

Original Research Article

Antimicrobial Susceptibility Profile of Coliforms from Bovine Mastitis Cases among Pastoral Herds in Parts of Kaduna State, Nigeria: Curbing the Environmental Health Risk

ABSTRACT

Consumption of raw milk from pastoral bovines have been identified as a major source of public and **e**Environmental **h**Health risk in developing countries. Antimicrobial resistance is a global health challenge threatening the lives of humans and animals. The indiscriminate use and abuse of antimicrobial agents among the pastoralists on commercial animals, especially for non-therapeutic purposes has been linked by various researchers to the development of resistant strains of potentially pathogenic bacteria which are being transferred from animals to humans. In this study, the antimicrobial susceptibility profile of coliform bacteria isolated from mastitis milk of pastoral herds was investigated. Out of 147 milk samples collected and screened for subclinical mastitis, 29 (19.7%) were positive. Out of the 29 mastitis positive samples, 13 (8.8%) were positive for coliforms (6 *E. coli* and 7 *K. pneumoniae*). All the coliform isolates in this study showed 100% resistance to Penicillin and Tetracycline, and were all 100% susceptible to Imipenem. -High multidrug resistance was expressed by all the isolates to Penicillin, Tetracycline and Erythromycin. All the isolates (100%) had MARI of 0.2 and above which is an indication of gross abuse of antibiotics within the studied population. However, antibiotics still effective against the coliform species tested were Imipenem (100%), Ciprofloxacin (92.3%), Gentamycin (92.3%), Chloramphenicol (84.6%), Amoxicillin/Clavulanic acid (84.6%) and Sulfamethoxazole/Trimethoprim **e** (69.2%).

Keywords: Coliforms, Antimicrobial, Susceptibility, Mastitis, Bovines, Pastoral herds

1. INTRODUCTION

Mastitis is the inflammation of mammary gland and is a complex and costly disease in dairy herds [1, 2]. It is characterized by physical, chemical and bacteriological changes in the milk, and pathological changes in the glandular tissue of the udder [3, 4]. The occurrence of disease is an outcome of interplay between three factors: infectious agents, host resistance, and environmental factors [5].

There is agreement among authors that mastitis is the most widespread infectious diseases in dairy cattle, and, from an economic aspect, the most damaging [6, 7, 8, 9, 10, 11]. It is a global problem as it adversely affects animal health, quality of milk and the economics of milk production. It affects every country, including developed ones and causes huge financial losses [12].

The two major forms of the disease are the clinical and subclinical mastitis [13]. Clinical mastitis results in alteration of milk composition and appearance, decrease milk production, and the presence of the cardinal signs of inflammation (pain, swelling and redness, with or without heat in infected mammary quarters). It is readily apparent and easily detected [13]. In

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-Check majuscule or minuscule typing

Comment [MH2]: Coliforms are naturally resistant to Penicillin. This has to be mentioned

30 the subclinical form of mastitis, detection of mammary quarters with the disease is more
31 difficult because signs are not readily apparent [13].

32 Furthermore, Somatic Cell Count (SCC) has been accepted as the best index to use to
33 predict udder infection in bovines, and has been used extensively as an indicator since the
34 1960s [13, 14]. Under field conditions, determination of somatic cell count in milk is usually
35 done using the California Mastitis Test (CMT); In fact, CMT scores are directly related to
36 average SCC [14]. CMT has the advantage of being inexpensive and is a test with real-time
37 result for selection of the quarters for subsequent bacteriological examination [13].

38 Indiscriminate use and continuous abuse of antibiotics among the pastoralists for both
39 therapeutic treatment of infections) and non-therapeutic (growth promoters) purposes on
40 dairy animals has resulted to the increasing emergence of resistant strains of pathogenic
41 bacteria, which is a great threat to human and animal health [11]. Hence, this study was
42 embarked on to investigate the antimicrobial susceptibility profile of coliform isolates from
43 mastitis milk samples of dairy cows among the pastoral herds in parts of Kaduna State,
44 Nigeria.

