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Antibiogram of Bacteria Isolated in Raw Milk Sold in Makurdi Metropolis, Benue State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The antibiogram profile of bacteria isolated from raw milk source in Makurdi, Benue State. Samples were collected in six different areas where raw milk are sold and were analyzed. The most frequently identified bacterium was *Escherichia coli*, which constituted 72(32.4%) followed by

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Staphylococcus aureus 42(18.92%), *Salmonella species* were the third most common, representing 30(13.51%), *Pseudomonas aeruginosa* and *Proteus species* both had 16(7.21%), *Bacillus species* 20(9.91%), and *Streptococcus species* 20(9.01%). The antibiogram results revealed that all isolated bacteria showed significant sensitivity to Chloramphenicol and Gentamicin. These findings highlight the need for government support for local milk producers to obtain resources and establish collection centers with adequate pasteurization facilities, thereby reducing the risks linked to the consumption of contaminated milk products. It is crucial that milk is pasteurized immediately after collection to lower bacterial loads, especially pathogenic strains. Further research is essential to devise effective strategies to tackle the issues Arelated to unpasteurized milk.

Keywords: Bacteria; antibiotics; raw milk.

1. INTRODUCTION

Raw milk is a locally produced dairy product that is commonly consumed in various African nations, including Nigeria, and it has been noted for its significant nutritional benefits (Beecher and Cookson, 2016). Proponents of raw milk contend that it provides benefits such as superior flavor, enhanced nutritional content, and bolstered immune support. However, the medical community has expressed concerns about its safety, particularly regarding the potential for infections (Smith et al*.,* 2012). The regulation and availability of raw or unpasteurized milk vary widely across different regions worldwide. In the United States, certain dairy producers have adopted low-temperature pasteurization methods, asserting that these techniques produce a product similar to raw milk (Smith et al*.,* 2012).

Raw milk is recognized as a highly nutritious food, specifically designed to support the growth of young individuals and to enhance the dietary balance of adults. It boasts a higher concentration of essential nutrients compared to many other individual food sources (Oliver et al*.,* 2005). Throughout history, milk and dairy products have been celebrated as "the most nearly perfect food" due to their impressive nutritional composition. Milk is an excellent source of calcium and phosphorus and is abundant in vitamins A, B1, B6, and B12, all of which are crucial for the development of bones and teeth (Oliver et al*.,* 2005). Nevertheless, both raw and processed milk can create a favorable environment for the growth of various microorganisms, which may result in spoilage or pose risks of infections and intoxications for consumers (Oliver et al*.,* 2005).

The existence of contaminating microorganisms, especially pathogenic bacteria, in milk presents serious public health issues. Poor hygiene practices among individuals handling raw milk can lead to the introduction of dangerous pathogens into the product. Given that raw milk is not processed further before it is consumed, it can pose health risks to consumers (Adeyemi and Umar, 1994). The rise of antibiotic-resistant pathogenic bacteria intensifies public health challenges globally, constituting a growing threat to health on an international scale (Levy, 2001).

2. MATERIALS AND METHODS

2.1 Study Area

Makurdi is the capital city of Benue State, Nigeria where this research was carried out. This city is situated between latitudes 7° 47' and 10° 00' North, and longitudes 6° 25' and 8° 81' East of the equator. It is bordered to the north by Guma Local Government Area, to the south by Gwer East Local Government, to the southwest by Gwer-West Local Government Area, and to the northwest by Doma Local Government Area in Nasarawa State. Makurdi lies within the Benue Valley along the banks of the Benue River. The town is a vital hub on the North-South transportation corridor, with access via both road and rail, connecting Nasarawa and Enugu States, and encompasses a total land area of approximately 810 square kilometers (National Population Commission, 2009; Mngutyo and Ogwuche, 2013; Olayinka et al*.,* 2013**).**

2.2 Sample Collection

Samples were collected in six different areas where raw cow milk are sold in Makurdi. The samples were collected from the following markets; North bank, Wuruku, wadata, High level, Fiidi and Kanshio. Approximately 100 mL of each sample was aseptically placed into sterile containers, utilizing a sample collector ice box kept at 4°C. Each sample was distinctly labeled for identification purposes and was swiftly transported to the Laboratory.

