Poor biosafety and biosecurity practices and haphazard antibiotics usage in poultry farms in Nepal hindering antimicrobial stewardship

Ajit Poudel[1, 2], Shreeya Sharma[1], Kavya Dhital[1], Shova Bhandari[2], Rajindra Napit[1, 2], Dhiraj Puri[1], Dibesh B. Karmacharya*[1, 2, 3]

Affiliation

[1] One Health Division, Biovac Nepal, Nala, Nepal

[2] One Health Division, Center for Molecular Dynamics Nepal, Kathmandu, Nepal

[3] Department of Biological Sciences, University of Queensland, Australia

*Corresponding Author – dibesh@biovacnepal.com;dibesh@cmdn.org

Abstract

Poultry industry in Nepal has experienced remarkable growth in the last decade, but farm biosafety and biosecurity measures are often overlooked by farmers due to lack of knowledge or to save cost. As a result, farms often suffer from sporadic and regular outbreaks of many zoonotic diseases such as highly pathogenic avian influenza (HPAI), impacting production and creating public health challenges. Poor farm management practices, including overuse of antibiotics for prophylaxis and therapeutics, can complicate the spread of poultry diseases by creating and enhancing antimicrobial resistance (AMR) that is threating to both, poultry, and human health.

We assessed biosafety, biosecurity risks and AMR stewardship in sixteen poultry farms located in four districts (Ramechhap, Nuwakot, Sindhupalchowk, and Kavre) surrounding densely populated Kathmandu valley. Risk assessment and AMR stewardship evaluation questionnaire were administered to formulate biosafety and biosecurity compliance matrix (BBCM). Risk assessment checklist assessed facility operations, personnel and standard operating procedures, water supply, cleaning and maintenance, rodent/pest control and farm record keeping. Oral and cloacal samples from the poultry were collected, pooled, and screened for eight poultry pathogens using Polymerase Chain Reaction (PCR) tests.

Based on BBCM, we identified one of the farms in Sindhupalchowk (Farm 4) having the most (BBCM score= 67%) and a farm in Kavre (Farm 3) to having the least (BBCM score= 12%) biosafety and biosecurity compliance. Although most of the farms (61.6%) followed general poultry farming practices, only half had clean and well-maintained farms. Personal safety standard procedure compliance (BBCM score = 42.4%) and rodent control (BBCM score = 3.1%) were the biggest gaps. At least one of either bacterial or viral pathogen was detected in all farms. *Mycoplasma gallisepticum* was the most common disease detected in all but one farm, followed by *Mycoplasma synoviae*. Although more than half of the farmers considered AMR a threat, over 26% of them used antibiotics as a preventive measure and 81% did not consider withdrawal period for antibiotics prior to processing of their meat products. Additionally, antibiotics classified as Watch and Restrict by the WHO were frequently used by the farmers to treat bacterial infections in their farms. Lack of awareness and inadequate enforcement of regulations have exacerbated the risk of disease transmission in farms and compromised antimicrobial stewardship.

Introduction

Nepal's poultry industry has seen a significant and rapid growth in the last decade, contributing more than 4% to the national gross domestic product (GDP) [1,2]. Majority of the poultry products are supplied by numerous commercial farms (54% of total poultry production) scattered throughout the country. Backyard poultry also accounts for significant proportion of the total poultry production (46%); poultry meat and eggs are an easy source for protein and livelihood [1,3]. Rapidly expanding commercial poultry is reared in 64 out of 77 districts of Nepal and has an annual growth rate of over 18% [1,4]. According to the latest poultry census by the Nepal Central Bureau of Statistics [5], majority of chicken reared in Nepal are broilers (87%), with only small number of farms keeping layer chickens (11%). Almost half of the poultry production (46%) come from the central region of the country. Chitwan, Kathmandu, and Kaski districts account for more than 85% of total meat and eggs production.

