



# **Biofilm Forming Ability and Antibiotic Susceptibility of Food-borne Pathogens Isolated from Common Dairy Products: Madara and Nono Vended in Makurdi Metropolis**

**Amina Ojochide Hassan<sup>1\*</sup>, Innocent Okonkwo Ogonna<sup>1</sup>  
and Victor Ugochukwu Obisike<sup>2</sup>**

<sup>1</sup>Department of Microbiology, University of Agriculture, Makurdi, Benue State, Nigeria.

<sup>2</sup>Department of Animal and Environmental Biology, Abia State University, Uturu, Abia State, Nigeria.

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors AOH and IOO designed the study. Authors VUO and AOH performed the statistical analysis. Authors AOH and IOO wrote the protocol and wrote the first draft of the manuscript. Authors AOH and VUO managed the analyses of the study. Author AOH managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Microbial resistance to antibiotics and biofilm formation ability of food-borne pathogens are major global health challenges. Most milk and milk products (Madara and Nono) could be vehicles for the transmission of multidrug resistant genes among any community. This study was aimed at determining the antibiotic susceptibility patterns and biofilm forming ability of some food-borne pathogens isolated from common dairy products: Madara and Nono in Makurdi metropolis. Two hundred and forty (240) samples comprising of one hundred and twenty (120) each of Madara (fresh raw milk from cow "FRM") and Nono (chance fermented cow milk "CFM") were examined for the presence of pathogens. Antibiogram of bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp. and *Klebsiella* spp.) using the disc diffusion method revealed

\*Corresponding author: E-mail: [ojochide.hassan@gmail.com](mailto:ojochide.hassan@gmail.com);

that susceptibility for Ampicillin (86.9%), Streptomycin (83.9%) and Ciprofloxacin (75.0%). Resistance was shown (26.7%) to Nalidixic acid, a commonly used antibiotic reflecting a public health concern. Most resistant isolates had a multiple antibiotics index of 0.3 (27.54%) with a least multiple antibiotics resistance index of 0.6 (0.85%). Detection of biofilm formation of isolates was done by Tube method. The study also revealed that out the total of 236 isolates tested for biofilm formation, 67 (28.4%) isolates were non or weak biofilm producers, 77 (32.6%) isolates were moderate biofilm producers and 92 (39%) isolates were strong biofilm producers. Findings of this research show high presence of a wide range of microorganisms, particularly enteric pathogens and enterotoxigenic strains of *S. aureus* which portrayed multidrug resistance and biofilm formation suggesting that FRM (Madara) and CRM (Nono) products might be important sources of food-borne infections and intoxication.

**Keywords:** Nono; Madara; microbial; resistance; antibiotics; biofilm.

## 1. INTRODUCTION

Dairy products are various products derived from cow's milk or that of other female mammals such as sheep, yaks, horses, camels and goats. These products include butter, yoghurt, Madara (fresh cow milk), Nono (fermented cow milk), cheese, whey, condensed and evaporated milk. Milk and milk products constitute important nutritional components for human diet and plays a prominent role in human nutrition [1]. Nono is a product formed when raw milk collected from cow's udder into a container is allowed to ferment naturally for 24h while Madara is the unfermented raw milk collected from cow's udder [2]. Nono is also more popular in public consumption, as Madara is not freely sold [2]. Madara is produced in homes especially in villages and producers could be ignorant of shelf-life and safety standards of the products. It is noticed that raw milk often contains microorganisms which may likely cause food-borne diseases [3]. Even when milk is fermented, the fermentation process may not eliminate these organisms (e.g. *Staphylococcus aureus*, *Escherichia coli* etc.) and may be carried to consumers.

