# ANTIBIOGRAM OF BACTERIA ISOLATED IN RAW MILK SOLD IN MAKURDI METROPOLIS, BENUE STATE, NIGERIA.


## ABSTRACT

Antibiogram profile of bacterial isolates from unpasteurized milk sold in selected markets in Makurdi metropolis was investigated. A total of 300 samples of unpasteurized milk were examined, out of which 222 (74%) were positive for some bacterial isolates. *Escherichia coli* had the highest percentage of 32.4% (72) followed by *Staphylococcus aureaus* 18.92% (42). *Salmonella species* came third with an occurrence of 13.51% (30), while *Pseudomonas aeruganosa* 7.21% (16) *Bacillus species* 9.91% (22), *Proteus species* 7.21% (16) and *Streptococcus species* 9.01% (20) were isolated in that order. The antibiogram profile revealed that all of the isolated bacteria were highly sensitive to Chloramphenicol and Gentamycin (>18mm, 17mm respectively). Inference from the research showed that government should endeavor to assist the poor local milk producers in buying and getting their products into collection centers where proper equipment for pasteurization are provided before the products gets to the consumers considering dangers inherent in consumption of contaminated milk products. Milk should be pasteurized immediately after collection to reduce bacterial load especially the pathogenic ones. Further research is needed to make the strategies of eliminating the challenges related to unpasteurized milk.



**Keywords:** Bacteria, Antibiotics, Raw milk


## Introduction

Raw milk is milk that has not been pasteurized, a process of heating liquid food to decontaminate them for safe drinking (Beecher and Cookson, 2016), while proponents of raw milk have stated that there are benefits to its consumption, including better flavor, better nutrition, and the building of a healthy immune system, the medical community has warned of the dangers, which include a risk of infection (Smith *et al.*, 2012). The availability and regulation of raw or unpasteurized milk vary around the world. In US, some dairies have adopted low-temperature pasteurization, which they say produces a product similar to raw milk (Smith *et al.*, 2012).

**Comment [nm1]:** Use 'FROM' instead IN

**Comment [nm2]:** Makurdi metropolis, Benue state, Nigeria

**Comment [nm3]:** *Pseudomonas aeruginosa*

**Comment [nm4]:** Though total positive 222 samples, it shows 218 positive in sum calculation; as well as instead of 100%, shows 98.17%. I think as per result- table 3, the occurrence number and percentage of *Salmonella species* should be corrected.

**Comment [nm5]:** There is no category of highly sensitive. It will be only sensitive

**Comment [nm6]:** The diameter of zone of inhibition was not mentioned in the result or discussion section. So it should be addressed in percentage (90-100)%

RawMilk is well recognized as a high quality nutritional food elaborated by nature to foster young ones and to maintain balanced diet by adult. It contains more essential nutrients in significant amount compared to other single foods (Oliver *et al.,* 2005).

Man has consumed milk and milk products from time immemorial and it has been considered nutritionally, as "the most nearly perfect food". Milk is an outstanding source of calcium and phosphorus and contains vitamin A, B1, B6, and B12 in significant amount which help in bone and teeth development (Oliver *et al.,* 2005). Raw milk or processed milk is a well-known good medium that supports the growth of several microorganisms with resultant spoilage of the product or infections and intoxications in consumers (Oliver *et al.*, 2005).

The presence of contaminant microorganisms, especially pathogenic bacteria in milk is of serious public concern. Poor hygiene practices by handlers of raw milk do lead to introduction of pathogenic microorganisms into the milk, and since raw milk do not undergo further processing before consumption, they may pose health risk to the consumers of milk (Adeyemi and Umar, 1994). Pathogenic bacteria that have become resistant to antibiotic drug therapy have increased the problems of public health all over the world, and it is an ever-increasing global health threat (Levy, 2001). Generally, the presence of antibiotic resistant bacteria in human foods such as milk has been implicated in contributing to the increasing drug resistance which often leads to failures in chemotherapy.

This investigation is therefore aimed at determining the antibiotic resistance pattern of bacteria isolated from raw milk being sold in Makurdi metropolis, Benue state, Nigeria.