In Nepal, in spite of burgeoning poultry industry, proper biosecurity measures are often overlooked [6]. Backyard poultry farmers often feel the burden of maintaining biosecurity due to lack of knowledge and perceived additional cost [7]. Biosafety encompasses measures to prevent transmission of infectious diseases, and biosecurity measures are meant to prevent introduction and spread of pathogens in farms. By implementing a proper biological containment (and exclusion) along with traffic control, segregation, and sanitation- an effective biosafety and biosecurity can be maintained [8]. Keeping healthy flocks not only guarantees financial security for the farmers, it can also prevent outbreaks of zoonotic diseases such as HPAI [2,9]. With increased commercial poultry production, maintaining biosecurity and biosafety measures in farms have been challenging [6]. Lack of government initiatives (and efforts), both in developing and developed countries alike, to raise awareness and implement regulations on biosecurity and biosafety have also grossly undermined proper safe farming practices [10]. Although Veterinary Standards and Drug Administration Office (VSDAO) in Nepal has developed a manual for proper poultry management including biosecurity guidelines, it isn't properly enforced and is often overlooked by farmers [11]. Poultry production that are primarily focused on profitability, with compromised biosafety and biosecurity practices for cost saving, will eventually face production loss and increased health risks to both birds as well as humans (and other animals) [8].

72 Despite increasing occurrence of disease outbreaks such as Avian Influenza (AI) in poultry farms, many farmers
73 in Nepal are unaware of the importance of biosecurity measures and their practices [7]. This lack of awareness,
74 along with inadequate enforcement of biosecurity regulations, is exacerbating the risk of AI transmission in the
75 country [12]. Poor farm management practices such as the use of unprocessed poultry waste as animal feed and
76 the overcrowding of birds due to poorly designed farm contribute to the spread of AI [13].

77 Disease outbreaks in poultry farms due to bacterial pathogens such as *Mycoplasma gallisepticum* (Mg),
78 *Mycoplasma synoviae* (Ms), *Escherichia Coli* (E. coli) and salmonella also occur due to lapses in biosafety and
79 biosecurity. Rampant and haphazard use of antibiotics in poultry farms in Nepal is a leading cause of
80 antimicrobial resistance (AMR) and a looming threat to human health [14]. There is a need to reduce and
81 promote responsible use of antibiotics in the poultry industry in Nepal [15,16]. Antimicrobial stewardship,
82 which is a coordinated program that promotes the appropriate use of antimicrobials (including antibiotics),
83 reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms,
84 has become one of the important aspects of a comprehensive biosecurity and biosafety practices [7].

85 Kathmandu is a densely-populated metropolitan capital city of Nepal with a population of over 2 million people
86 [17]. Any emerging, re-emerging and diseases of human health concern originating from animal production
87 sites, such as poultry farms, can rapidly spread in a city like Kathmandu. Since disease outbreak, transmission
88 and spread dynamics are directly linked to biosafety and biosecurity status of farms, it is vital to understand the
89 current status of the farms located close to the city. We conducted a comprehensive risk assessment and status
90 evaluation of biosafety, biosecurity and AMR stewardship in sixteen poultry farms located in four districts with
91 high poultry production (Ramechhap, Nuwakot, Sindhupalchowk, and Kavre) surrounding the Kathmandu
92 valley.

93

Methodology

Study Site and Data collection

Four farms (small and medium sized; poultry <2000 per farm) in each of the four districts (Kavre, Sindhupalchowk, Ramechhap and Nuwakot) were selected for the study (Figure 1). Risk assessment checklist (Supplementary Table 1) and AMR stewardship questionnaire (Supplementary Table 2) were used to collect data. Risk assessment checklist assessed facility operations, personnel and standard operating procedures, water supply, cleaning and maintenance, rodent/pest control and farm record keeping.

