

# Prevalence and Antibiotic Susceptibility Patterns of *Campylobacter* Species in Locally Pasteurized Milk Product (Nunu) Sold in Zaria Metropolis, Kaduna State, Nigeria

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**Abstracts:** Contaminated milk and milk-products are regarded as vehicles for the transmission of Campylobacteriosis, infectious diarrhoea caused by *Campylobacter* but the prevalence of this bacterium in nunu had not been established. This study aimed at determining the prevalence of *Campylobacter* species in locally pasteurized milk product (nunu) sold in Zaria metropolis and establish the antibiotic resistance pattern of the isolates. A total of 180 nunu samples were collected and cultured for *Campylobacter* species using membrane filtration method, characterized using biochemical testing and API campy kits. Susceptibility of the isolates was carried out using the conventional agar diffusion method and the MAR indexing was calculated respectively. Out of the 180 nunu samples, 29 samples were positive for *Campylobacter* species giving a prevalence of 16.1%. *Campylobacter coli* were mostly isolated with isolation rate of 10.5% while *C. jejuni* were 5.6%. Imipenem were found to have 100% efficacy against all the *Campylobacter* species followed by Gentamicin (95%) while the isolates were resistant to Erythromycin (100%). Altogether, 39% of the *Campylobacter* species were resistant to three and more class of antibiotics and this is mostly implicated among strains of *Campylobacter coli* (47%) compared to *C. jejuni* (31%). The highest MAR index of 0.63 observed in this study is of public health importance. In this study, all the isolates had a MAR index greater than 0.2. Thus, *Campylobacter* contamination of the milk product is likely to be from a high risk source. With the increasing trend in *Campylobacter* resistance, it is therefore recommended that the use of antibiotic in animal food production and human therapy had to be controlled.

**Keywords:** Prevalence, Antibiotic, *Campylobacter* Spp, MAR Index, Multidrug Antibiotic Resistance, Nunu

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## 1. Introduction

Milk is known to be nutritious by helping the body fight against diseases but raw milk and contaminated milk have been implicated in most outbreak and sporadic cases of Campylobacteriosis [2, 4]. Milk in its original state does not contain microbes, but become contaminated during unhygienic processing. Thus, the health of the dairy animals

as well as the environment where the milking is taking place affects the microbiological quality of milk [32]. Raw or unpasteurized milk contains myriad of foodborne pathogens that are potential threat to milk consumers. In Nigeria, sales of milk, either raw or pasteurized are legal but must meet the Milk and Dairy Products Regulation [18]. However, the regulation do not certifies that raw milk is free of all pathogens. Sour milk locally called “nunu” is a temperature stable product of milk produced mainly by the Fulani.

However, their unsanitary milking methods have made the microbiological quality of this product (nunu) questionable.

Pasteurization removes the intrinsic danger that is related to consumption of milk and other heat sensitive food products of animal origin [4]. Conventional low temperature pasteurization such as heating the milk at 63- 66°C for 30 minutes is commonly used during Milk processing in Nigeria. This temperature is able to kill all non-pathogenic and non-spore forming pathogenic microbes that could be present during milking. However, most locally pasteurized milk still contains good number of pathogenic microbes that could cause disease to the consumers. This could be as a result of inadequate pasteurization or post-pasteurization contamination by the milk handlers through the use of contaminated water and unsanitary milking methods. Pasteurized milk has been implicated in most outbreaks of diseases associated with milk due to contamination in the milking system [21]. Gastroenteritis is the major foodborne disease associated with consumption of raw milk with diarrhoea reported in over 90% of such cases [27].

*Campylobacter* species are the major causes of acute bacterial diarrhoea worldwide [8] with a prevalence of 17% in Nigeria [6]. Two species of *Campylobacter* were known for years to be responsible for human gastrointestinal diseases with no clear understandable knowledge of the roles of other *Campylobacter* species in diarrhoea. This is because; the antimicrobial content of the selective medium used for the isolation of these organisms inhibit the growth of these emerging *Campylobacter* species. However, the evolution of media that are selective to *Campylobacter* with low antimicrobial contents, had enable clinicians to isolate *Campylobacter* species that are not *C. jejuni* and *C. coli*; leading to their discovery as agent of human gastrointestinal diseases. Unhygienic preparation of animal food products make these organisms to be found in animal population [32]. They are of both food and medical important causing the disease Campylobacteriosis. Poultry meats that are not cooked sufficiently and not pasteurized milk are unique in the transmission of Campylobacteriosis. Its low infective dose is a concern to the public health especially among raw milk consumers. We were able to isolate *Campylobacter* from the locally pasteurized milk product (nunu) sold in Zaria metropolis, characterized them using API Campy kits and determine the antimicrobial susceptibility pattern to some commercial antibiotics based on antibiotic commonly used in the area as well as recommended by CLSI, [7] for treatment of bacterial diarrhoea.