## MATERIALS AND METHODS.

### Study Area

The study was carried out in Makurdi town, the capital city of Benue State, Nigeria. Makurdi town is located at Latitudes 7° 47[1]and 10° 00[1] North and Longitudes 6° 25[1] and 8° 8[1] east of the equator. It is bounded by Guma Local Government Area to the North, Gwer East Local Government to the South, Gwer-West Local Government Area to the South-West and Doma Local Government Area of Nasarawa State to the North-West. It is situated in the Benue Valley on the bank of river Benue. The town is strategically located on the North-South transportation network by road and by rail respectively, between Nasarawa and Enugu States with the total land area of about 810 square kilometer (National Population Commission, 2009; Mngutyo, and Ogwuche, 2013; Olayinka *et al*., 2013).

**Study Population**

Local milk handlers (especially Fulani women) who sell cow milk in Makurdi were patronized and unpasteurized milk was collected from Makurdi metropolis. A minimum of two samples of raw cow milk was obtained from each milk seller.

**Sample Collection**

A total of 300 samples of raw milk were collected among milk handlers in some selected areas within Makurdi (North bank market, Wurukum market, Wadata market, High level Market, Kanshio market, and Fiidi market) in Makurdi metropolis. About 100ML of each raw milk sample bought was aseptically dispensed into sterile sample containers using a sample collector ice box at $4^0$C. Samples were well labeled for easy identification and were transported to the Research laboratory of Medical Microbiology and Parasitology at Benue State University Teaching Hospital without delay.

**Comment [nm7]:** was bought and

**Determination of Total Microbial Counts in Milk**

Standard plate count method was used to determine the Total Colony Counts of microorganisms in thesamples of raw milk: Raw milk (Sanders, 2012). Aserial dilution of the samples from each of the different locations was performed and plated out on Plate Agar using pour plate technique. The plates were incubated at 37oC for 24 h. The averagemicrobial loads of each of the raw milk from the different locations were obtained andexpressed as Colony Forming Units per milliliter [CFU/ml] (Harrigan and McCance, 1976).

Comment [nm8]: avoid repitition

**Isolation and identification of bacterial isolates**

Standard bacteriological methods were employed for the isolation of bacteria as recommended by Cheesbrough (2006). Serially diluted samples were also inoculated by pour plate technique in MacConkey Agar, Chocolate Agar, Eosin Methylene Blue (EMB) Agar, Mannitol Salt Agar (MSA) and Salmonella-Shigella Agar (SSA) in order to commence the preliminary process of identification of the isolates. McConkey Agar was used to isolate lactose fermenting gram negative bacteria, Chocolate agar was used to isolate fastidious bacteria, Eosin Methylene Blue was used for the selective isolation of enteric coliforms, Manitol Salt agar was for the selective

Isolation of salt-tolerant bacteria and Salmonella-Shigella agar was used for the isolation of enteric bacilli. All plates were incubated at 37oC for 24 h, and identification was based on cultural, morphological and biochemical characteristics as recommended by Holt (1994).

**Antibiogram Profile**

Antibiotic susceptibility testing was performed by the Kirby-Bauer disc diffusion method. Antibiotic susceptibility tests were performed on bacterialisolates to determine their antibiotic-resistance and susceptibility profiles (Kirby *et al*., 1966). Bacteriafrom the agar slants were

inoculated in tryptose soy broth and incubated at 37°C. Fresh overnight cultures were used for antibiotic sensitivity tests. An aliquot (0.5ML) from each isolate suspension was spread plated by sterilized swab on Mueller Hinton agar (Oxoid, England). Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar (Oxoid, England) to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18 to 24 hours (CLSI, 2019). Antimicrobial susceptibility test was conducted using 9 antibiotics. The antibiotics used were erythromycin (15 µg), penicillin G (10 IU), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), vancomycin (30 µg), tetracycline (30 µg), cephoxitin (30 µg) and ciprofloxacin (5 µg). The susceptibility of the bacterial isolates (Inhibition zone diameters) to each antibiotic agent were measured and the results were categorized as either susceptible, intermediate or resistant based upon interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI) to antimicrobials (CLSI, 2019).