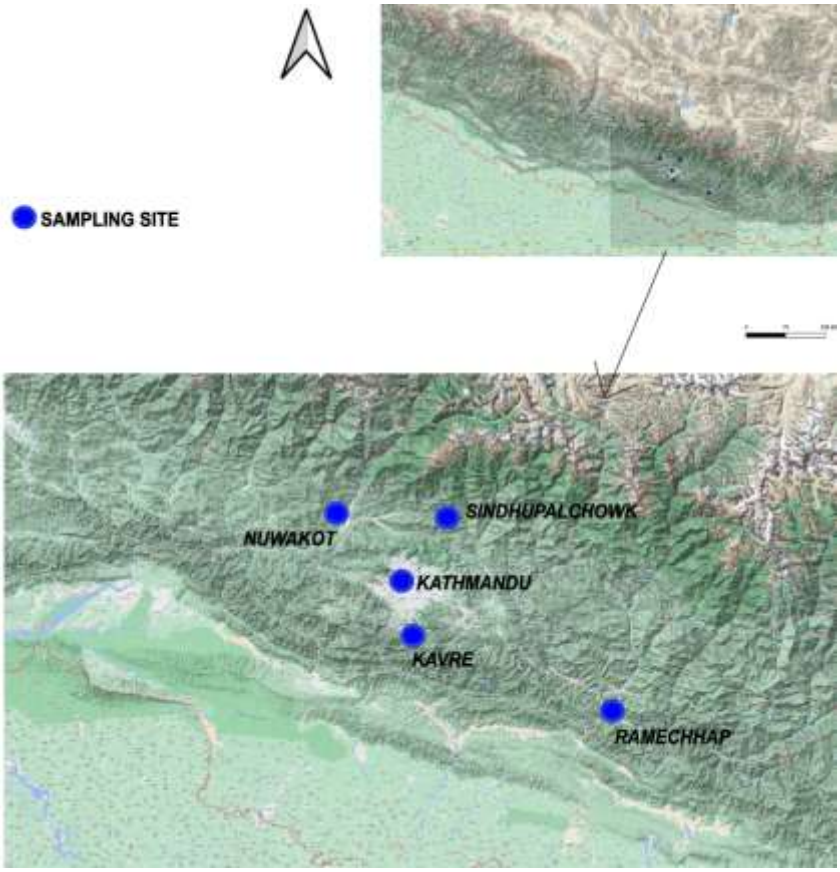

Figure 1: Selected poultry farms in the districts (Kavre, Sindhupalchowk, Ramechhap and Nuwakot) surrounding Kathmandu valley. The top map shows location of the districts in Nepal (shown in red border) and the bottom map shows their location in respect with Kathmandu Valley (shown just for reference, not a sampling site). The map was generated using QGIS software, Version 3.30.0 [18].

107 **Biological Sample Collection**

108 Oropharyngeal (n=1) and cloacal (n=1) swabs from each chicken were collected from selected farms (n= 16
109 farms; 30 birds per farm) and stored in viral transport media (VTM). All samples were stored in ice boxes (2-
110 8^0 C) during sample collection and transported in liquid nitrogen container (-196^0 C) to BIOVAC's lab in
111 Kathmandu. The samples were then pooled (n=10) from each farm (n= 6 pooled samples; pooled oropharyngeal
112 swabs=3 and pooled cloacal swabs=3). These pooled samples were screened for eight poultry pathogens-
113 Newcastle disease virus (NDV), Influenza A Virus (IAV), Infectious Bronchitis Virus (IBV), Infectious Bursal
114 Disease (IBD), *Mycoplasma synoviae (Ms)*, *Mycoplasma gallisepticum (Mg)*, Marek's Disease Virus-1
115 (MDV1) and Marek's Disease Virus-2 (MDV2) using PCR.

116 **Nucleic Acid Extraction and PCR**

117 The nucleic acid (DNA/RNA) from pooled samples were extracted using automated nucleic acid extractor
118 (abGenix™ AITbiotech, Singapore) following manufacturer's instructions. The pooled swab samples were
119 stored at -20°C. PCR for detection of the eight pathogens were performed using SuperScript™ III Platinum™
120 One-Step qRT-PCR Kit w/ROX (Invitrogen, Catalog number 11745500). The primers for NDV, IBV, IBDV,
121 Mg, Ms, MDV1 and MDV2 were designed using NCBI PrimerBlast® (Table 1).

