 28 March 2023; revised on 13 May 2023; accepted on 15 May 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.2.0820

Abstract

The research entailed the isolation and antibiotic susceptibility analysis of retailed powdered milk from Orie Uga market in Aguata Anambra State. The increasing rate of morbidity and mortality from diarrhoea among the populace motivated the research. Samples of powdered milk were aseptically collected from six (6) different sellers in the market and analyzed using standard microbiological procedures. The pH of the samples were measured using a pH meter while their temperature were checked using a thermometer; serial dilution and growth on nutrient agar followed by Gram stain alongside biochemical tests were carried out; total viable counts were performed on all the samples which indicated that sample A harboured the lowest number of microbial count of 4.5.0x108cfu/g leaving sample C with the high number of counts- 7.5x108cfu/g precisely; susceptibility tests were done on all the samples with the use of three (3) antibiotics namely Ampicillin, Co-trimoxazole and Tetracycline. The results of the study indicated that the average pH of the samples was acidic while the average temperature was found to be normal. The isolated bacteria were majorly *Staphylococcus* species. The susceptibility tests revealed that the isolates were resistant to Ampicillin but sensitive to Co-trimoxazole and Tetracycline which then demands that the preferred drugs for treating any health complication resulting from the consumption of the sampled products were co-trimoxazole and tetracycline. This study is meant to enlighten all stakeholders in food security/consumption and health management.

Keywords: Microbiology; Milk; Antibiotics; Food Security/Consumption; Diarrhoea

1. Introduction

Food powders are consumable substances in crystal forms whose shelf-lives are controlled by the availability of water (Hedegaard *et al.,* 2014).

Reduction of the water presence in milk helps to reduce microbial presence in milk and also help to minimize the bulkiness for economy.

Microorganisms are ubiquitous and then do enter milk through the environment especially during the handling processes (Ezenweinyinya, 2018; Ezendianefo, 2022). It then increases in their numbers rapidly. It is better to minimize microbial presence than controlling their growth.

Statement of Problem

Researches have revealed that there is microbial presence in the milk powder (Ezenweinyinya, 2018; Ezendianefo, 2022).

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So, this study was meant to either support or dispute the fact that there are possible contaminations in the milk powder which has led to diverse health issues like diarrhea.

More so, these health issues have defied medical interventions leading to wastage of human resources used in changing medications and in extreme cases, has culminated into death.

Aim of the Study

This study aims at the isolation and antibiotic susceptibility analysis of retailed powdered milk from Orie Uga market in Aguata Anambra State.

Objective of the Study

- The following objectives were delineated for the study:
- To collect milk powder from retailers in Orie Uga market.
- To analyze milk powder for the presence of bacteria.
- To investigate the susceptibility of the isolated bacteria to available antibiotics.

1.1. Research Questions

The following research questions guided the study:

Are there bacteria in the retailed milk powder sold in Orie Uga markets?

What were the antibiotic susceptibility profiles of the bacteria isolated?

1.2. Area of Study

This research was done in Orie Uga market. The market is found in Aguata Local Government of Anambra State, Nigeria. Anambra is one of the thirty-six (36) states of Nigeria and located in the South-Eastern region of the country.

The location of Anambra in Nigeria is $6^{o}20'N$ $7^{o}00'E$.

Scope of Study

The scope of this study was limited to the prevalence of bacteria and their antibiotic susceptibility profiling in retailed milk powders that were obtained from Orie Uga market in Anambra State, Nigeria.

Limitation of Study

In the course of carrying out this study, the following limitations were observed:

Finance- This is the bedrock of limitations to the study as several areas of the research proceedings like transportation and laboratory materials were money-intensive

2. Material and methods

2.1. Sample and Sampling Techniques

For this study, a total of six (6) samples of milk powders were randomly selected from the population of the study.

Simple Random Sampling (SRS) technique was employed for the selection.