## RESULTS

The isolates were confirmed using different biochemical test as shown in table 1. The occurrences of bacterial isolates in unpasteurized milk gotten from samples collected from different markets in Makurdi metropolis showed that, out of 300 samples screened, 222 (74%) were positive for bacterial isolates. Wadata MKT had the highest percentage of bacterial isolates 44 (88%), followed by North Bank MKT 42 (84%), High Level MKT 38 (76%), Wurukum MKT 36 (72%), Fiidi MKT 32 (64%), while Kanshio MKT had the least percentage 30 (60%) shown in table 2.

**Comment [nm9]:** In materials and method-
1. The total microbial load of each raw milk was counted but no result was written.

2. Microbial identification was based on cultural, morphological and biochemical characteristics. But here addressed only biochemical.

Table 3 shows different prevalence of various bacterial isolates in unpasteurized milk gotten from different markets in Makurdi metropolis. *Escharichia coli* in North bank MKT had highest percentage of (42.9%), followed by Wadata MKT (36.4%), while Fiidi MKT had the least prevalence of (25.0%). *Staphylococcus aureus* in North Bank MKT (23.8%) was highest in prevalence, followed by Wadata MKT (22.7%) while Fiidi MKT had the least prevalence of (12.5%) of bacterial isolates.*Salmonellaspecies* in Fiidi MKT was highest with prevalence of (25.0%), followed by Kanshio MKT (20.0%), while North bank MKT had the least prevalence of (9.5%). *Pseudomonas aeruginosa* had the highest prevalence of (11.11%) in Wurukum MKT, followed by (10.5%) in High Level MKT while Wadata MKT had the least prevalence of (4.5%).*Bacillus cereus* was found high in Kanshio MKT (13.33%), followed by Wurukum MKT (11.11%) while Fidi MKT had the least prevalence of (6.25%). *Proteus species* was found high in Fidi MKT (12.5%), followed by High Level MKT (10.5%) while Wadata MKT had the least prevalence percentage of 4.5%. *Streptococcus species* was found high in Fidi MKT (12.5%), followed by Wurukum MKT (11.11%) while North bank MKT had the least prevalence of (4.8%).

Out of 222 bacterial isolates, antibiotic susceptibility test were performed on 72(32.4%) isolates of *E.coli.Escherichia coli* isolates were found to be highly susceptible to Ciprofloxacin (100%), followed by Gentamycin (86.1%). However, resistance to Penicillin (96.1%), followed by Cephoxitin (58.33%) shown in table 4. *Staphylococcus aureus* were found to be highly susceptible to Ciprofloxacin (100%), followed by Gentamycin, erythromycin and vancomycin (83.33%). However, Resistance to Cephoxitin (88.09%), followed by Penicillin (71.4%) shown in table 5. *Salmonella Spp* isolates were found to be highly susceptible to Ciprofloxacin (100%), followed by Gentamycin (86.7%). However, resistance to Tetracycline(86.7%),followed by

Vancomycin (83.3%) as shown in table 6.*Pseudomonas aeruginosa* isolates were found to be highly susceptible to Ciprofloxacin (100%), followed by Chloramphenicol (87.5%). However, resistance to Vancomycin and Penicillin (100%), followed by followed by Cephoxitin (68.7%) shown in table 7. *Bacillus cereus* isolates were found to be highly susceptible to Ciprofloxacin, Trimethoprim-sulfamethoxazole and Gentamycin (90.91%), followed by Chloramphenicol (86.4%) while Erythromycin (81.82%) was the fourth antibiotics that are susceptible to *Bacillus Spp* isolates. However, resistance to Cephoxitin (90.91%), followed by Penicillin (86.4%) and Vancomycin (81.82%) merged the third antibiotics that were resistance to *Bacillus cereus* shown in table 8.*Proteus Spp* isolates were found to be highly susceptible to Ciprofloxacin (100%), followed by Chloramphenicol (87.5%) while Gentamycin and Trimethoprim-sulfamethoxazole (75.0%) came third. However, resistance to Vancomycin (75.0%), Cephoxitin (75.0%), Tetracycline (75.0%) and Penicillin (75.0%) shown in table 9.*Streptococcus Spp* isolates were found to be highly susceptible to Ciprofloxacin and Gentamycin, Chloramphenicol (90.0%) followed by Trimethoprim-sulfamethoxazole (80.0%) However, resistance to Penicillin, Cephoxitin and Vancomycin (90.0%), followed by Tetracycline (85.0%) shown in table 9