Aside from milk and its products harbouring food-borne pathogens, some of these pathogens (*E. coli*, *S. aureus*, *Salmonella* spp., *Shigella* spp. etc.) could be resistant to different antibiotics. The recent re-emergence of antimicrobial resistance patterns among several microorganisms continue to generate serious challenges to the public health practitioners particularly in the food industry [4]. Bacteria in these milk products exists as either planktonic cells or as cell-forming biofilms. Most biomass of microorganisms exists as biofilms [5]. Many, if not all bacteria are able to form biofilms, which are communities of cells organized within a

permeable extracellular matrix. Biofilms are complex bioactive structures composed of one or more bacterial species protected by a matrix of extracellular polysaccharides [6]. Bacteria living within the biofilm are more resistant to antibiotics than their planktonic counterparts [5]. In the medical setting, bacteria in biofilms are responsible for most chronic infections in humans.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

Nono samples (120) were collected from North bank, Wadata and Modern market areas of Makurdi town in Benue State, Nigeria (40 samples per location) and labelled A, B and C respectively and Madara samples (120) were also collected (40 samples per market area) and labelled D, E and F respectively. These samples were maintained in an ice-packed container immediately after collection and transferred to the laboratory where analyses were carried out within 1-2 hours of collection. All samples were collected in sterile containers.

### 2.2 Isolation of Bacteria

Bacteria colonies were isolated from the samples by serial dilutions and spread plate techniques making use of different media which were prepared according to the manufacturer's instructions. The media employed for the isolation of organisms included: Nutrient Agar (Himedia Laboratories Pvt Ltd, India), Mannitol Salt Agar (MSA) (Oxoid Ltd, England) for *Staphylococcus aureus*, Eosin methylene blue (EMB) Agar (Himedia Laboratories Pvt Ltd, India) for *Escherichia coli*, *Salmonella Shigella* Agar (SS Agar) (Himedia Laboratories Pvt Ltd, India) for *Salmonella* and *Shigella* and Mac Conkey

Agar (Oxoid Ltd, England) for Coliforms. Media were sterilized by autoclaving at 121°C for 15 minutes except for SS Agar. Inoculated media were incubated at 37°C for 24h. Predominant bacteria colonies were sub-cultured and purified for identification.

### 2.3 Identification of Bacteria

For the initial characterization of the isolates, morphological examinations of the bacterial species were used. The pure cultures of isolates were further classified by cultural and biochemical techniques, thereafter compared with standard reference organisms. The following tests were performed: Catalase, oxidase, motility, urease, indole, coagulase, citrate, triple sugar iron utilization and Gram stain/ morphology examination.

### 2.4 Antibiotic Susceptibility Testing

Antibiotics susceptibility testing were carried out on the isolates using the disc diffusion method according to Bauer et al. [7]. The antibiotics used were: Pefloxacin, Ciprofloxacin, Chloramphenicol, Ampicillin, Nalidixic acid, Gentamycin, Augmentin, Ceporexlin, Ofloxacin and Streptomycin. National Committee for Clinical Laboratory Standards criteria was used to interpret diameter of inhibition zone with specified potencies [8]. The disc diffusion for Gram-positive and Gram-negative bacteria was performed on Nutrient Agar prepared according to manufacturer's instruction and sterilized by autoclaving at 121°C. Well isolated colonies (4 to 5) of the same morphological types were selected from agar plate and streaked across nutrient agar plates evenly. Discs were placed evenly (not closer than 24 mm from centre to centre) on the surface of the agar plate by using sterile forceps. These plates were inverted and placed in an incubator at 35 ± 2°C for 16 to 18 hours within 15 min after placing the discs. Each plate was examined the next day and zone diameter was measured and incorporated as susceptible or resistant [7,8].

#### 2.4.1 Multiple antibiotic resistance (MAR) and multiple antibiotic resistance index (MARI) of Isolates

Multiple antibiotic resistance (MAR) for this study was defined as resistance of isolate to three or more antibiotics [9,10]. Multiple antibiotic resistance index (MARI) was calculated according to Furtula et al., [11] as the ratio of

number of antibiotics to which an organism is resistant, to the total number of antibiotics to which an organism is exposed to. The following equation was used,

$$\text{MARI} = a/b \text{ [11]}$$

Where a 'represents the number of antibiotics to which isolates were resistant, b 'represents the number of antibiotics to which isolates were exposed.