Table 1 Names of the Retailers and their Samples' Identifications

S/N	Names of Retailers	Identifications
1	Uche	A
2	Nkiru	B
3	Nnebe	C
4	Oge	D
5	Amaka	E
6	Mgbedi	F

2.2. Sample Collection

Milk powders were bought from six (6) different sellers and were labeled properly (A to F). They were aseptically transported to the laboratory kitchen of Tansian University for analysis within 48h.

Aliquots of the samples were analyzed using standard microbiological procedures.

2.3. Materials

The materials used include six (6) different samples of milk powder, a nutrient agar (NA) media, sterile Petri dishes, bijou bottles, aluminum foil, distilled water, and cotton wool. Other materials are those found in the well-equipped microbiology's laboratory of Tansian University.

2.3.1. Sterilization of Materials

The environments were thoroughly sterilized with bleach. All equipment were washed with detergent and rinsed severally in distilled water.

As well, the nutrient agar (NA) media was sterilized as follows:

2.3.2. Sterilization of Nutrient Agar

This medium was prepared according to its manual's specifications by measuring 7.0g of the agar powder on a "Metlar" electronic balance MT-2000 and poured into a conical flask. Then, distilled water was used to dissolve it and make up to 250ml of a homogenized mixture in the conical flask.

The conical flask was sealed with cotton wool and aluminum foil and then put into an autoclave for sterilization at a temperature of 121^{o}C for 15 minutes.

After autoclaving, it was cooled to a temperature that is tolerable by the human cheek at about 45^{o}C. It was then poured into sterilized Petri dishes, covered and allowed to solidify.

2.4. Physical Parameters' Analysis

2.4.1. Procedure for pH and Temperature

The following analyses were done on three aliquots of each sample and the average results noted.

Then, the pH and temperature were measured using standard procedures.

The pH of each water sample was measured using a pH meter and temperature was measured using a mercury thermometer.

2.4.2. Identification and Confirmation of Bacteria

The identity of bacterial isolates present on the milk powders were realized and counted through microbiological analysis using serial dilution method (Cullen *et al.,* 2016) and then growth on Nutrient agar using the pour plate method

while the confirmation of the bacterial isolates were achieved by biochemical tests (Arora *et al*., 2012) and Gram stain method (Bauer *et al.,* 1999).

2.5. Serial dilution method

2.5.1. Procedure

This research followed the procedure as described by Cullen *et al.,* 2016 as follows:

Dilution was made in the 1st tube by taking 2ml normal saline in a tube and inoculating the desired culture in it; 10 tubes and plates were labelled 1, 2, 3……..,, 10; 9 ml of normal saline was added in each test tube; 1 ml (known volume) of the culture was transferred from the previously made dilution into the 1st tube having 9ml normal saline; 1ml (known volume) was transferred from 1st tube into 2nd test tube and repeated steps till 10th test tube; 1ml was discarded from the 10th test tube and lastly, the remaining diluted 10th test tube was covered for further analysis.

2.5.2. Inoculation

0.1ml inoculums collected from the test tubes labeled 10^{-5} and 10^{-6} from each of the stock solutions labeled A-E were used to inoculate the nutrient agar plates.

Pour plate method was employed in the process.

Inoculums placed on nutrient agar plates (for bacteria growth) were incubated at 30^{o}C for 24 hours.

2.6. Total Viable Count

This was performed after the incubation period by counting the distinct colonies formed on the Petri dishes. Then, the mean colony for the two dishes (10^{-5} and 10^{-6}) was obtained and denoted as N. Firstly, viable count was obtained by dividing the mean colony (N) with the product of the dilution used (0.1) and the volume plated (example is 10^{-5}). This was done for the two dishes (10^{-5} and 10^{-6}) and the two results were added together to get the total viable count. This was carried out on the six samples.

2.6.1. Sub-Culturing

Inoculums grown on nutrient agar plates were sub cultured into the bijou bottles after the 24hours of incubation.

2.6.2. Identification of the Bacterial Isolates

This identification protocol was carried out in accordance with the process described by Arora *et al*., 2012.