122 For IAV, the primers IAV ISO_F and IAV ISO_R were used (Table 1) [19].

123

124 **PCR Condition**

125 PCR for each pathogen test was done in a 25ul reaction containing 4 µL of extracted RNA (for NDV, IAV, IBD,
126 IBV) and 4 µL of extracted DNA (for MG, MS, MDV1 and MDV2), 1 µL each of respective 10pm forward and
127 reverse primers, 12.5 µL of 2X Mastermix with ROX, 0.2 µL of SuperScript™ III Platinum™ enzyme and 6.3
128 µL of Nuclease free water. All eight PCR were carried out with enzyme activation at 45°C for 15 minutes
129 followed by one cycle of initial denaturation at 95°C for 5 minutes. PCR for RNA viruses consisted of 45 cycles
130 of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds and extension at 72°C for 20 seconds.
131 PCR for MS, MG, MDV1 and MDV2 consisted of 10 cycles of denaturation at 95°C for 30 seconds, annealing

132 at 63°C for 40 seconds and extension at 72°C for 20 seconds followed by 35 cycles of denaturation at 95°C for
133 30 seconds, annealing at 60°C for 40 seconds and extension at 72°C for 20 seconds. The final extension for all
134 eight PCR were carried out at 72°C for 2 minutes. All the amplified PCR products were visualized using 1.5%
135 Agarose Gel (Supplementary Figures).

136

137

138 **Table 1: PCR primers for each of the pathogen designed using PrimerBlast®**

Pathogen	Gene	Primer Sequence (5'- 3')	PCR amplicon size
NDV	Matrix Gene	CTCAATGTCACTATTGAYGTGG CTGAGGAGARGCATTKGCTAT	316 bp
IAV	Matrix Gene	CTTCTAACCGAGGTCGAAACG GGTGACAGGATTGGTCTTGTC	156 bp
IBV	N Gene	GGTGATGACAAGATGAWYGAGGA CTCCTCATCTGAGGTYAATGC	387 bp
IBDV	VP2 gene	CCTGAACTAGCAAAGAACCTG CAAGACGGTCCCTCTCACT	97 bp
MS	16S rRNA	CGTTCTCAGTTCGGATTGTAGTC GTCGTCTCCGAAGTTAACAAACC	170 bp
MG	MGC2 Protein	GCTGGGTTGATTGTTGTTTCTT TCTTCACGTTCTTGGATCATCAT	95 bp
MDV1	Glycoprotein B	GCTACCTCATTGTCACGATTGC TTGCACTCGCTGTAGCGCT	256 bp
MDV2	Glycoprotein B	GTGACCGCCGTGTCTACTTG CTCTTTCGTGTAGACCGACAG	378 bp

139

140

141

142 **Biosafety and Biosecurity Risk Assessment**

143 **Biosafety and Biosecurity Compliance Matrix (BBCM) Score**

144 We created a Biosafety and Biosecurity Compliance Matrix (BBCM) score based on risk assessment checklist
145 which included criteria such as facility operations, personal and standard operating procedures, water supply,
146 cleaning and maintenance, rodent/pest control and farm record keeping (Supplementary Table 1). General
147 practices relate to infrastructure of or within the farm that aids in its biosecurity. Personnel standards and
148 procedures include hygiene practices, use of disinfectants and precautionary measures taken to minimize
149 pathogen contamination in farms. Water supply assessed whether the water given to poultry is adequately
150 disinfected and clean. We also investigated preventive measures taken by farms to control rodents. Poultry
151 rearing area and equipment cleanliness practices implemented by the farm personnel were also evaluated under
152 cleaning and maintenance criteria. And finally, under record keeping criteria, we assessed practices of keeping
153 records of daily activities, material usage/ consumption and any breaches detected in the farm.

154 For each selected criteria complied, one point was given for every activity implemented and a BBCM score was
155 tallied for each category in every farm. A final farm BBCM score was then calculated and converted into
156 percentage. We categorized farms that had >90% BBCM score as high, 60-89% as medium and <60% as low.
157 Scores received by each farm are shown in

158 Table ***2*** and visualized in Figure 2.

159

160 Results

161 Biosafety and Biosecurity Risk Assessment

162 Analysis of overall Biosafety and Biosecurity of all farms surveyed showed low compliance (average BBCM
163 score= <41%). The highest BBCM rating was scored by a farm in Sindhupalchowk (Farm 4, BBCM= 67%) and
164 the lowest was by a farm in Kavre (Farm 3, BBCM= 12%). At district level, Sindhupalchowk had the most
165 Biosafety and Biosecurity compliance (BBCM= 53%) whereas Ramechhap had the least (BBCM= 32%). Of all
166 the assessed criteria, rodent control was the most neglected (BBCM= 3.1%). Only two farms (Ramechhap Farm
167 2 and Sindhupalchowk Farm 4) had implemented one out of four record keeping practices. General poultry
168 farming practice was the most biosafety and biosecurity compliant criteria fulfilled by all farms (BBCM=
169 61.6%) (Table 2).