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46 2. MATERIAL AND METHODS

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48 2.1 Study Area

49 The study was carried out in Giwa, Igabi, Chikun, Soba, Zaria, Sabongari and Birnin Gwari
50 Local Government Areas (LGA) of Kaduna State, Nigeria (Fig. 1). These are seven out of
51 the 23 LGAs in the state. The state lies between latitude 9.00° and 11.52° North and
52 longitude 6.08° and 8.83° East and is 608m above sea level. The number of LGAs studied
53 was limited by the serious security challenge in the Northern part of Nigeria. The study area
54 has distinct wet and dry seasons within the Guinea Savannah and part of the Sudan
55 Savannah in Nigeria. Agriculture is the main ~~stay-occupation-of-the-economy~~ of Kaduna
56 State with about 80% of the people actively engaged in farming. Another major occupation of
57 the people is animal rearing and poultry farming. The animals reared include cattle, sheep,
58 goats and pigs [15]. Pastoralism, Agro-pastoralism and intensive dairy farming are the
59 predominant dairy production systems in Kaduna State. The pastoralists move around with
60 their herds in search of fresh pasture land or grazing areas. Agro-pastoralism is practiced by
61 farmers who grow food crops and keep livestock, while the intensive dairy farmers use part
62 or all of their land to grow fodder crops for their dairy cattle [16].

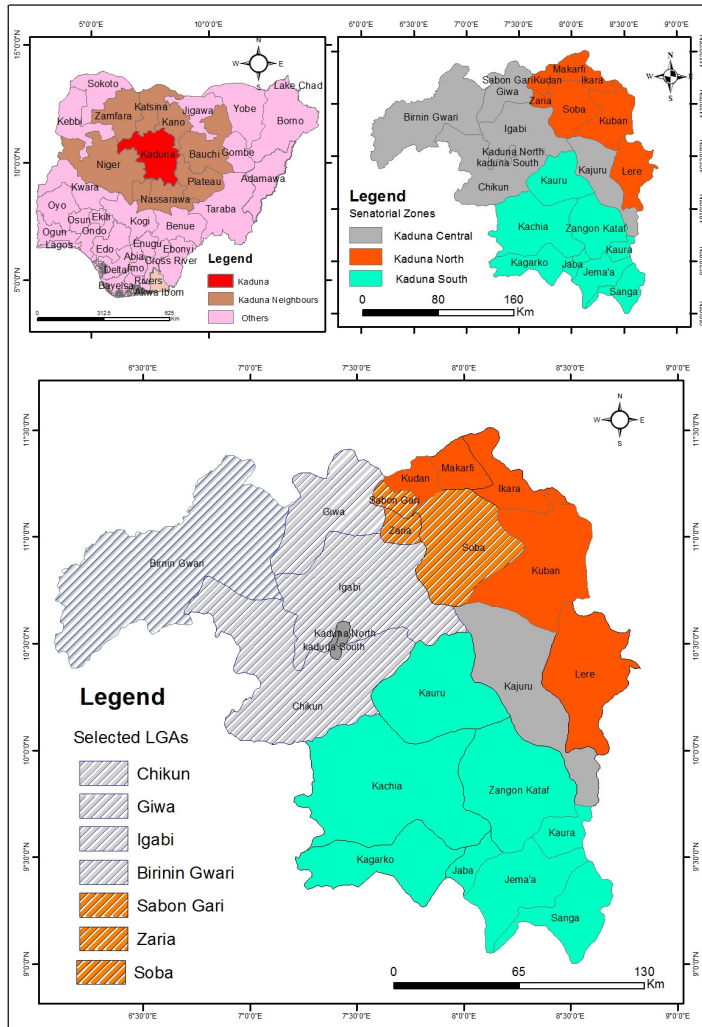


Fig. 1: The map of Nigeria and Kaduna State showing the study LGAs
Adapted from the Administrative Map of Nigeria [17, 18]

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66 **2.2 Study Design**

67 A cross-sectional study was carried out among 147 lactating bovines from 30 herds spread
 68 across seven Nomadic settlements within seven LGAs in Kaduna state between May, 2017
 69 and July 2018 using quantitative methods of data collection.