#### 2.4.2 Determination of biofilm forming ability (Tube Method)

Using the method described by Christensen et al. [12], a loopful of the isolates were inoculated into 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes were then incubated at 37°C for 24 h. After incubation, the test tubes were decanted and washed with phosphate buffer saline (pH 7.3) and then dried. These test tubes were then stained with crystal violet (0.1%). Excess stain was rinsed off with deionized water. Thereafter, tubes were dried in inverted positions. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as 1-weak/none, 2-moderate and 3-high/strong. This was performed in triplicate and repeated three times.

## 3. RESULTS

### 3.1 Morphological and Cultural Characterization of Bacteria Isolated from Madara and Nono in Makurdi Metropolis

Table 1 shows the cultural characteristics of the organisms on the different growth media used, it also displays the Grams reactions of the isolates.

### 3.2 Biochemical Characterization of Bacteria Isolated from Madara and Nono in Makurdi Metropolis

The biochemical characteristics of presumptive *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., *Salmonella* spp., *Klebsiella* spp. and *Bacillus* spp. isolates are displayed Table 2. *E. coli* isolates were negative to oxidase, urease, citrate, coagulase tests but were positive to catalase and indole tests. They did not produce H<sub>2</sub>S but fermented glucose, lactose and sucrose with gas production on TSI agar.

*Staphylococcus aureus* isolates were catalase and Coagulase positive. *Salmonella* spp. isolates fermented only glucose and produced H<sub>2</sub>S differentiating them from all other Enterobacteriaceae. *Shigella* spp isolates fermented only glucose without producing H<sub>2</sub>S production. *Klebsiella* spp were citrate positive, produced gas and fermented glucose, lactose and sucrose but were negative to oxidase, urease, indole, coagulase, and catalase tests. *Bacillus* spp were motile and negative to indole and coagulase tests and fermented only glucose and sucrose without gas formation as shown in Table 2.

### 3.3 Antibiotic Susceptibility of Isolates

The antibiotic susceptibility and sensitivity pattern of organisms isolated was done using ten different antibiotic agents. A total of 102 *E. coli*, 90 *S. aureus*, 10 *Salmonella* spp, 8 *Shigella* spp, 20 *Klebsiella* spp and 6 *Bacillus* spp isolates were tested against these antibiotics (Table 8).

The isolates had 43.2% and 40.7 % susceptibility to Ceporex and Septrin respectively. They were highly susceptible to Ampicillin (86.9%), Streptomycin (83.9%), Ciprofloxacin (75%) and Ofloxacin (72.2%). Susceptibility to Pefloxacin was at 64.0% while to Augmentin was at 39.4%. Isolates were least susceptible to Nalidixic acid (26.7%) as shown in Table 3.

### 3.4 Susceptibility Patterns of Bacterial Isolates from Madara Samples

Table 4 shows the susceptibility pattern of bacterial isolates from Madara samples, based on zone of inhibition produced against test antibiotics. A total of 146 isolates were tested against ten (10) antibiotics. Antibiotics susceptibility of bacteria isolates from Madara shows that *E. coli* was more susceptible to Streptomycin with 85.9% and Ofloxacin 83.1% while it was least susceptible to Nalidixic acid (28.2%). *S. aureus* was totally susceptible to Ampicillin (100%), whereas to Streptomycin,