170 General practices, personal standards and procedure, and cleaning and maintenance were the only categories
171 implemented in all sixteen farms (Table 2, Figure 2). Only two farms (Ramechhap 2 and Sindhupalchowk 4)
172 implemented rodent control, these were also the only farms that implement some activities of all six criteria.
173 Water supply was implemented in all but two farms (Nuwakot 1 and Kavre 3). Similarly, record keeping was
174 implemented in all but four farms (Kavre 3, Kavre 4, Ramechhap 1, and Ramechhap 4).

175 Across all 16 farms, general practices received the highest criteria average (61.6%), followed by cleaning and
176 maintenance (50%), water supply (43.8%), record keeping (42.6%), personal standards and procedure (42.4%),
177 and rodent control (3.1%).

178

179 **Table 2: Total number and percentage of BBCM criteria met by the farms as per our assessment**
180 **checklist.** Overall average BBCM score was calculated by dividing the sum of all BBCM fulfilled criteria (n)
181 and calculating the percentage. The highest BBCM score (Green cell- Sindhupalchowk Farm 4) was 67%, and
182 the lowest BBCM score was 12% (Orange cell- Kavre Farm 3). The total number of activities assessed in each
183 criterion are listed within parenthesis. Details of the activities assessed are shown in Supplementary Table 1.

184 Mg – Mycoplasma gallisepticum, Ms – Mycoplasma synoviae, IBD – Infectious Bursal Disease, IAV –
185 Influenza A Virus

Location	General Practices (17)		Personal Standards & Procedure (15)		Water Supply (4)		Rodent Control (4)		Cleaning & Maintenance (10)		Record Keeping (6)		Overall Average	Diseases Detected
	n	%	n	%	n	%	n	%	n	%	n	%	%	
Nuwakot Farm 1	11	65	11	73	0	0	0	0	4	40	3	50	38	Mg
Nuwakot Farm 2	12	71	6	40	3	75	0	0	5	50	3	50	48	IBD
Nuwakot Farm 3	9	53	5	33	2	50	0	0	4	40	2	33	35	IBD
Nuwakot Farm 4	14	82	8	53	2	50	0	0	5	50	2	33	45	Mg
												District Average (%)	**41.5**	
Kavre Farm 1	13	76	10	67	2	50	0	0	8	80	5	83	59	Mg
Kavre Farm 2	14	82	6	40	3	75	0	0	7	70	2	33	50	Mg
Kavre Farm 3	5	29	2	13	0	0	0	0	3	30	0	0	12	Mg
Kavre Farm 4	6	35	3	20	2	50	0	0	4	40	0	0	24	Mg
												District Average (%)	**36.25**	
Ramechhap Farm 1	8	47	4	27	1	25	0	0	4	40	0	0	23	Mg & Ms
Ramechhap Farm 2	13	76	3	20	3	75	1	25	6	60	3	50	51	Mg & Ms
Ramechhap Farm 3	7	41	5	33	1	25	0	0	5	50	1	17	28	Mg & Ms
Ramechhap Farm 4	10	59	4	27	1	25	0	0	4	40	0	0	25	IAV & Mg
												District Average (%)	**31.75**	

Sindhupalchowk Farm 1	9	53	6	40	1	25	0	0	4	40	5	83	40	Mg
Sindhupalchowk Farm 2	13	76	8	53	3	75	0	0	8	80	5	83	61	IAV
Sindhupalchowk Farm 3	10	59	10	67	1	25	0	0	3	30	5	83	44	Mg
Sindhupalchowk Farm 4	14	82	11	73	3	75	1	25	6	60	5	83	67	Mg
												District Average (%)	**53**	
Criteria Average (%)	***61.6***		***42.4***		***43.8***		***3.1***		***50***		***42.6***			

At least one of either screened bacterial or viral pathogen was detected in all farms. Mg was the most common disease detected, in all but one farm, followed by Ms. Farms in Ramechhap district were most affected, with Mg detected in all four farms, Ms in three, and IAV in one. Two different poultry diseases (IAV and Mg) were detected in Sindhupalchowk (Farm 2). IBD (Farm 2 and 3) and Mg (Farm 1 and 4) were detected in Nuwakot district (Table 2). Results of PCR tests detecting the diseases (gel images) are shown in Supplementary Figures.