70 **2.3 Inclusion and Exclusion Criteria**

71 The study population constitutes all the lactating bovines of the Nigerian indigenous breeds
 72 within the study area. All farmers/pastoralist who declined consent as well as regions within

73 the state that have been identified as volatile security spots were not included in this study.
74 The animals were selected from herdsman settlements in parts of Kaduna State, Nigeria.
75 More so, only lactating bovines that are not currently on treatment were included in this
76 study, while those currently undergoing any form of treatment were excluded.

77 2.4 Sample Size Determination and Sampling Technique

78 The sample size was calculated using the formula of Sarmukaddam and Gerald [19]
79 expressed by Eq. 1. Mbuk *et al.* [11] recorded a prevalence of 10.3% for bovine coliform
80 mastitis in Kaduna state, Nigeria which was used for sample size estimation in this study.

$$n = \frac{Z^2 p(1-p)}{L^2} \quad (1)$$

81

82 Where:

83 n = is the number of samples

84 Z = is the standard normal distribution at 95% confidence interval = 1.96

85 p = is the prevalence of previous study = 10.3% = 0.103

86 L = is the allowable error, which is taken at 5% = 0.05

87 Therefore, sample size,

$$n = \frac{1.96^2 \times 0.103 \times (1 - 0.103)}{0.05^2} = 142$$

88

89 A sample size of 142 was estimated at 5% level of significance. A sample size of 147 was
90 however used for ease of proportionate distribution.

Comment [MH3]: ??

91 A multi-stage sampling technique was used in this study. The seven LGAs were purposively
92 selected out of 23 LGAs in Kaduna state, being the LGAs with less security risks and were
93 accessible at the time of this study. This was followed by the purposive selection of a
94 settlement from each of the seven LGAs (total of seven settlements) based on the
95 availability of lactating bovines that are not currently on treatment, willingness of the
96 farmers/pastoralists to participate in the study, and accessibility of the location in order to
97 easily transport samples collected to the laboratory for further analysis. Finally, 147 bovines
98 were randomly but and proportionately selected from all herds within the seven settlements.
99 Bovine listing and enumeration was done to a total of 50, 30, 39, 27, 55, 40 and 68 for
100 Settlements A, B, C, D, E, F and G, respectively out of which 24, 15, 19, 12, 26, 19 and 32
101 were respectively selected. A herd of bovines whose owner consented was sampled and in
102 the event that he or she declined, the next contiguous herd of bovines was sampled.
103 Computer generated list of random numbers from Minitab 14.2 statistical software was used
104 to select the bovines for each of the settlements in this study.

105 2.5 Sample Collection and Screening for Subclinical Mastitis

106 Strict aseptic procedures was followed to prevent contamination with microorganisms
107 present on the skin of udder and teats, hands of samplers and barn environment according
108 to the methods of National Mastitis Council Guidelines described by Middleton *et al.* [20].

109 Prior to milk sample collection, udders and teats were cleaned using a disposable paper
110 towel immersed in 70% ethyl alcohol and dried to avoid presence of faecal debris
111 contamination in the milk as it could interfere with the interpretation of CMT result. Foremilk
112 (first jets) was discharged to reduce the contamination of teat canal. Sterile universal bottles
113 with tight fitting cups were used. The bottles were labelled appropriately with permanent
114 marker before sampling. To reduce contamination of teat ends during sample collection, the
115 near teats were sampled first and then followed by the far ones. About 8mls of raw milk was
116 aseptically collected from each bovine (2mls from each quarter). The California Mastitis Test
117 (CMT) Reagent was used according to the manufacturer's instructions on the field to identify
118 samples with subclinical mastitis. 2mls of milk samples was collected directly from each
119 quarter of the udder and mixed together. 2mls of the composite milk sample was then added
120 to 2mls of CMT reagent on the test paddle and mixed gently to observe reaction. The result
121 was graded as described by various authors [21, 22]. All samples that tested positive for
122 subclinical Mastitis were properly labelled and immediately transported to the Bacteriological
123 Analysis Laboratory of the Department of Microbiology, Faculty of Life Science, Ahmadu
124 Bello University, Zaria in an ice box for processing.