**Table 1. Morphological and Cultural Characteristics of Bacterial isolates from Madara and Nono in Makurdi**

Bacteria	Gram's reaction	Cultural characteristics on media
<i>E. coli</i>	Gram negative short rods	Colonies showing green metallic on EMB agar
<i>S. aureus</i>	Gram positive cocci (in clusters)	Golden yellow colonies on MSA
<i>Salmonella</i> spp	Gram negative rods	Non-lactose fermenting pale coloured colonies with black centers on SS agar
<i>Shigella</i> spp	Gram negative rods	Non-lactose fermenting pale coloured colonies on SS agar
<i>Klebsiella</i> spp	Gram negative long rods	Large, moist, shiny and mucoid pink colonies on MacConkey agar
<i>Bacillus</i> spp	Gram positive rods	Large grey white granular colonies with less wavy edge

**Table 2. Biochemical Characterization of Bacteria Isolated from Madara and Nono in Makurdi Metropolis**

CA	CI	CO	ID	M	OX	UR	SLP	BT	H <sub>2</sub> S	Gas	G	L	S	Probable Bacteria
+	-	-	+	+	-	-	Y	Y	-	+	+	+	+	<i>E. coli</i>
+	+	+	-	-	-	+	Y	Y	-	-	+	+	+	<i>S. aureus</i>
-	-	=	-	-	-	-	R	Y	-	-	+	-	-	<i>Shigella</i> spp
-	+	=	-	-	-	-	R	Y	+	=	+	-	-	<i>Salmonella</i> spp
+	+	NT	-	-	-	+	Y	Y	-	+	+	+	+	<i>Klebsiella</i> spp
+	+	NT	-	+	-	NT	Y	Y	-	-	+	-	+	<i>Bacillus</i> spp

Key: OX = Oxidase test, UR = Urease test, CI = Citrate test, ID = Indole test, CA = Catalase test. CO = Coagulase test, TSIA = Triple Sugar Iron Agar test, SLP = Slope, BT = Butt, H<sub>2</sub>S = Hydrogen Sulphide Production, G = Glucose Fermentation, L = Lactose Fermentation, S = Sucrose Fermentation, Y = Yellow Colour (Acid Production), R = Red Colour (Alkaline Production), + = Positive test, - = Negative test, NT = Not Tested

Ciprofloxacin, Ofloxacin, Septrin it had 90.9% susceptibility each. *S. aureus* was least susceptible to Nalidixic acid as shown in Table 4. *Shigella* spp. were totally susceptible (100.0%) to all the antibiotics used except Ofloxacin, Nalidixic acid, and Ceporexin, while *Bacillus* spp. were totally resistant to Septrin.

### 3.5 Multiple Antibiotic Resistance Patterns of Bacteria Isolates from Madara Samples

Multiple Antibiotic Resistance patterns of Bacteria isolates from Madara samples as shown in Table 5 revealed that 20 (28.2%), 8 (11.3%), 2 (92.8%) and 1 (1.4%) of *E. coli* isolates were resistant to a combination of 3, 4, 5 and 6 antibiotics respectively. For *S. aureus*, 13 (29.6%), 4 (9.1%) were resistant to a combination of 3 and 4 antibiotics respectively. *Salmonella* and *Klebsiella* spp followed the same pattern 2 (20) and 1 (10) respectively for each of the organism. *Bacillus* spp 1 (33.3%) was

resistant to 3 antibiotics with as shown in Table 5.

### 3.6 Susceptibility Patterns of Bacterial Isolates from Nono Samples

Table 6 captures the antibiotic susceptibility of bacteria isolates from Nono samples. *E. coli* showed 90.3% susceptibility to Gentamycin, and Ampicillin, 77.4% to Ofloxacin and had the least susceptibility to Augmentin (23.3%). *S. aureus* were totally susceptible to Ampicillin (100%), 93.5% for Ciprofloxacin while Ceporexin and Nalidixic acid had the least effect (3.2%) on the isolates as shown on Table 6. *Klebsiella* spp were totally susceptible to Gentamycin and Pefloxacin. They showed 10% resistance to Augmentin and Ceporexin at and recorded the least susceptibility to Nalidixic acid. *Bacillus* spp isolates were highly susceptible to Ciprofloxacin, Augmentin, Ceporexin, Ampicillin and Streptomycin at 100% each while all its isolates were resistance to Septrin and Nalidixic acid as shown in Table 6.