**Table 1: Biochemical tests for the identifications of isolates obtained from Unpasteurized Milk in Makurdi Metropolis.**

| Biochemical Tests | Isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Escherichia Coli* | *Staphylococcus aureus* | *Salmonella typhi* | *Pseudomunas aeruginosa* | *Bacillus cereus* | *Proteus spp* | *Streptococcus pyogenes* |
| Catalase test | + | + | + | + | + | + | — |
| Coagulasetest | _ | + | _ | — | _ | _ | _ |
| Citrate utilization test | — | + | — | + | + | + | _ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MR test | + | + | + | — | — | + | — |
| VP test | — | + | — | — | + | — | |
| Indole test | + | — | — | — | — | — | _ |
| Oxidase test | — | — | — | + | — | — | _ |
| TSI A test Glu | + | + | + | — | + | + | + |
| Lactose | + | + | — | — | — | — | + |
| Urease test | — | + | — | — | _ | + | — |

Key: — Negative, + Positive

**Table 2: Occurrence Frequency of Bacterial Isolates from Unpasteurized Milk purchased from Selected Markets in Makurdi metropolis in percentage (%).**

| Markets | Sample size | No. of sample positive for bacteria isolation in unpasteurized milk |
|---|---|---|
| North Bank MKT | 50 | 42 (84%) |
| High Level MKT | 50 | 38 (76%) |
| Wadata MKT | 50 | 44 (88%) |
| Kanshio MKT | 50 | 30 (60%) |
| Fidi MKT | 50 | 32 (64%) |
| Wurukum MKT | 50 | 36 (72%) |

Key: MKT- Market

**Table 3: Prevalence of different bacterial isolated from unpasteurized Milk samples with respect to Markets in Makurdi metropolis in percentage.**

| | | | | Bacterial | Pathogens | | | |
|---|---|---|---|---|---|---|---|---|
| Markets | No. of isolates | *E.coli* | *S. aureus* | *Salm. spp* | *p. aeruginosa* | *Bacillus cereus* | *Proteus spp* | *Srept. spp* |
| NBMKT | 42 | 18(42.9%) | 10(23.8%) | 4(9.5%) | 2(4.8%) | 4(9.5%) | 2(4.8%) | 2(4.8%) |
| HLMK | 38 | 10(26.3 | 8(21.1%) | 4(10.5 | 4(10.5%) | 4(10.5%) | 4(10.5 | 4(10.5%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| T | | %) | | %) | | | %) | |
| WAMKT | 44 | 16(36.4 %) | 10(22.7 %) | 6(13.6 %) | 2(4.5%) | 4(9.1%) | 2(4.5%) | 4(9.1%) |
| KMKT | 30 | 10(33.3 %) | 4(13.3%) | 6(20.0 %) | 2(6.7%) | 4(13.33 %) | 2(6.7%) | 2(6.7%) |
| FMKT | 32 | 8(25.0%) | 4(12.5%) | 8(25.0 %) | 2(6.25%) | 2(6.25%) | 4(12.5 %) | 4(12.5%) |
| WUMKT | 36 | 10(27.8 %) | 6(16.7%) | 6(16.7 %) | 4(11.11%) | 4(11.11 %) | 2(5.6%) | 4(11.11 %) |

Key: NBMKT- North bank Market, HLMKT- High Level Market, WAMKT- Wadata Market, KMKT – Kanshio Market, FMKT- Fidi Market, WUMKT- Wurukum Market and *spp*- species.