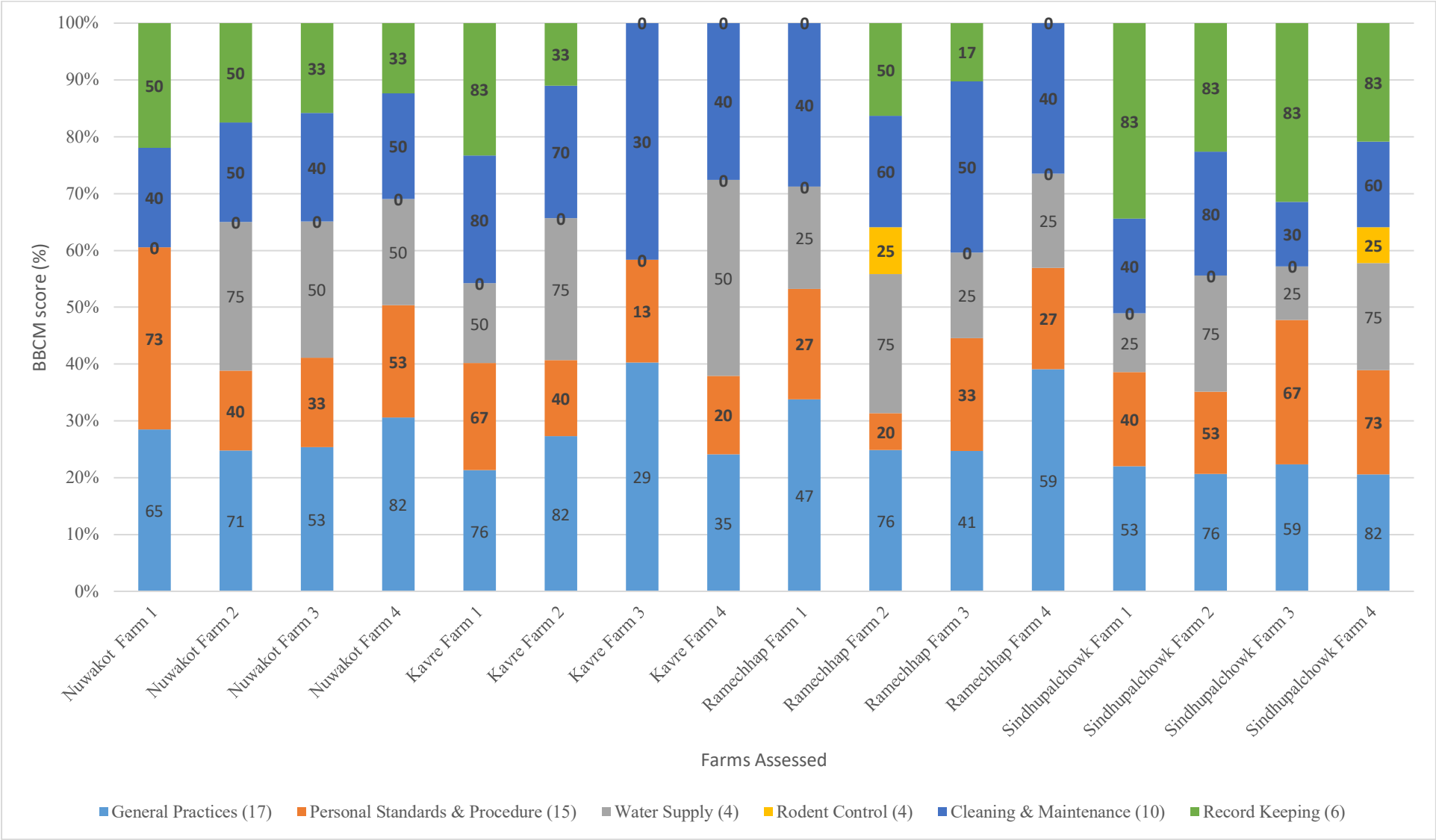

Figure 2: Graphical representation of BBCM scores received by all 16 farms for various biosafety & biosecurity parameters. Scores (percentage) received by all farms assessed in this study using BBCM score. Numbers in parenthesis in the legend refers to total number of activities assessed within each criterion.