2.7. Microscopical Examination

2.7.1. Gram Staining Techniques

This was done in accordance with Bauer *et al.,* 1999).

2.7.2. Motility Test

This is done to ascertain whether an organism can move or not. The medium used is nutrient agar and the concentration is about 0.3% after sterilization to allow for free spread of organisms.

Inoculation was done by a single line stab on the agar. After overnight incubation, movement away from the stab line or a hazy appearance throughout the medium indicates a positive test.

2.8. Confirmation of the isolates

2.8.1. Biochemical Tests

Catalase Test

This is used to know whether the isolate can produce the enzyme, catalase. A 24 hour old culture was smeared on a clean slide with distilled water, 2-3 drops of hydrogen peroxide was added on the smears. Bubbling reaction indicated positive test while absence of bubbles indicated negative reaction.

Coagulase Test

This is based on the ability of some isolate to coagulate human and rabbit plasma enzymatically. A loopful of normal saline was placed on a clean slide and small quantity of the test organism was emulsified on the normal saline, a drop of human plasma was added on the suspension and mixed for about 5 – 10 seconds, clumping of organisms with this time indicated positive test.

Citrate Utilization Test

This confirms the ability of the isolates to utilize citrate 'as the sole carbon source'. About 2.4g of Simmon's citrate agar was dissolved in 100ml of distilled water, sterilized by autoclaving at 121^{o}C for 15 minutes, dispensed in test tubes and allowed to set in slants, inoculated and incubated at 37^{o}C for 96 hours. Blue colour and streak showed positive test while the original green colour and no growth indicates negative test.

Indole Test

This test shows the ability of an organism to produce indole from nitrogen. About 1.5g of peptone water and 1g of glucose were dissolved in 150ml of water, poured in test tubes, autoclaved at 121 ^{o}C for 15 minutes. The medium was inoculated with the test organism and incubated at 37^{o}C for 48 hours for optimum accumulation of indole, Kovac's reagent was added to each test tube and mixed. Development of red colour indicates positive reaction.

Methyl Red Test

About 1.5g of peptone water and 1g of glucose were dissolved in 100ml of water, poured in test tubes, inoculated and incubated for 48hours, about 5 drops of methyl red indicator reagent was added in each test tube and mixed. Positive result was bright red and negative result was yellow.

Voges Proskauer Test

About 1.5g of peptone water and 1g of glucose were dissolved in 100ml of water, dispensed in test tubes, autoclaved at 121 ^{o}C for 15 minutes. The medium was inoculated with the test organism and incubated at 30 ^{o}C for 48 hours. 0.5ml of O' rMera reagent was added and mixed. A pink colour indicates positive reaction.

Sugar Fermentation Test

This shows the ability of organisms to produce acid and or gas from carbohydrates. 1g of each sugar (sucrose, lactose, glucose and maltose) was put in a test tube. About 1.5g of peptone water was dissolved in 100ml of distilled water and poured in the test tubes containing the sugars. 1ml of bromocrepsol blue was added before dispensing in test tubes. This was autoclaved with inverted Durham's tubes at 115 ^{o}C for 10 minutes. Colour change from blue to yellow indicated acid production, presence of bubbles and empty space at the bottom of the inverted Durham's tube indicated gas production.

2.8.2. Antibiotic susceptibility profile of the bacteria

Procedure of Kirby-Bauer disc diffusion

All procedures and results were as described in the Medical Laboratory Manual for tropical countries (Cheesbrough, 2005) and with reference to the Bergey's Manual of Systematic Bacteriology (Krieg *et al.*, 1994); the results were analyzed in accordance with the British Society for Antimicrobial Chemotherapy- BSAC (Andrew, 2007) and Clinical Laboratory Standard Institute.

Three (3) antibiotics were employed in this study. The antibiotics and their needed concentrations were as follows: Ampicillin (AMP)- 10µg, Co-trimoxazole (COT)- 250µg, Tetracycline (TET)- 30µg.