125 **2.6 Bacteriological Analysis of CMT Positive Milk Samples**

126 **2.1.1 Inoculation of Raw Milk Samples**

127 The CMT positive milk samples were inoculated on MacConkey agar (Oxoid, England) by
128 streak method as described by Mekonnin *et al.* [23]. A loop full of milk sample was streaked
129 on the agar plates aseptically using quadrant method for each sample. The plates were
130 incubated at 37°C and examined after 24 hours for growth.
131

132 **2.1.2 Primary Isolation of Coliform Bacteria**

133 Bacteriological analysis was focused only on the identification and isolation of Coliform
134 bacteria. Hence, pink to red distinct colonies resulting from the utilization of lactose on
135 MacConkey agar were presumptively considered as Coliform bacteria. The suspected
136 isolates were sub-cultured to get pure isolates. The pure isolates were cultured on Eosin
137 Methylene Blue Agar (EMB) which is selective and differential for Coliform bacteria. Isolates
138 that showed metallic green sheen on EMB were presumptively considered as *E. coli*, while
139 those with coloured appearance were considered to be other Coliform bacteria. The
140 suspected Coliform isolates were stored in Nutrient Agar slant for further characterization
141 and identification using the conventional biochemical tests and Microgen A+B ID Kits (UK).
142

143 **2.1.3 Biochemical Characterization**

144 All suspected coliform bacterial isolates that stained red with Gram reaction were subjected
145 to Conventional biochemical tests. The tests conducted were: Indole, Methyl Red, Voges-
146 Proskauer and Citrate Utilization (IMViC). The suspected coliform bacterial isolates from the
147 tests were identified up to species level using Microgen A+B Kit (UK) in accordance with the
148 manufacturer's instructions.
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150 **2.1.4 Antibiotic Susceptibility Testing**

151 Antibiotic susceptibility testing was conducted for all the isolated Coliform species using disk
152 diffusion method according to the criteria of the Clinical and Laboratory Standard Institute

153 [24]. Direct colony suspension of the isolates was adjusted to a turbidity equivalent to a 0.5
 154 McFarland standard and was aseptically inoculated on Mueller-Hinton agar (Oxoid, UK)
 155 using spread plate technique. The antibiotic impregnated disks (Oxoid, UK) were aseptically
 156 fixed on the solid agar surface 15mm apart using a dispenser (Oxoid). The plates were
 157 incubated at 37°C for 24 hours.

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 159 Commercially available antibiotics (Oxoid, UK) recommended as drugs of choice against
 160 *enterobacteriaceae* and those frequently used in the treatment of human and animal
 161 infections within the study area were selected. Thus, a total of ten antibiotics were used in
 162 this study. The antibiotic disks used with their various concentrations were: Amoxicillin-
 163 Clavulanic acid (30µg), Imipenem (10µg), Ciprofloxacin (5µg), Gentamycin (30µg),
 164 Chloramphenicol (30µg), Trimethoprim/Sulphamethoxazole (25µg), Erythromycin (15µg),
 165 Penicillin (10µg), Streptomycin (30µg) and Tetracycline (30 µg). Chloramphenicol was still
 166 included in this analysis because it is still being used for animal [clinical treatment](#) in Nigeria
 167 notwithstanding the global recommendation for its removal from animal [clinical treatment](#).

168
 169 Furthermore, the diameters of the zones of inhibition around the disks were measured to the
 170 nearest millimeter using caliper. The isolates were classified as susceptible, intermediate
 171 and resistant according to the interpretive standards of Clinical and Laboratory Standard
 172 Institute [24]. Moreover, isolates that showed resistance to two or more classes of antibiotics
 173 were considered as multidrug resistant [25, 26, 27, 28].