**Table 3. Percentage susceptibility**

Drug Family	Antibiotics	% Susceptibility
Quinolones	Pefloxacin	64.0
Quinolones	Ciprofloxacin	75.0
Quinolones	Ofloxacin	72.2
Aminoglycosides	Gentamycin	52.5
Cephalosporins	Ceporexin	43.2
Aminoglycosides	Streptomycin	83.9
Sulfonamide	Septrin	40.7
Penicillin	Ampicillin	86.9
Penicillin/ Beta-lactams	Augmentin	39.4
Quinolones	Nalidixic acid	26.7

**Table 4. Antibiotic Susceptibility Patterns of Bacteria Isolates from Madara Samples**

Antibiotics	EC n= 71	SA n= 44	SH n= 8	SM n= 10	KB n= 10	BA n= 3
Pefloxacin	30 (42.3)	39 (88.6)	8 (100)	10 (100)	10 (100)	2 (66.7)
Ciprofloxacin	36 (50.7)	40 (90.4)	8 (100)	9 (90)	9 (90)	3 (100)
Ofloxacin	36 (50.7)	40 (90.9)	6 (75)	10 (100)	8 (80)	3 (100)
Septrin	59 (83.1)	40 (90.9)	8 (100)	10 (100)	5 (50)	0 (0)
Augmentin	25 (32.2)	18 (40.9)	8 (100)	8 (80)	10 (100)	3 (100)
Nalidixic acid	23 (32.4)	1 (2.3)	4 (50)	7 (70)	4(40)	2(66.7)
Ceporexin	20 (28.2)	4 (9.1)	7 (87.5)	10 (100)	9 (90)	3(100)
Gentamycin	40 (56.3)	5 (11.4)	8 (100)	10 (100)	10 (100)	2 (66.7)
Ampicillin	46 (64.8)	44 (100)	8 (100)	9 (90)	10 (100)	2 (66.7)
Streptomycin	61 (85.9)	40 (90.9)	8 (100)	10 (100)	9 (90)	3 (100)

Key: EC = *E. coli*, SA= *S. aureus*, SH= *Shigella* spp, SM = *Salmonella* spp, KB= *Klebsiella* spp, BA= *Bacillus* spp

**Table 5. Multiple Antibiotic Resistance Patterns of Bacteria Isolates from Madara Samples**

NA	Resistance Pattern	EC n= 71	SA n= 44	SH n= 8	SM n= 10	KB n= 10	BA n= 3	MARI
3	PeGeTa, PeAuNa, CeNaGe, SePeTa, CiStTa, AmGeCi	20 (28.2)	13 (29.6)	1 (12.5)	2 (20)	1 (33.3)		0.3
4	PeGeTaNa, AmSeTaAu, GeAuCeNa, StGeNaTa	8 (11.3)	4 (9.1)	-	1 (10)	1 (10)	-	0.4
5	CiSeAuCeNa, PeAuGeNaCe, AuAmCeSeCi	2 (2.8)	-	-	-	-	-	0.5
6	SeTaAuNaCeSt	1 (1.4)	-	-	-	-	-	0.6
<b>Total</b>	<b>(%)</b>	<b>31 (43.7)</b>	<b>17 (38.6)</b>	<b>1 (12.5)</b>	<b>3 (30)</b>	<b>3 (30)</b>	<b>1 (33.3)</b>	

Key: EC = *E. coli*, SA= *S. aureus*, SH= *Shigella spp.*, SM = *Salmonella spp.*, KB= *Klebsiella spp.*, BA= *Bacillus spp.*, Pe= Pefloxacin, Ci=Ciprofloxacin, Ta=Ofloxacin, Se=Septrin, Au=Augmentin, Na=Nalidixic acid, Ce=Ceporexin, Ge=Gentamycin Am=Ampicillin, St=Streptomycin. MARI=Multiple Antibiotics Resistance Index. NA=Number of Antibiotics