206 **Table 4: Antibiotic Sensitivity Pattern of *Escherichia coli* (n=72) from Unpasteurized Milk**
207 **obtained from Selected Markets in Makurdi metropolis in percentage (%).**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 60(83.33%) | 6(8.33%) | 6(8.33%) |
| Penicillin | 10(13.9%) | - | 62(86.1%) |
| Gentamycin | 62(86.1%) | - | 10(13.9%) |
| Trimethoprim-sulfamethoxazole | 55(76.4%) | 5(6.9%) | 12(16.7%) |
| Chloramphenicol | 50(69.44%) | 10(13.9%) | 12(16.7%) |
| Vancomycin | 60(83.33%) | - | 12(16.7%) |
| Tetracycline | 55(76.4%) | 2(2.8%) | 15(20.8%) |
| Cephoxitin | 30(41.7%) | - | 42(58.33%) |
| Ciprofloxacin | 72(100%) | 0(0%) | 0(0%) |

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**Table 5: Antibiotic Sensitivity pattern of *Staphylococcus aureus* (n=42) from Unpasteurized Milk obtained from Selected Markets in Makurdi metropolis in percentage (%).**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 35(83.33%) | 2(4.8%) | 5(11.9%) |
| Penicillin | 6(14.3%) | 6(14.3%) | 30(71.4%) |
| Gentamycin | 35(83.33%) | - | 7(16.7%) |
| Trimethoprim-sulfamethoxazole | 27(6436%) | 5(11.9%) | 10(23.8%) |
| Chloramphenicol | 30(71.4%) | 4(9.5%) | 8(19.05%) |
| Vancomycin | 35(83.33%) | - | 7(16.7%) |
| Tetracycline | 30(71.4%) | (7.14%) | 9(21.43%) |
| Cephoxitin | 5(11.91%) | - | 37(88.09%) |
| Ciprofloxacin | 42(100%) | 0(0%) | 0(0%) |

**Comment [nm10]:** 64.36%

**Comment [nm11]:** 3(7.14%)

222 **Table 6: Antibiotic Sensitivity Pattern *of Salmonella Spp*(n=30) from Unpasteurized Milk**

223 **obtained from Selected Markets in Makurdi metropolis.**

Comment [nm12]: From Table 3. It was calculated 34. But why 30 in here?

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 20(66.9%) | 2(6.7%) | 8(26.7%) |
| Penicillin | 7(23.33%) | - | 23(76.7%) |
| Gentamycin | 26(86.7%) | - | 4(13.3%) |
| Trimethoprim-sulfamethoxazole | 20(66.7%) | 5(16.7%) | 5(16.7%) |
| Chloramphenicol | 25(83.3%) | - | 5(16.7%) |
| Vancomycin | 5(16.7%) | - | 25(83.3%) |
| Tetracycline | 4(13.3%) | - | 26(86.7%) |
| Cephoxitin | 6(20.0%) | 3(10.0%) | 21(70.0%) |
| Ciprofloxacin | 30(100%) | 0(0%) | 0(0%) |

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**Table 7: Antibiotic Sensitivity Pattern of *Pseudomonas aeruginosa* (n=16) from Unpasteurized Milk obtained from Selected Markets in Makurdi metropolis in percentage (%).**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 12(75.0%) | 4(25.0%) | 0(0%) |
| Penicillin | 0(0%) | - | 16(100%) |
| Gentamycin | 10(62.5%) | - | 6(37.5%) |
| Trimethoprim-sulfamethoxazole | 7(43.75%) | 4(25.0%) | 5(31.25%) |
| Chloramphenicol | 14(87.5%) | - | 2(12.5%) |
| Vancomycin | 0(0%) | - | 16(100%) |
| Tetracycline | 7(43.75%) | 4(25.0%) | 5(23.25%) |
| Cephoxitin | 5(31.25%) | - | 11(68.75%) |
| Ciprofloxacin | 16(100%) | 0(0%) | 0(0%) |

**Comment [nm13]:** It will be 31.25%

239 **Table 8: Antibiotic Sensitivity Pattern *Bacillus cereus* (n=22) from Unpasteurized Milk**