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175 3. RESULTS AND DISCUSSION

176 3.1 Prevalence of Subclinical Mastitis and Coliforms in the Study Area

177 In this study, out of 147 milk samples from pastoral herds, 29 (19.73%) were positive for
 178 subclinical Mastitis out of which only 13 (8.84%) species of coliforms were isolated (six *E.*
 179 *coli* and seven *K. pneumoniae*). This implies that the prevalence of coliform mastitis in the
 180 study area (Parts of Kaduna state) is 8.84%. Samples from Birnin-gwari LGA harboured the
 181 highest number of coliforms 4 (2.7%) while no coliform bacteria were isolated from samples
 182 collected from Soba Local Government Area (Table 1). *K. pneumoniae* and *E. coli* were the
 183 species associated with mastitis milk (Table 2 and Table 3).

Comment [MH4]: If You have 147 samples one decimal digit is enough.

184

185 **Table 1: Prevalence of subclinical mastitis and associated coliform**
 186 **bacteria among the bovines studied**

S/N	Local Government Area/ Settlements/Herds	No. of Lactating Bovines	No. of Bovines Examined	No.(%) of samples positive for Subclinical Mastitis	No.(%) of samples positive for coliform bacteria
1.	Giwa	50	24	6(4.1)	3(2.0)
2.	Igabi	30	15	5(3.4)	2(1.4)
3.	Chikun	39	19	3(2.0)	1(0.7)
4.	Soba	27	12	1(0.6)	0(0.0)
5.	Zaria	55	26	4(2.7)	1(0.7)
6.	Sabongari	40	19	3(2.0)	2(1.4)
7.	Birnin Gwari	68	32	7(4.8)	4(2.7)
	Total	309	147	29(19.73%)	13(8.84%)

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190 **Table 2: Biochemical Characterization (IMVIC) Of Isolates**

Suspected Coliform Isolates	Indole Test	Methyl Red Test	Vogues Proskauer Test	Citrate Utilization Test	Probable Organism
C1	+	+	-	-	<i>Escherichia</i> sp
C2	+	+	-	-	<i>Escherichia</i> sp
C3	+	+	-	-	<i>Escherichia</i> sp
C4	+	+	-	-	<i>Escherichia</i> sp
C5	+	+	-	-	<i>Escherichia</i> sp
C6	+	+	-	-	<i>Escherichia</i> sp
C7	-	-	+	+	<i>Klebsiella</i> sp
C8	-	-	+	+	<i>Klebsiella</i> sp
C9	-	-	+	+	<i>Klebsiella</i> sp
C10	-	-	+	+	<i>Klebsiella</i> sp
C11	-	-	+	+	<i>Klebsiella</i> sp
C12	-	-	+	+	<i>Klebsiella</i> sp
C13	-	-	+	+	<i>Klebsiella</i> sp

191 **Key:** C1-C5 = Probable *Escherichia* species, C6-C13 = Probable *Klebsiella* species

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Table 3: Microgen Tests for the Identification of the Isolates up to Species level

Presumptive Isolates	Octal number	Final Identification	Percentage Probability
EC1	04600570	<i>Escherichia coli</i> inactive	96.658%
EC2	05604520	<i>Escherichia coli</i> inactive	90.24%
EC3	04604420	<i>Escherichia coli</i> inactive	86.546%
EC4	04405421	<i>Escherichia coli</i> inactive	88.326%
EC5	07600570	<i>Escherichia coli</i>	49.876%
EC6	07601370	<i>Escherichia coli</i>	92.64%
KP1	47523766	<i>Klebsiella pneumonia</i>	99.71%
KP2	47523666	<i>Klebsiella pneumonia</i>	95.107%
KP3	47523777	<i>Klebsiella pneumonia</i>	95.2%
KP4	47523757	<i>Klebsiella pneumonia</i>	99.3%
KP5	47555777	<i>Klebsiella pneumonia</i>	87.34%
KP6	47544776	<i>Klebsiella pneumonia</i>	65.13%
KP7	47544777	<i>Klebsiella pneumonia</i>	57.767%