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out in some parts of Zaria metropolis, Nigeria. The parts were Zaria City, Tudun-Wada, Sabon-Gari, Samaru, Kgwagilila and PZ. Zaria Metropolis is situated among the high plains of Northern Nigeria, on latitudes 11°07'N to 11°51'N and longitudes 7°43'E to 7°45'E

[29].

#### 2.1.1. Sample Collection

In this study, a total of 180 nunu samples were collected uniformly using sterile plastic containers with cap from different Fulani women located in Samaru, PZ, Kgwagilila, Sabo, Tudun-Wada and Zaria city all in Zaria metropolis and capped immediately. The samples were transported in an ice-packed container to the Department of Microbiology Laboratory, Ahmadu Bello University.

#### 2.1.2. Isolation of *Campylobacter* Species

Upon arrival at the Laboratory, the samples were vortex in a high speed for 10 sec and then, 25 ml of the samples were aseptically added onto 500 ml sterile conical flask containing 250 ml Bolton broth (Oxoid) and incubated initially at 37°C for 4 h and latter at 42°C for 2 days in a microaerophilic condition; after which a sterile 0.5 ml syringe was used to aseptically collect 0.2ml of the enriched culture and spread onto a *Campylobacter* blood agar plate (Oxoid) supplemented with 7% hydrolysed sheep blood and incubated at 37°C for 5 days in a 3.5L anaerobic jar under a microaerophilic condition (6% O<sub>2</sub> and 10% CO<sub>2</sub> in 84% N<sub>2</sub>) using a Campy-Gen gas generating sachet (Campygen N25 Oxoid).

#### 2.1.3. Identification of the Isolates

Suspected colonies were subcultured onto Columbia blood agar plate supplemented with 7% hydrolysed sheep blood. The plates were transferred into a 3.5L anaerobic jar where Campy-Gen gas generating sachet (Campygen N25 Oxoid) was introduced to keep the microaerophilic condition (6% O<sub>2</sub> and 10% CO<sub>2</sub> in 84% N<sub>2</sub>) and incubated at 37°C for 24 h. Isolates were characterised using standards method based on morphological characteristics and biochemical testing. Positive isolates were further analysed by API Campy kits (bioMérieux, France) and interpreted using the APIWEB to identify the isolates to species levels.

### 2.2. Antibiotics Susceptibility Testing

This was carried out using the Conventional agar diffusion method on a Mueller-Hinton agar plate supplemented with 5% hydrolysed sheep blood. The plate was inoculated with standard suspension (0.5 MacFarland) of the isolates using a sterile swab stick and swabbed to ensure even distribution. It was allowed to stand for few mins, after which, Antibiotic discs such as Tetracycline (30µg), Gentamicin (10µg), Nalixidic acid (30µg), Amoxicillin (25µg), Azithromycin (15 µg), Erythromycin (30 µg), Imipenem (10 µg) and Ciprofloxacin (5 µg) were placed on its surface using sterile forceps and incubated at 37°C for 24 h under microaerophilic condition, after which, area of clear growth were measured and interpreted standardly [7].