240 **obtained from Selected Markets in Makurdi metropolis.**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 18(81.82%) | 2(9.09%) | 2(9.09%) |
| Penicillin | 3(13.6%) | - | 19(86.4%) |
| Gentamycin | 20(90.90%) | - | 2(9.09%) |
| Trimethoprim-sulfamethoxazole | 20(90.90%) | 1(4.5%) | 1(4.5%) |
| Chloramphenicol | 19(86.4%) | - | 3(13.6%) |
| Vancomycin | 2(9.09%) | 2(9.09%) | 18(81.82%) |
| Tetracycline | 4(18.18%) | 1(4.5%) | 17(77.3%) |
| Cephoxitin | 2(9.09%) | - | 20(90.91%) |
| Ciprofloxacin | 20(90.91%) | - | 2(9.09%) |

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**Table 9: Antibiotic Sensitivity Pattern *of Proteus Spp* (n=16) from Unpasteurized Milk obtained from Selected Markets Makurdi metropolis.**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 11(68.75%) | 2(12.5%) | 3(18.75%) |
| Penicillin | 4(25.0%) | - | 12(75.0%) |
| Gentamycin | 12(75.0%) | - | 4(25.0%) |
| Trimethoprim-sulfamethoxazole | 12(75.0%) | 2(12.5%) | 2(12.5%) |
| Chloramphenicol | 14(87.5%) | - | 2(12.5%) |
| Vancomycin | 4(25.0%) | - | 12(75.0%) |
| Tetracycline | 2(12.5%) | 2(12.5%) | 12(75.0%) |
| Cephoxitin | 4(25.0%) | - | 12(75.0%) |
| Ciprofloxacin | 16(100%) | 0(0%) | 0(0%) |

**Table 10: Antibiotic Sensitivity Pattern *of Streptococcus Spp* (n=20) from Unpasteurized Milk obtained from Selected Markets in Makurdi metropolis.**

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Erythromycin | 14(70.0%) | 4(20.0%) | 2(10.0%) |
| Penicillin | 2(10.0%) | - | 18(90.0%) |
| Gentamycin | 18(90.0%) | - | 2(10.0%) |
| Trimethoprim-sulfamethoxazole | 16(80.0%) | 2(10.0%) | 2(10.0%) |
| Chloramphenicol | 18(90.0%) | - | 2(10.0%) |
| Vancomycin | 2(10.0%) | - | 18(90.0%) |
| Tetracycline | 2(10.0%) | 1(5.0%) | 17(85.0%) |
| Cephoxitin | 2(10.0%) | - | 18(90.0%) |
| Ciprofloxacin | 18(90.0%) | - | 2(10.0%) |

## DISCUSION

Pathogenic bacteria have been a major concern to the public all over the world. The fact that milk contains a lot of nutrients has made it possible for growth and development of most microorganisms including the pathogenic ones (Saeed *et al.,* 2009). Pareke and Subhash (2008), asserted that animal health, milking utensil and the environment are contributors to contamination of fresh raw milk.

The results obtained from this study indicated that the samples were heavily contaminated by bacteria. This agreed with Syed *et al*. (2014) who recorded similar high results in unpasteurized milk in Beed City. The results of unpasteurized milk is consistent with that reported in Keffi by Makwin*et al.* (2014) and contrary to the one reported by Laba and Udonsek (2013) in Ilorin.

In this study, the incidence of bacterial isolates obtained from different Markets in Makurdi metropolis, 50 samples were collected in markets namely, North Bank market, High Level market, Wadata market, Kanshio market, Fiidi market and Wurukum market, and the percentage of positive bacterial isolates were 42 (84%), 38 (76%). 44 (88%), 30 (60%), 32 (64%) and 36 (72%) respectively.

**Comment [nm14]:** Each market

The results reported in this study are likewise high when compared to those documented, by Alian*et al.* (2012), Shitanidi and Sternesjo (2004), Gundogen*et al.* (2006) and that improper hygiene and poor farm management practices contributed to the presence of high microbial growth in the milk, consistent with that reported by Jyoti *et al*. (2014) who reported the microbial content of unpasteurized milk from different dairy shops of Allahabad city.