**Table 6. Antibiotic Susceptibility Patterns of Bacteria Isolates from Nono Samples**

Antibiotics	EC n= 31	SA n= 46	KB n= 10	BA n= 3
Pefloxacin	12 (38.7)	28 (60.9)	10 (100)	2 (66.7)
Ciprofloxacin	20 (64.5)	43 (93.5)	6 (60)	3 (100)
Ofloxacin	24 (77.4)	12 (38.7)	4 (40)	2 (66.7)
Septrin	5 (29.4)	19 (61.3)	5 (50)	0 (0)
Augmentin	7 (23.3)	4 (12.9)	9 (90)	3 (100)
Nalidixic acid	21 (40.2)	1 (3.2)	3 (30)	0 (0)
Ceporexin	16 (51.6)	1 (3.2)	9 (90)	3(100)
Gentamycin	28 (90.3)	3 (9.4)	10 (100)	2 (66.7)
Ampicillin	28 (90.3)	46(100)	5 (50)	3 (100)
Streptomycin	31 (100)	25 (54.3)	8 (80)	3 (100)

Key: EC = *E. coli*, SA= *S. aureus*, KB= *Klebsiella spp.*, BA= *Bacillus spp.*

**Table 7. Multiple Antibiotic Resistance Pattern of Bacteria isolates from Nono Samples**

NA	Resistance pattern	EC n= 31	SA n= 46	KB n= 10	BA n= 3	MARI
3	PeAuSe, CeGeNa, SeNaGe, SePeTa, CiAuTa, AmGeCi, AuSePe, NaCeAu	10 (32.3)	13 (28.3)	2 (20)	1 (33.3))	0.3
4	PeAuTaNa, AmSeTaAu, GeAuCeNa, SeGeNaTa	4 (12.9)	5 (10.8)	0 (0)	0 (0)	0.4
5	CiSeAuCeNa, PeAuGeNaCe, AuAmCeSeCi	3 (9.6)	2 (4.3)	0 (0)	0 (0)	0.5
6	SeTaAuNaCePe	1 (3.2)	0 (0)	0 (0)	0 (0)	0.6
<b>Total</b>	<b>(%)</b>	<b>17 (54.9)</b>	<b>20 (43.5)</b>	<b>2 (20)</b>	<b>1 (33.3)</b>	

Key: EC = *E. coli*, SA= *S. aureus*, KB= *Klebsiella spp.*, BA= *Bacillus spp.*, Pe= Pefloxacin, Ci=Ciprofloxacin, Ta=Ofloxacin, Se=Septrin, Au=Augmentin, Na=Nalidixic acid, Ce=Ceporexin, Ge=Gentamycin Am=Ampicillin, St=Streptomycin. MARI=Multiple Antibiotics Resistance Index. NA=Number of Antibiotics

### 3.7 Multiple Antibiotic Resistance Patterns of Bacteria Isolates from Nono Samples

Table 7 showed the Multiple Antibiotics Resistance Patterns of Bacteria from Nono sample, 10 (32.3%), 4 (12.9%), 3 (9.6%) and 1 (3.2%) of the *E. coli* isolates were resistant to 3, 4, 5 and 6 antibiotics respectively as shown Table 4.8b. Isolates of *S. aureus* [13] (28.3), 5 (10.8) 2 (4.3)] were resistant to 3, 4 and 5 antibiotics respectively. *Klebsiella* and *Bacillus* had 2 (20%) and 1 (33.3%) isolates showing resistance to 3 antibiotics respectively as shown in Table 7.

### 3.8 Biofilm Formation

Table 8 showed the biofilm formation pattern. From the total of 236 isolates tested for biofilm formation, 67 (28.4%) isolates were non or weak biofilm producers, 77 (32.6%) isolates were moderate biofilm producers and 92 (39 %) isolates were strong biofilm producers. Some

isolates of *E. coli*, *S. aureus*, *Klebsiella* spp and *Bacillus* spp showed strong biofilm formation while all isolates of *Shigella* spp did not produce biofilm as shown in Table 8. No statistical significance was observed.