### 2.3. Determination of Multiple Antibiotic Resistance (MAR) Index

Multi-resistance of the *Campylobacter* isolate was

determined using the MAR indexing method as described by Krumperman, [15].

$$\text{MAR index} = \frac{\text{Number of antibiotic to which an isolate was resistant}}{\text{Total number of antibiotics to which the isolate was exposed}}$$

### 3. Results

#### 3.1. Prevalence and Distribution of *Campylobacter* Species in the Nunu Samples

Out of 180 nunu samples collected, 29 samples were positive for *Campylobacter* species giving a prevalence of 16.1%; 10 (5.6%) were *Campylobacter jejuni* while 19 (10.5%) were *Campylobacter coli* (table 1)

**Table 1.** Prevalence and distribution of *Campylobacter* species in the nunu sample.

| <i>Campylobacter</i> species (n = 180) | No. isolated | Percentage (%) |
|--|--------------|----------------|
| <i>C. coli</i>                         | 19           | 10.5           |
| <i>C. jejuni</i> subsp <i>jejuni</i>   | 7            | 3.9            |
| <i>C. jejuni</i> subsp <i>doylei</i>   | 3            | 1.7            |
| Total                                  | 29           | 16.1           |

N- Number of samples

#### 3.2. Antibiotic Resistance Pattern of *Campylobacter* Species Isolated From Nunu

Among the antibiotics tested, Imipenem had a 100% efficacy against all the *Campylobacter* species isolated while Erythromycin had the least efficacy (0%). Gentamicin had 90%, activity on all *Campylobacter jejuni* followed by Azithromycin with 80%, Ciprofloxacin with 80%, Nalixidic acid with 60%, Tetracycline with 60%, and then Amoxicillin with 50% activity. Gentamicin were found to have 100% activity against all *C. coli* followed by Ciprofloxacin with

79%, Azithromycin with 58%, Amoxicillin with 36% activity and then Nalixidic acid and Tetracycline with 32 and 21% respectively (table 2).

**Table 2.** Resistance patterns of the *Campylobacter* species (%S/R) to selected antibiotics.

| Antibiotics | <i>Campylobacter jejuni</i> |    | <i>Campylobacter coli</i> |     |
|-------------|-----------------------------|----|---------------------------|-----|
|             | %S                          | %R | %S                        | %R  |
| AZM (15 µg) | 80                          | 20 | 58                        | 42  |
| IPM (10 µg) | 100                         | 0  | 100                       | 0   |
| NA (30 µg)  | 60                          | 40 | 32                        | 68  |
| TE (30 µg)  | 60                          | 40 | 21                        | 79  |
| CN (10 µg)  | 90                          | 10 | 100                       | 0   |
| CIP (5 µg)  | 80                          | 20 | 79                        | 21  |
| AML (25 µg) | 50                          | 50 | 37                        | 63  |
| E (10 µg)   | 30                          | 70 | 0                         | 100 |

Key: S: Susceptibility; R: Resistance; AZM: Azithromycin; IPM: Imipenem; TE: Tetracycline; NA: Nalixidic acid; CN: Gentamicin; CIP: Ciprofloxacin; E: Erythromycin; AML: Amoxycillin

#### 3.3. Resistance Profile of *Campylobacter* Species Isolated From Nunu

The MAR index of the *Campylobacter* isolates in this study is shown in Table 3 below. *Campylobacter jejuni* in this study exhibited 12 antibiogram while *C. coli* had 9. The MAR indices of the *Campylobacter* isolates range from 0.38 to 0.88. The highest MAR index (0.88) was observed among the *C. jejuni* strains that were resistant seven antibiotics. The MDR Multidrug resistance (MDR) in this study was defined as resistance to at least three different classes of antibiotics. *Campylobacter* isolates showed 39% MDR with *C. coli* 47% while *C. Jejuni*