**Comment [nm15]:** hygienic

This study revealed the prevalence of bacterial pathogen in raw milk within Makurdi city. *Echerichia coli* in North bank market had the highest prevalence of (42.9%), followed by Wadatamarket (36.4%) while Fiidi market had the least (25.0%) as the most prevalent bacteria similar to the finding of Jyoti *et al.* (2014) and Kewler*et al*. (1992) and contrary to that of Makwin*et al*. (2014) in Keffi and higher than that of Olatunji *et al.* (2013). The isolation of *Staphylococcus aureus* was higher in North bank market (23.8%), followed by wadata market (22.7%) and Fiidi market had the least (12.5%) in this study which agrees with that of Makwin*et al*. (2014), Olatunji *et al.* (2013), Jyoti *et al*. (2014) and Anklo and Sterneojo (2006) who isolated 30%, 26.7%, 33.33%, 20.4% and 15% respectively from unpasteurized milk. The implication of the presence of *Salmonella species* is that it causes tyhoid, which is a serious health problem to the consumers. The prevalence of *Salmonella species* (25.0%) in Fiidi market was closely followed in Kanshio market (20.0%) and North bank market had the least (9.5%) which is in agreement with the findings of Jyoti *et al.* (2014) and Makwin*et al*. (2014). The lower percentages were obtained among *P. aerugunosa* (11.11%), *Bacillus spp* (13.33%), *Proteus spp* (12.50%) and *Streptococcus spp* (12.5%) which agreed with Olatunji *et al.* (2013), Donkor *et al.* (2007), Laba and Udonsek (2013) and Makwin*et al*. (2014).

**Comment [nm16]:** According to result section-table 3. These percentages are higher for these individual organism. So do correction of it.

A total of 9 antibiotics were used to determine Antibiotics susceptibility pattern of the isolates from Unpasteurized Milk from selected Markets. Nine (9) antibiotics were tested against 72 isolates of *E. coli*, 42 isolates of *S. aureus*, 30 isolates of *Salmonella species*, 16 isolates of *P. aerugunosa*, 22 isolates of *Bacillus species*, 16 isolates of *Proteus species*, and 20 isolates of *Streptococcus species*.

The study shows high susceptibility percentages of antibiotic to the 72 isolates of *Echerichia coli* were found to be highly susceptible to Ciprofloxacin (100%), followed by Gentamycin (86.1%), Vancomycin and Erythromycin (83.33%), Tetracycline and Trimethoprim-sulfamethoxazole (76.4%). However, they were resistances to Penicillin (86.1%) and Cephoxitin (58.33%). The highest resistance observed against penicillin in this study is similar to that of Sileshi and Munees (2016) in Ethiopia, Martha *et al.* (2016) in Tanzania. These results are also similar to the findings by Idriss*et al*. (2014) in Slovakia, Belaynehe*t al*. (2014) in Ethiopia. The high sensitivity found in case of Ciprofloxacin, Gentamycin, Erythromycin and Trimethoprim-sulfamethoxazile is contrary to the findings by Mukta and Manir (2016), Islam *et al*. (2010) and similar to the findings of Sileshi and Munees (2016) and Martha *et al.*, (2016).

*Staphylococcus aureus* isolates were found to be susceptible to Ciprofloxacin (100%), Gentamycin, Vancomycin and Erythromycin (83.33%), Chloramphenicol and Tetracycline (71.4%). However, they showed resistance to Cephoxitin (88.09%) and Penicillin (71.4%). The high resistance to Cephoxitin and Penicillin in this study agreed with the findings of Thaker*et al*. (2013) in India, where *Staphylococcus aureus* isolates (100%) was resistant to Penicillin-G. The sensitivity pattern of the bacterial species isolated to the antibiotic tested is comparable to the report of earlier workers (Inyang (2009), Udo *et al.* (2001), Tagoe*et al.* (2011), and Mukut*et al.* (2013)) in Nigeria.

*Salmonella Species* isolates were found to be susceptible to Ciprofloxacin (100%), Gentamycin (86.7%), Chloramphenicol (83.3%), and Erythromycin (66.9%) and Trimthoprim-sulfamethoxazole (66.7%). However, there was resistance observed with Tetracycline (86.7%), Vancomycin (83.3%), Penicillin (76.7%) and Cephoxitin (70.0%). The high resistances of these

antibiotics are similar to the study conducted by Makwin*et al*. (2014) in Keffi town and the susceptibility of some of these antibiotics to *Salmonella* Species is similar to the work conducted by Sileshi and Munees (2016), Martha *et al*. (2016) and Mukta and Manir (2016).