## 4. DISCUSSION

### 4.1 Antibioqram and Multidrug Resistance

Antibiotics susceptibility assessment of bacteria isolated in this study showed varying degrees of bacterial resistance as well as multiple antibiotics resistances in bacterial isolates. The result showed that most of the isolates were susceptible to the antibiotics which are in contrast with earlier reports in other studies on milk and milk products [13] *E. coli* isolated were more susceptible to Streptomycin, Ofloxacin, Gentamycin and Ampicillin. Nalidixic acid (26.7%) was revealed to be the least effective antibiotic in this study while Ampicillin (86.9%) had the highest antibacterial susceptibility effect.

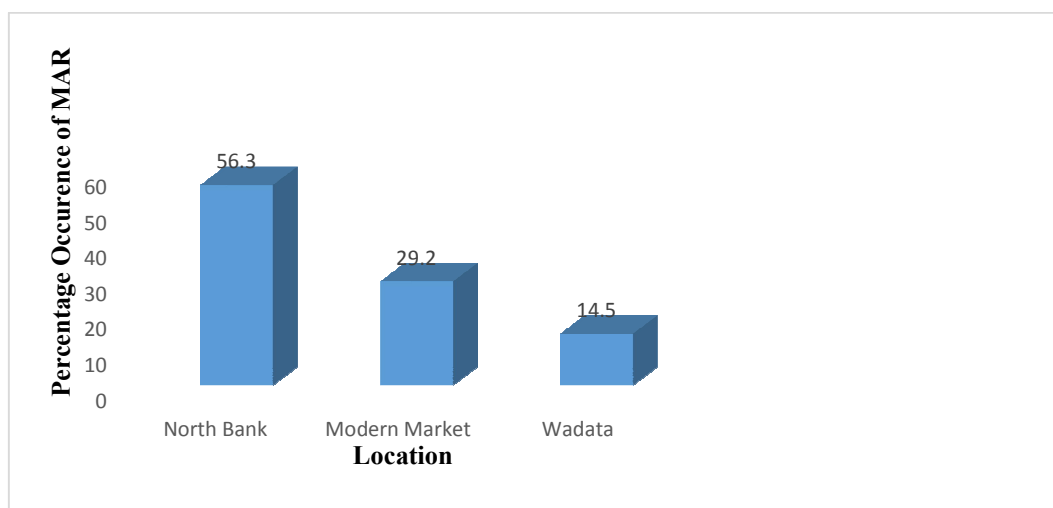


Fig. 1. Distribution of MAR Isolates in Selected Locations

Table 8. Biofilm formation

Organisms	Negative 1	Moderate 2	Strong 3
<i>E. coli</i>	29	37	36
<i>S. aureus</i>	40	27	23
<i>Shigella</i> spp	8	0	0
<i>Salmonella</i> spp	6	6	0
<i>Klebsiella</i> spp	8	7	5
<i>Bacillus</i> spp	1	2	3
Total	92	77	67

$$\chi^2 = 3.92, P \leq 0.05, df = 10$$

Antibiotic resistance of isolated bacteria from Nono samples and Madara samples may be traced to abuse in the use of antibiotics. Many antibiotics have been reported to be persistent in the environment and have been isolated from ground water [14] which could probably be used at times in the preparation of Nono and Madara products. This could enhance the emergence and spread of bacterial resistance among people who may consume these products. In this study, isolates of *Shigella* spp were 100% susceptible to all the antibiotics tested.

Multidrug resistance for this study is defined as resistance of isolate to three or more antibiotics as adapted from previous studies [9,10]. MAR index was calculated as the ratio of number of antibiotics to which an organism is resistant to total number of antibiotics to which an organism is exposed [11]. MAR index values greater than 0.2 indicates high risk source of contamination where antibiotics are often used [11]. In the present study, high index of 0.6 for both madara and nono samples is a serious concern. Multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [15].