2.3 Total Microbial Counts in Raw Milk

Total microbial counts in raw milk samples were assessed through the standard plate count method (Sanders, 2012). A series of dilutions was conducted on samples collected from different locations, and the diluted samples were then plated on Plate Agar utilizing the pour plate technique. The plates were incubated at 37°C for a duration of 24 hours to evaluate the average microbial loads present in each sample.

2.4 Isolation and Identification of Bacteria Isolates

Standard bacteriological techniques were utilized for bacteria isolation, following the guidelines set forth by Cheesbrough (2006). Samples were serially diluted and subsequently inoculated using the pour plate method on agars to initiate the preliminary identification of the isolates. MacConkey Agar facilitated the isolation of lactose-fermenting gram-negative bacteria, while Chocolate Agar was employed for the cultivation of fastidious organisms. Eosin Methylene Blue Agar was specifically used for the selective isolation of enteric coliforms, Mannitol Salt Agar targeted salt-tolerant bacteria, and Salmonella-Shigella Agar was designated for the isolation of enteric bacilli. All plates were incubated at 37°C for 24 hours, with identification based on cultural, morphological, and biochemical characteristics as outlined by Holt (1994).

2.5 Antibiotic Sensitivity Assessment

Bacteria isolated were subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method. This testing aimed to assess the antibiotic resistance and susceptibility profiles of bacteria isolates (Kirby et al*.,* 1966). Bacteria were taken from agar slants and inoculated into tryptose soy broth, followed by incubation at 37°C. Fresh overnight cultures were utilized for the antibiotic sensitivity assessments. An aliquot of 0.5 mL from each isolate suspension was evenly spread on Mueller Hinton agar (Oxoid, England) using a sterilized swab. Antibiotic discs were then carefully placed on the inoculated agar to ensure proper contact with the surface, and the plates were incubated aerobically at 37°C for a duration of 18 to 24 hours (CLSI, 2019). The antimicrobial susceptibility test involved nine different antibiotics: erythromycin (15 µg), penicillin (10µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), vancomycin (30 µg), tetracycline (30 µg), cefoxitin (30 µg), and ciprofloxacin (5 µg). The susceptibility of the bacterial isolates was determined by measuring the inhibition zone diameters for each antibiotic, with results classified as susceptible, intermediate, or resistant according to the interpretive criteria established by the Clinical and Laboratory Standards Institute (CLSI, 2020).

2.6 Statistical Analysis

The data collected were examined utilizing the Statistical Package for Social Sciences (SPSS) version 20.0 software (SPSS, 2012). The analysis included the calculation of averages, proportions (percentages), two-way analysis of variance, and the Least Significant Difference test to differentiate between means.

3. RESULTS

Antibiotic susceptibility testing was performed on all bacteria isolates. *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 1. *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 2. *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 3. *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 4. *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 5. *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 6. *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%) as shown in Table 7.

Table 1. Antibiotic sensitivity assessment of *Escherichia coli* (n=72)

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%)

Table 2. Antibiotic Sensitivity Assessment of *Staphylococcus aureus* (n=42)

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%)

Table 3. Antibiotic Sensitivity Assessment *of Salmonella Spp* (n=30)

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%)

Table 4. Antibiotic sensitivity assessment of *Pseudomonas aeruginosa* (n=16)

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%)

Table 5. Antibiotic Sensitivity Assessment of *Bacillus cereus* (n=22)

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%)

Table 6. Antibiotic sensitivity assessment *of Proteus Spp* (n=16)

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 7. Antibiotic sensitivity assessment *of Streptococcus Spp* (n=20)

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%)

4. DISCUSSION

Pathogenic bacteria represent a considerable risk to global public health. The nutrient-dense nature of milk supports the growth of a variety of microorganisms, including harmful strains (Saeed et al*.,* 2009). Pareke and Subhash (2008) indicate that factors such as the health of the animals, the cleanliness of milking equipment, and environmental conditions play a role in the contamination of fresh raw milk.