*Pseudomonas aerugunosa* was highly susceptible to Ciprofloxacin (100%), Chloramphenicol (87.5%), Erythromycin (75.0%), and Gentamycin (62.5%). However, there was high resistance in Vancomycin and Penicillin (100%) and Cephoxitin (68.75%), while *Bacillus species* was highly susceptible to Ciprofloxacin and Gentamycin (90.90%), Chloramphenicol (86.4%) and Erythromycin (81.82%) and Trimethoprim-sulfamethoxazole (90.90%). However, there was also some high resistance of *Bacillus species* isolates to Cephoxitin (90.91%), Vancomycin (81.82%), Penicillin (86.4%) and Tetracycline (77.3%). *Proteus Species* were highly susceptible to Ciprofloxacin (100%), Chloramphenicol (87.5%), Gentamycin (75.0%), Erythromycin (68.75%) and Trimethoprim-sulfamethoxazole (75.0%). However, the *Proteus Species* isolates were highly resistant to Vancomycin, Penicillin, Cephoxitin and Tetracycline (75.0%). *Streptococcus species* were highly susceptible to Ciprofloxacin, Gentamycin, and Chloramphenicol (90.0%), Trimethoprim-sulfamethoxazole (80.0%) and Erythromycin (70.0%). However, some of the *Streptococcus species* isolates were highly resistant to Penicillin, Cephoxitin and Vancomycin (90.0%) and Tetracycline (85.0%). The sensitivity pattern of these bacterial isolates isolated is comparable to the reports of earlier researchers Inyang (2009), Udo *et al*. (2001), Tagoe*et al.* (2011), Makut*et al.* (2013), Makwin*et al.* (2014), Muktar and Manir (2016), Sileshi and Munees (2016) and Martha *et al.* (2016). All the isolates were susceptible to Ciprofloxacin and Gentamycin and resistance to Penicillin and Cephoxitin which is in agreement with the findings by Idress*et al*. (2014), Belayneh*et al.* (2018) and Martha *et al.* (2016).

The prevalence of resistance of some strain of *Echerichia coli, S. aureus, Salmonella species, P. aerugunosa, Bacillus species, Proteus species* and *Streptococcus species* in unpasteurized milk is the reflection of the use and misuse of antibiotic in the society. This is not surprising because there is indiscriminate use of antibiotics by the Nigeria public of which Makurdi is a part. The public health implication of this investigation is that antimicrobial resistance strains of pathogenic bacteria may colonize humans through consumption of contaminated unpasteurized milk obtained in selected markets in Makurdi metropolis and this would lead to failure of chemotherapy among human consumers of unpasteurized milk.

## CONCLUSION

Higher incidences of different pathogenic microorganisms were observed in unpasteurized milk sold in different markets in Makurdi metropolis. Bacterial isolates observed in this study are suspected to contaminate the samples from various sources, which could be due to poor handling and storage of milk collection. The environment, utensils used the state of hygiene of the animal from which the milk was collected and the sanitary conditions of the milk collectors are all possible source of contamination. The research has established that Ciprofloxacin antibiotic was more highly susceptible to the isolates with 100% efficacy, what hitherto was not known. Vancomycin and Penicillin showed total resistance.

**Comment [nm17]:** (90-100)%

The present study also gives us a redirection to sensitivity profile of *Echerichia coli, Staphylococcus aureus, Salmonella species, Pseudomonas aerugunosa, Bacillus species, Proteus species and Streptococcus species,* isolated from unpasteurized milk samples against nine (9) commonly used antibiotics in Makurdi and also depict the multi-drugs resistance of some bacterial isolates in unpasteurized milk samples which ultimately gives a pointer to the

indiscriminate use of antibiotics in Makurdi. It is a great matter of concern for human health also because a good percentage of the people consume this milk. Moreover, these multi-drugs resistant bacteria may no longer be treated with conventional therapeutic drugs, while they are also capable of spreading their resistant gene to other bacterial genera.

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