196 **Key:** EC1-EC6 = *Escherichia coli* (6); KP1-KP7 = *Klebsiella pneumoniae* (7)

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3.2 Antimicrobial Susceptibility

The coliform isolates in this study showed 100% resistance to Penicillin and Tetracycline, and were all 100% susceptible to Imipenem. High multidrug resistance was expressed by all the isolates to Penicillin, Tetracycline and Erythromycin. All the isolates (100%) had MARI of 0.2 and above which is an indication of gross abuse of antibiotics within the studied population which was further buttressed by the 100% resistance displayed against penicillin and tetracycline. However, antibiotics still effective against the coliform species tested were Imipenem (100%), Ciprofloxacin (92.3%), Gentamycin (92.3%), Chloramphenicol (84.6%), Amoxicillin/Clavulanic acid (84.6%) and Sulfamethoxazole/Trimethoprim (69.2%) (Table 4).

Comment [MH5]: Coliform are naturally 100% resistant to Penicillin. Highlight You finding of 100% resistance to tetracycline

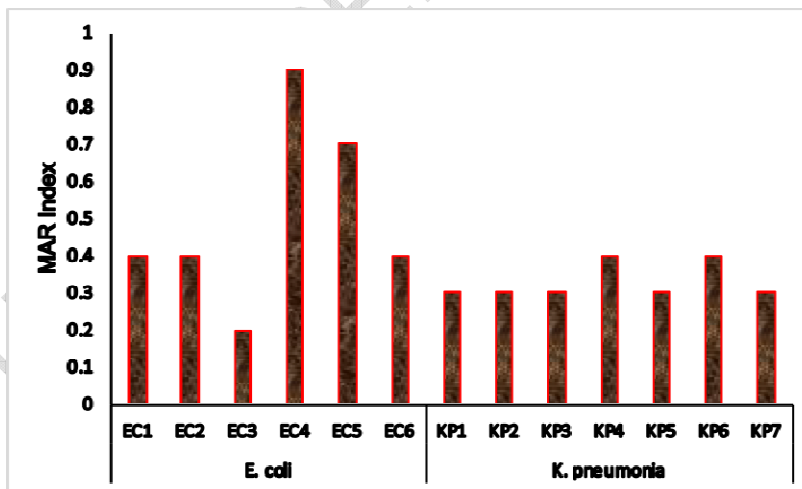
210 **Table 4: Antimicrobial Susceptibility Profile of Coliform Bacterial**
 211 **Isolates obtained from Mastitis Milk Samples (n=13)**

S/N	Antibiotic Generic Name	Discs Concentration (µg/ml)	No. (%) of Resistance	No. (%) of Intermediate Resistance	No. (%) of Susceptibility	Total (%)
01.	Erythromycin (E)	15.0	12(7.7)	0(0)	1(7.7)	13(100)
02.	Ciprofloxacin (CIP)	5.0	1(7.7)	0(0)	12(92.3)	13(100)
03.	Penicillin (PEN)	10.0*(I.U)	13(100)	0(0)	0(0.0)	13(100)
04.	Imipenem (IPM)	10.0	0(0.0)	0(0)	13(100)	13(100)
05.	Tetracycline (TET)	30.0	13(100)	0(0)	0(0.0)	13(100)
06.	Sulfamethoxazole/Trimethoprim (SXT)	25.0	4(30.8)	0(0)	9(69.2)	13(100)
07.	Chloramphenicol (C)	30.0	1(7.7)	1(7.7)	11(84.6)	13(100)
08.	Streptomycin (S)	10.0	3(23.0)	4(30.8)	6(46.2)	13(100)
09.	Amoxicillin/Clavulanic acid (AMC)	30.0	1(7.7)	1(7.7)	11(84.6)	13(100)
10.	Gentamycin(GN)	30.0	1(7.7)	0(0)	12(92.3)	13(100)

212 *Penicillin is given in International Units (IU).