**Table 3.** Multiple antibiotic resistance (MAR) index of the *Campylobacter* species.

| <i>Campylobacter</i> species | Antibiotic resistant profiles | No of Isolate | MAR index | Percentage of Isolates (%) |
|------------------------------|-------------------------------|---------------|-----------|----------------------------|
| <i>C. jejuni</i>             | AZM-NA-E                      | 2             | 0.38      | 2.70                       |
|                              | AZM- CN-CIP                   | 1             | 0.38      | 1.35                       |
|                              | NA-E-TE                       | 4             | 0.38      | 5.40                       |
|                              | AZM-AML-E-TE                  | 2             | 0.50      | 2.70                       |
|                              | AZM-NA-E-AML                  | 2             | 0.63      | 2.70                       |
|                              | AZM-TE-E- CN                  | 1             | 0.50      | 1.35                       |
|                              | AZM- TE- E- AML- NA           | 2             | 0.63      | 2.70                       |
|                              | AZM -CIP- E- AML- TE          | 2             | 0.63      | 2.70                       |
|                              | AZM- NA- E- AML- CIP          | 2             | 0.63      | 2.70                       |
|                              | AZM-CIP-E-AML-NA-TE           | 2             | 0.75      | 2.70                       |
|                              | AZM-CIP-E-AML-NA-CN           | 1             | 0.75      | 1.35                       |
|                              | AZM-CN-E-AML-NA-TE-CIP        | 1             | 0.88      | 1.35                       |
| <i>C. coli</i>               | AZM-NA-E                      | 8             | 0.38      | 10.8                       |
|                              | AZM-CIP-NA                    | 4             | 0.38      | 5.40                       |
|                              | AZM-E-AML                     | 8             | 0.38      | 10.8                       |
|                              | AZM-AML-E-TE                  | 8             | 0.50      | 10.8                       |
|                              | AZM-TE-E-NA                   | 8             | 0.50      | 10.8                       |
|                              | AZM-CIP-E-AML-TE              | 4             | 0.63      | 5.40                       |
|                              | AZM-CIP-E-AML-NA-TE           | 4             | 0.75      | 5.40                       |
|                              | E-TE-CIP-NA-AML               | 4             | 0.63      | 5.40                       |
|                              | AZM-AML-NA-TE-CIP             | 4             | 0.63      | 5.40                       |

## 4. Discussion

### 4.1. Prevalence and Distribution of *Campylobacter* Species in the Nunu Samples

The consumption of contaminated milk and milk product has been implicated as vehicle for transmission of human Campylobacteriosis [25]. In this study, the prevalence of *Campylobacter* species was investigated by isolating *Campylobacter* species from nunu sold in Zaria metropolis and 16.1% was observed. This high isolation rate could be due to cross contamination of the milk products, use of contaminated water during the preparation, unhygienic milking conditions and post-pasteurization contamination. When compared with result of the prevalence of *Campylobacter* in raw milk from other places, it was observed to show a higher occurrence. For instance, [14], in Tanzania reported prevalence of 13.4%, [12] reported 10.4% in Pakistan while [26] reported 4.8% at Sokoto. These discrepancies in the prevalence studies on *Campylobacter* in milk and milk product could be due to differences in sampling and isolation methods.

*Campylobacter coli* were mostly isolated with prevalence of 10.5% while *C. jejuni* had a prevalence of 5.6%. This high occurrence of *Campylobacter coli* could be due to proven facts that *C. coli* are more resistant to antimicrobial agents than *C. jejuni* [22, 27]. This was supported by the findings of [10], who reported that *C. coli* were more prevalence than *C. jejuni* in Leipzig, Germany. This work also agrees with the work of [1] and [11] who also reported a higher prevalence of *C. coli* than *C. jejuni*. However, this observation was not in accordance with previous studies that *Campylobacter jejuni* is the most common thermotolerant *Campylobacter* species [23, 25, 30]. These differences may be due to different methods of identification used. In their work, species confirmation was only based on primary biochemical test which may overestimate the *Campylobacter jejuni* strains [22, 10].

Campylobacteriosis are usually self-limiting and treatment involves replacing the loss of electrolyte but antibiotics such as tetracycline, macrolides, and fluoroquinolones may be taken in severe cases. However, the trend in antibiotic resistance in *Campylobacter* species has made these antibiotics ineffective in the treatment of Campylobacteriosis [27]. This problem is more complicated by the use of antibiotics as prophylaxis, indiscriminate use of antibiotics in food animal production and misuse of antibiotics.