196 **AMR Stewardship**

197 A little over half (52%) of the surveyed farmers considered AMR as a real threat (Table 3). Almost all farmers
198 (93%) had not attended any programs or campaigns related to AMR. Very few farmers (12%) received training
199 on AMR stewardship; these farmers received training/information on AMR from local animal health centres
200 (21%), veterinarians (29%), vet technician (17%) or from vet suppliers (21%). Majority of the farmers (81%)
201 did not consider implementing "withdrawal period" for antibiotics use prior to selling their meat products. Most
202 of the famers trusted veterinarians (40%) and vet technician (14%) on receiving consultation on antibiotics use.
203 Farmers used various antibiotics for prophylaxis (26%) and therapeutics (76%) needs.

204 **Table 3: Knowledge and perception of AMR among poultry farmers**. Responses (in percentage) obtained
205 from farmers on AMR-related questions.

AMR Questions		Percentage
Is AMR a threat?	Yes	52
	No	48
Where did you get AMR knowledge from?	Local Animal Health Center	21
	Veterinarian	29
	Vet Technician	17
	Vet Suppliers & Shops	21
	Self	12
Who do you most trust for consultation?	Veterinarians	40
	Vet Technician	14
	Self	2
	Local Animal Health Center	26
	Shops & Sales Team	14
	Local Animal Health Center	2
Do you consider withdrawal period after antibiotics use?	Yes	12
	No	81
	Don't Know	7
Withdrawal period prior to culling?	Yes	14
	No	79

	Don't Know	7
Any programs/campaigns attended on AMR?	Yes	5
	No	93
	Don't Know	2
Antibiotics used for prophylaxis?	Yes	26
	No	74
Antibiotics used for therapeutics?	Yes	76
	No	24

The farmers were also inquired about various types and proportion of antibiotics they used (Figure 3). Tetracycline was the most used (36%) antibiotics, followed by Colistin (14%), Quinolone (12%), Aminoglycoside (12%) and Macrolide (9%). Penicillin (3%) and Beta-lactam (2%) were the least used antibiotics.