North Bank location had the highest percentage occurrence of MAR (56.3%) (Fig. 1) suggesting area of high and indiscriminate usage of antibiotics [11] This observation in North Bank suggests that the high antibiotic consumption rate in the area may have arisen by no other factor other than increases in animal population. Similar trends of increased livestock-antibiotics usage had been reported by Adesokan *et al.* (2015) [16]. Recent researches [17,16] on global antibiotics consumption and antimicrobial usage in livestock animals in south-western Nigeria respectively have shown substantial increase in antibiotics consumption in humans and livestock in developing countries. This is a matter of concern for livestock disease management and livestock production in general owing to the existing emergence of bacterial strains resistant to major antibiotics. This pattern of antibiotic consumption is usually due to lack of regulatory control in the sales of veterinary drugs in most developing countries. The high percentage occurrence of multiple antibiotics resistance (MAR) index (41%) among the bacterial isolates in this study might have arisen due to common practices such as abuse of and over the counter usage of antibiotics. In addition to this is

continued administration of antibiotics repeatedly against infections that appear nonresponsive to the normal dose given earlier [18]. The residues of antibiotics have been reported in tissues of food animals and their products [19,20].

*E. coli* had highest occurrence of MAR (50%) among the isolates. Madara samples had higher percentage occurrence of MAR than Nono obviously because the product had the highest number of *E coli* occurrence.

#### 4.2 Biofilm Formation

The ability of food-borne pathogens to form biofilms helps them to survive in hostile environments within the host. This is considered to be responsible for chronic or persistent infections [21]. Biofilm analysis of the bacterial isolates reviewed showed that of all the six bacterial species isolated from the samples, thirty-six isolates of *E. coli*, twenty-three of *S. aureus*, three of *Klebsiella* spp and three of *Bacillus* spp showed strong biofilm formation while thirty-seven isolates of *E. coli*, twenty-seven of *S. aureus*, four of *Salmonella* spp, seven of *Klebsiella* spp and two of *Bacillus* spp were moderate biofilm producers. All isolates of *Shigella* spp were non-biofilm producers. In previous studies, bacteria such as *Bacillus cereus*, *Escherichia coli*, *Shigella* spp and *Staphylococcus aureus* were also reported to produce biofilm in dairy processing plants [22]. Biofilms formed in food-processing environments are of special importance as they have the potential to act as a persistent source of contamination that may lead to food spoilage or transmission of diseases [23]. Biofilms can modify the microenvironment to enhance microbial survival on the internal surface of milk tankers. According to the National Institutes of Health (NIH), more than 65 to 80 percent of all microbial infections are now caused by biofilm-producing bacteria. Recent studies indicate that biofilm-producing bacteria move freely in the blood system until they encounter a solid surface [24]. It's this ability to "feel" something solid that converts mobile bacteria to a stationary form that essentially finds a place to camp and produce the bacterial biofilm shield [24]. Biofilm-anchored bacteria show much greater resistance to antibiotics (as much as a thousand times more) than their free-moving more vulnerable counterparts [24]. As the bacteria community grows inside the mucous-like mass, individual cells chemically talk to each other (quorum sensing) regarding the presence of antibiotics.



When the antibiotics are no longer a threat, the bacteria break off a piece of the bacterial biofilm and ride in it like a spaceship to other areas to spread the infection.

## 5. CONCLUSION

The high mean total viable counts of microorganism and the presence of a wide range of organisms, particularly enteric pathogens isolated from the Madara and Nono showed that these milk products sold in Makurdi metropolis could be an important source of food-borne infections.

Bacterial isolates formed biofilms on the surfaces of containers bearing madara and nono and can consequently act as persistent sources of contamination threatening the microbiological quality and safety of madara and nono. This can lead to food-borne disease and economic losses.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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