This research identified 72 isolates of *Escherichia coli* that displayed notable susceptibility to various antibiotics, showing complete sensitivity to Ciprofloxacin (100%), followed by Gentamycin at 86.1%, and both Vancomycin and Erythromycin at 83.33%. Tetracycline and Trimethoprim-sulfamethoxazole had a susceptibility rate of 76.4%. In contrast, resistance was observed against Penicillin (86.1%) and Cephoxitin (58.33%). The significant resistance to penicillin aligns with findings from Sileshi and Munees (2016) in Ethiopia and Martha et al*.* (2016) in Tanzania, as well as observations by Idriss et al. (2014) in Slovakia and Belayneh et al. (2014) in Ethiopia. The high sensitivity to Ciprofloxacin, Gentamycin, Erythromycin, and Trimethoprim-sulfamethoxazole contrasts with the results reported by Mukta and Manir (2016) and Islam et al*.* (2010), while being consistent with the findings of Sileshi and Munees (2016) and Martha et al. (2016).

Isolates of *Staphylococcus aureus* demonstrated complete susceptibility to Ciprofloxacin (100%) and significant sensitivity to Gentamycin, Vancomycin, and Erythromycin (83.33%), as well as Chloramphenicol and Tetracycline (71.4%). However, these isolates showed resistance to Cephoxitin (88.09%) and Penicillin (71.4%). The high levels of resistance to Cephoxitin and Penicillin in this study support the findings of Thaker et al. (2013) in India, where *Staphylococcus aureus* isolates exhibited complete resistance to Penicillin-G.

Antibiotic sensitivity assessment of *Salmonella Species* 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%).

Antibiotic sensitivity assessment of *Bacillus species* were 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 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%).

Antibiotic sensitivity assessment of *Streptococcus species* clearly indicate 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 bacteria 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). The bacteria isolates were susceptible to Ciprofloxacin and Gentamycin and resistance to Penicillin and Cephoxitin. This is in agreement with the findings by Idress et al. (2014), Belayneh et al. (2018) and Martha et al. (2016).

The occurrence of antibiotic-resistant strains of *Echerichia coli, S. aureus*, *Salmonella species, P. aeruginosa, Bacillus species*, *Proteus species*, and *Streptococcus species* in unpasteurized milk highlights the consequences of both the appropriate and inappropriate use of antibiotics within society. This situation is particularly concerning given the widespread and indiscriminate use of antibiotics among the Nigerian population, including those in Makurdi. The implications of this research to public health suggest that antimicrobial-resistant strains of pathogenic bacteria could potentially infect humans through the consumption of contaminated unpasteurized milk sourced from selected markets in the Makurdi area, which may ultimately result in treatment failures for individuals consuming this milk.

5. CONCLUSION

In the Makurdi metropolis, unpasteurized milk sold in various markets exhibited higher levels of pathogenic microorganisms. The bacteria isolates identified in this research are believed to have contaminated the milk samples from multiple sources, likely due to inadequate handling and storage practices during milk collection. Factors contributing to this contamination include the environment, the cleanliness of utensils, the hygiene of the animals providing the milk, and the sanitary conditions maintained by the milk collectors. The research findings indicate that Ciprofloxacin demonstrated a remarkable susceptibility to the isolates, achieving 100% efficacy, a fact that was previously unknown. In contrast, Vancomycin and Penicillin exhibited complete resistance. This study also provides insights into the sensitivity profiles of *Staphylococcus aureus, Salmonella* species, *Pseudomonas aeruginosa Echerichia coli, Bacillus* species, *Proteus* species, and

Streptococcus species isolated from unpasteurized milk samples, tested against nine commonly used antibiotics in Makurdi. Additionally, it highlights the issue of multi-drug resistance among certain bacterial isolates in these milk samples, which raises concerns about the indiscriminate use of antibiotics in the region. This situation poses significant risks to human health, as a considerable portion of the population consumes this milk. Furthermore, these multi-drug resistant bacteria may become untreatable with standard therapeutic drugs and have the potential to transfer their resistance genes to other bacterial species**.**

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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