213 More so, all the isolates tested exhibited six resistance patterns (A-F) according to their
 214 resistance to different antimicrobial groups (Table 5). All the isolates tested were considered
 215 multiple drug resistant (MDR) as they showed resistance to more than two classes of
 216 antibiotics tested. However, *Escherichia coli* isolate coded EC4 had the highest MAR Index
 217 of 0.9 (resistant to nine out of ten antibiotics tested), followed by EC5 that had 0.7 MAR
 218 Index (resistant to seven out of 10 antibiotics tested). *Escherichia coli* isolates coded EC1
 219 EC2 and EC6 and *Klebsiella pneumonia* isolates coded KP4 and KP6 had MAR Index of 0.4
 220 (resistant to four out of ten antibiotics tested). *Klebsiella pneumonia* isolates coded KP1,
 221 KP2, KP3, KP5 and KP7 had MAR Index of 0.3 each (resistant to three out of ten antibiotics
 222 tested, while *Escherichia coli* isolate with code EC2 had the least MAR Index of 0.2
 223 (resistant to two out of ten antibiotics tested) (Fig.2).
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225 **Fig. 2: Multiple Antibiotic Resistance (MAR) Index of coliform species investigated**

226 The species of coliforms isolated in this study were *Klebsiella pneumoniae* and *Escherichia*
 227 *coli*. *Klebsiella pneumonia* was the dominant species associated with bovine mastitis in this
 228 study. -This [finding](#) is in agreement with the work of Mbuk *et al.* [11] who isolated similar
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231 species of these organisms in Kaduna State where *Klebsiella pneumonia* was the highest,
232 but *Escherichia coli* was not isolated in their study. These findings also agree with Hogan
233 and Smith [29] who reported that *Klebsiella pneumoniae* and *Escherichia coli* are the
234 species of coliforms most frequently isolated from cases of bovine mastitis. The dominance
235 of *Klebsiella pneumoniae* in this study agreed with the report of Podder *et al.* [30] who
236 reported that *Klebsiella pneumoniae* is well adapted to survive in the udder and usually
237 establishes subclinical mastitis infection of long duration which can be shed in milk,
238 facilitating transmission to healthy animals mainly during milking process. Generally, the
239 presence of these Coliform bacterial species in the milk is an indication of faecal and
240 environmental contamination resulting from poor hygienic practices of rearing and milking
241 the animals as there are no established mastitis control practices employed among the
242 herdsmen.

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244 The results of antimicrobial susceptibility test in this study showed that all the species of
245 coliforms tested were sensitive to Imipenem followed by decreasing sensitivity to
246 Ciprofloxacin, Amoxicillin/Clavulanic acid, Streptomycin, Chloramphenicol, Gentamycin and
247 Sulfamethoxazole/Trimethoprim. This agrees with the report of Mbuk *et al.* [11] and Lira *et al.*
248 [31] that showed similar susceptibility pattern. Susceptibility of Imipenem to all coliform
249 species tested has proven that Carbapenems still retain considerable potency against
250 Enterobacteriaceae. This agrees with the recommendation of CLSI [24], where this class of
251 antibiotics was among the recommended antibiotics for treatment of infections caused by
252 Enterobacteriaceae. High level of susceptibility to Imipenem in this study might be due to its
253 rare use and abuse in cattle. However, it is worthy of note here that the Coliform species
254 tested showed 1.4% intermediate resistance and 5.1% resistance to some CLSI [24]
255 recommended antibiotics (Ciprofloxacin, Amoxicillin/Clavulanic acid, Streptomycin,
256 Chloramphenicol, Gentamycin and Sulfamethoxazole/Trimethoprim). Therefore, irrational
257 prescriptions and indiscriminate use of these drugs may lead to complete resistance in the
258 future [32].

259
260 However, all the species of Coliform bacteria tested were completely resistant to Penicillin
261 and Tetracycline. This is similar to the reports of previous studies of high resistance to these
262 same antibiotics [11, 31, 33]. The high degree of resistance observed in this study might be
263 due to prolonged and indiscriminate usage of those antibiotics which could lead to possible
264 resistance development in humans and animals [34, 35].

Comment [MH6]: Here a sentence has to be inserted about the natural resistance of Coliforms to Penicillin.