The antibiotics were placed on the discs. Using sterile forceps, the antibiotic impregnated paper discs were then aseptically placed on the surface of the Nutrient Agar (NA) plates at equal distance to each other; 0.1ml of the broth culture of each isolate was uniformly inoculated on the nutrient agar plates through a streak-plate method over the entire plates, in at least three planes, using a swab stick.

The plates were then allowed to dry for 10mins and were incubated at 37^{o}C for 24h; after incubation, diameters of clear zone of inhibitions were observed and measured in millimeter.

2.9. Method of Data Analysis

In this study, the inferential statistics was employed during the data analysis whose goal was to draw conclusions and make inferences in order to generalize them to a population

3. Results

3.1. Physical Parameters

The outcomes of the pH measurement and temperature carried out on the samples were enumerated on the table below.

Table 2 shows the results of the physical parameters; Table 3 shows the total viable count of bacterial isolates while table 4 shows the characteristics of the bacterial isolates respectively.

Table 2 Results of the Physical Parameters

S/N	Sample	pH	Temperature (^oC)
1	A	4.5	34
2	B	6.5	35
3	C	5.5	30
4	D	6.5	37
5	E	6.0	36
6	F	4.0	38

Table 3 Total viable count of Bacteria in the Samples

S/N	Sample	Bacteria
1	A	4.5×10^8
2	B	5.5×10^8
3	C	7.5×10^8
4	D	6.5×10^8
5	E	7.0×10^8
6	F	6.5×10^8

Table 4 Confirmatory Characteristics of Bacterial Isolates

Characteristics	Samples					
	A	B	C	D	E	F
Gram stain	+	+	+	+	+	+
Microscopy	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase test	+	+	+	+	+	+
Coagulase test	+	+	+	+	+	+
Motility test	-	-	-	-	-	-
Indole test	-	-	-	-	-	-
Methyl red test	+	+	+	+	+	+

Voges proskauer	+	+	+	+	+	+
Citrate test	+	+	+	+	+	+
Glucose test	+	+	+	+	+	+
Lactose test	+	+	+	+	+	+
Sucrose test	+	+	+	+	+	+
Maltose test	+	+	+	+	+	+
Inference:	A	A	A	A	A	A

Key: + = positive; - = negative; A = *Staphylococcus* species

3.2. Antibiotic Susceptibility Test

The isolates were subjected to antibiotic susceptibility tests to determine their sensitivity and resistance patterns and from the results, these were revealed in the varying level of response each isolate gave toward the tested antibiotics.

Table 5 The Results of the Antibiotic Susceptibility Test (mm)

Isolate Identity	**Antibiotics**		
	AMP	**COT**	**TET**
ISOLATE A	12	13	15
ISOLATE B	15	15	11
ISOLATE C	10	16	10
ISOLATE D	13	12	07
ISOLATE E	14	10	13
ISOLATE F	10	14	12

KEY: Ampicillin (AMP)- 10µg, Co-trimoxazole (COT)- 250µg, Tetracycline (TET)- 30µg

4. Conclusion

The morbidity and mortality rate among the populace following their consumption of retailed milk powder called for this research.

Susceptibility tests revealed that the isolates were resistant to Ampicillin but sensitive to Co-trimoxazole and Tetracycline which then demands that the preferred drugs for treating any health complication resulting from the consumption of the sampled products were co-trimoxazole, metronidazole and tetracycline (Ezendianefo, 2022). The un-hygienic dispensing and packaging processes of the powdered milk by the retailers inside the market should be discouraged as such should have been done under strict aseptic conditions preferably in the production environment. This study is meant to enlighten all stakeholders in food security/consumption and health management.

Therefore all stakeholders should, as a matter of urgency, be ready to put up measures that will help to curtail the ill health of the people after the consumption of the ill-fated milk powder.

Recommendations

The following suggestions were proffered:

- The selling of milk powders in retailing cups inside the market must be prohibited but should be restricted to only the factory packages made under sanitized production environment.
- Good Manufacturing Practices (GMPs) should be adequately followed during and after the production of milk powder.

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