### 4.2. Antibiotic Resistance Pattern of *Campylobacter* Species Isolated From Nunu

*Campylobacter* resistance to Macrolides used in the study (Erythromycin and Azithromycin) is could be due to point mutation in the peptidyl-encoding region in the domain V of the 23S rRNA gene leading to modification of the target ribosomal proteins while their resistance to Tetracycline could be due to protection of the ribosomal binding sites of

tetracycline and mutations in the 16S rDNA mediated by the *tet* (O) plasmids [31, 9]. *Campylobacter* resistance to Fluoroquinolones used in the study (Nalixidic acid and Ciprofloxacin) is due to mutation in the *gyrA* gene encoding subunits of DNA *gyrase* and Topoisomerase IV subunit, *parC* while their resistance to Amoxycillin could be due to the high production of  $\beta$ -lactamase by *Campylobacter* species that break the  $\beta$ -lactam ring of the antibiotics [31, 9]. The *Campylobacter* species in this study showed varying sensitivity to selected antibiotics. Imipenem had 100% activity against all *Campylobacter* species. Its mechanism of action involves binding to penicillin-binding proteins, thereby stopping the formation of the bacterial cell wall. This low resistance observed in Imipenem could be due to the fact that this Carbapenem is able to resist hydrolysis by  $\beta$ -lactamases, modification in penicillin-binding proteins and decreased in porin uptake leading to inhibition of *Campylobacter* cell wall synthesis [3]. This finding was in agreement with [20, 17, 13] who reported a 100% susceptibility of all *Campylobacter* species to Imipenem.

The *Campylobacter jejuni* isolates in the study have 90% susceptibility to Gentamicin, followed by Azithromycin (80%), Ciprofloxacin (80%), Tetracycline (60%), Nalixidic acid (60%) and Amoxycillin (50%). Erythromycin had the least efficacy on *C. jejuni* with a 30%. This high resistance of *C. jejuni* to Erythromycin agrees with the findings of [20, 19, 24, and 13] where a high resistance was reported to Erythromycin. A higher resistance (79%) and (100%) of *C. jejuni* to Erythromycin were reported by [5, 28] respectively.

The *Campylobacter coli* isolates in the study were highly susceptible to Gentamicin (100%) and Resistant to Erythromycin. Ciprofloxacin has some degree of activity (79%), followed by Azithromycin (58%) and Amoxicillin (37%) on *C. coli*. Nalixidic acid and Tetracycline have the least activity with a 32% and 21% respectively. This high susceptibility of *C. coli* to Gentamicin could be due to the fact that this antibiotic is not commonly used in animal production hence not subject to much abuse. This was in line with the findings of [19] who reported a 0% resistance of *C. coli* to Gentamicin. The study also agrees with [14] who reported a low resistance (18%) of *Campylobacter* species to gentamycin in Tanzania. The high resistance of *Campylobacter coli* to Erythromycin (100%) in this study agrees with [13] who reported a 100% resistance of *C. coli* to Erythromycin.

### 4.3. Resistance Profile of *Campylobacter* Species Isolated From Nunu

The occurrence of multiple antibiotic resistant strains among *Campylobacter* species is a problem in the treatment of Campylobacteriosis. In this study, *Campylobacter* species exhibited different antibiotic resistance patterns; *Campylobacter jejuni* isolates showed 12 while *C. coli* showed 9. Altogether, 39% of the positive *Campylobacter* isolates demonstrated multiple antibiotic resistances which were more pronounced among *Campylobacter coli* 47% than

*C. jejuni* 31%. This problem is further compounded by the circulation of substandard drugs, use of antibiotics in livestock production and misuse of antibiotic. The most important is the high resistance of *Campylobacter* species to Erythromycin, which has been used to treat human Campylobacteriosis.

The *Campylobacter* isolates in this study had a high MAR index of 0.88 (1.35%) with the susceptibility patterns showing resistance to seven antibiotics while the lowest MAR index of 0.38 with susceptibility patterns showing resistance to three antibiotics. In this study, all the isolates had a MAR index greater than 0.2. An isolate with MAR index less than 0.2 is believed to have originated from a low risk source of contamination where antimicrobial is not commonly used while an isolate with MAR index greater than 0.2 is known to have originated from high risk source of contamination such as animal faecal material [16].

## 5. Conclusion

The findings from this study showed a relatively high prevalence (16.1%) of *Campylobacter* species in locally pasteurized milk product (nunu) sold in Zaria metropolis. The results obtained showed that Imipenem, Azithromycin and Gentamicin should be recommended as first drugs of choice in the management of Campylobacteriosis. The increasing level of *Campylobacter* resistance in the treatment of Campylobacteriosis has great implication in the public health, and so, measures have to be set in place to curb this menace.

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## Conflict of Interest

The authors declared they had no conflict of interests regarding the publication of this paper.

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