265
266 Moreover, all the *Klebsiella pneumonia* and *Escherichia coli* species in this study exhibited
267 multidrug resistance, as they were consistently resistant to two or more classes of antibiotics
268 among others used especially Erythromycin, Penicillin and Tetracycline. This finding agreed
269 with the previous reports where Coliform species tested displayed multidrug resistance to
270 Erythromycin, Penicillin and Tetracycline [11, 31]. These findings however, contradict the
271 report of Memom *et al.* [33] where coliform species were completely resistant to
272 Ciprofloxacin, Gentamycin, Amoxicillin and Sulfamethoxazole/Trimethoprim. However,
273 based on this study, antibiotics still effective against the coliform species tested were
274 Imipenem (100%), Ciprofloxacin (92.3%), Gentamycin (92.3%), Chloramphenicol (84.6%),
275 Amoxicillin/Clavulanic acid (84.6%) and Sulfamethoxazole/Trimethoprim (69.2%). Although
276 Chloramphenicol is prohibitive prohibited for use in animal clinical treatments, it is still being
277 applied in Nigeria since there is no legal framework for its prohibition yet. Since, this study
278 has recorded high sensitivity of coliforms to Imipenem, Ciprofloxacin, Amoxicillin/Clavulanic
279 acid, Streptomycin better than Chloramphenicol, this study joins voice the global practice to
280 discourage the use of Chloramphenicol in animal clinical treatment in Nigeria.

Comment [MH7]: Check if all *K. pneumoniae* are still multi-resistant according to definition if Penicillin is skipped from the list.

281
282 Furthermore, the result of susceptibility pattern of Coliform bacterial species obtained in this
283 study affirms that some of CLSI [24] recommended antibiotics of choice against the
284 treatment of infections caused by Enterobacteriaceae are increasingly becoming ineffective

285 | within the studied population. Therefore, it is always very important to ~~always~~ conduct
286 antimicrobial sensitivity tests before empirical therapy is initiated to avoid resistance
287 development to other sensitive antibiotics in future. However, based on the degree of
288 susceptibility pattern obtained in this study, Imipenem is recommended as first line drug of
289 choice where infection by *K. pneumonia* and *E. coli* respectively is suspected within the
290 | studied area.

291

292 **4. CONCLUSION**

293

294 | This study concludes that the prevalence of subclinical mastitis in Kaduna State is 19.73 %
295 while the prevalence of Coliform Mastitis is 8.84%. A low prevalence of Coliform mastitis was
296 observed in this region, but the presence of *Klebsiella pneumonia* and *Escherichia coli* in
297 raw milk samples of the studied bovine constitute serious environmental health risk to the
298 consumers as the milk obtained from these herds are widely circulated and consumed
299 without any form of treatment. They are also among the list of organisms classified as
300 dangerous biological agents that have the potential to pose a severe threat to public health
301 and safety by United States Public Health Services. The species of coliforms isolated in this
302 study showed decreased sensitivity to the majority of recommended antibiotics of choice by
303 Clinical and Laboratory Standard Institute (CLSI). This phenomenon could result to complete
304 resistance development in future if not properly handled. The high level of resistance to
305 some of the commonly used antibiotics by the herdsmen imply that the selection pressure
306 imposed by the use of these antibiotics whether therapeutically in veterinary medicine or as
307 prophylaxis in the animal production is a key driving force in the selection of antimicrobial
308 resistance.

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312 **COMPETING INTERESTS**

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314 None

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317 **CONSENT**

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319 Ethical consent was obtained from the Postgraduate Board of the Department of
320 Microbiology, Faculty of Life Sciences, Ahmadu Bello University for, Zaria to undertake the
321 study and to publish this report and accompanying images. A copy of the written consent is
322 available for review by the Editorial office/Chief Editor/Editorial Board members of this
323 journal.

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326 **ETHICAL APPROVAL**

327

328 All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.
329 85-23, revised 1985) were followed, as well as specific national laws where applicable. All
330 experiments have been examined and approved by the appropriate ethics committee

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