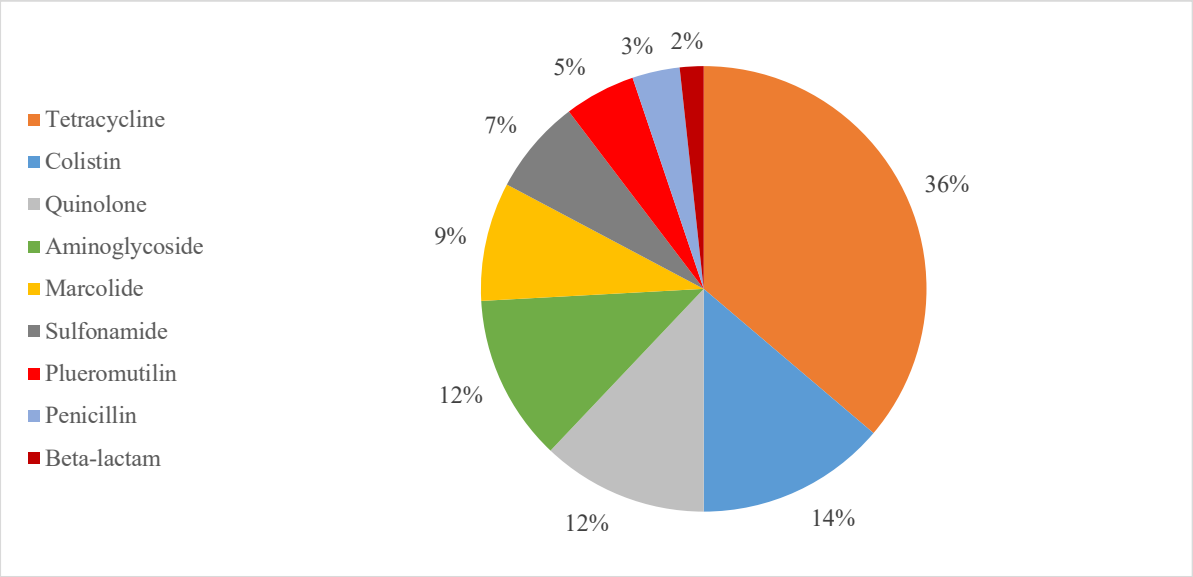

Figure 3: Various types of antibiotics used (in %) in the surveyed poultry farms.

Discussion

Poor biosafety and biosecurity measures on poultry farms can lead to introduction and spread of bacterial and viral diseases. Regular monitoring and testing, an important component of implementing strict biosecurity and biosafety measures, are essential for detecting and containing infections in farms. Poultry farms with compromised biosafety and biosecurity compliance often suffer from various prevalent bacterial and viral infections such as Mycoplasma, IBD and IAV- affecting overall poultry health and lowering production.

Mg and Ms are bacterial diseases which spread through direct contact, respiratory secretions, or contaminated materials [20]. IBD, on the other hand, is a viral disease that can cause damage to the immune system of young chickens, often leading to mortality [21]. It is transmitted through contact with contaminated sources, such as faeces. IAV in poultry is primarily transmitted through direct contact with infected birds or through contact with contaminated surfaces, feed, or water. Wild birds, particularly waterfowl, are the natural reservoirs for the virus and are thought to be one of the major sources of avian influenza outbreaks [22,23].

Introduction of infected birds, equipment, or materials in farms can result in the spread of diseases [24,25]. Movement of people and animals on and off the farm can also contribute to the spread of disease [26]. Birds that are housed in crowded or unsanitary conditions are susceptible to diseases; maintaining proper biosecurity and biosafety measures in such farms are often challenging [27]. Implementation of these measures were severely lacking in the farms that we surveyed in our study. Only one farm scored medium biosafety and biosecurity compliance rating (Sindhupalchowk Farm 4, BBCM= 67%). None of the farms received a high score of >90%, and not surprisingly, diseases were detected in all farms (Table 2). Moreover, out of all sixteen farms, only two (Ramechhap 2 and Sindhupalchowk 4) had evidence of conducting activities pertaining all six different categories of the assessment. Rest of the farms had activities/practices missing from an entire category.

Biosecurity measures, such as controlling the movement of people and animals onto the farm, implementing disinfection procedures, and providing protective clothing, can significant improve poultry production by reducing disease outbreaks and spread [28,29]. We detected Mg and Ms in most of the farms, these bacterial infections have low mortality but associated morbidity directly affects egg and meat production [30]. Overall,

241 the farms in Ramechhap district scored poorly in BBCM, something the district animal health authority needs
242 to be aware of and make effort to improve.

243 Although majority of the farms focused on general practices, cleaning and maintenance, and personal standards
244 and procedures, except for two farms (Ramechhap 2 and Sindhupalchowk 4) most had completely ignored
245 rodent control (Table 2). Rodents are a major pest and a disease vector of poultry farms [31]. Rodents are also
246 known to cause damage to farm infrastructure and feed, kill young chicks and eat eggs [32].

247 Poultry farms often resort to using medications to treat and salvage their flock only after disease outbreaks. We
248 detected viral pathogens (IBD and IAV) and bacterial infections (Mg and Ms) in all but one farm (out of 16).
249 Antibiotics was rampantly used, both as prophylaxis and therapeutics, by the farmers. Antibiotics such as
250 Tetracycline, Colistin, Quinolone, Aminoglycoside, and Macrolide were the most used antibiotics by the
251 farmers- consistent with national trend [33,34]. More than a quarter (26%) of the farmers used antibiotics as
252 prophylaxis as a preventive measure and 76% of farmers used them as therapeutics to treat diseases. Detection
253 of bacterial infections in the farms even after the use of all these antibiotics is deeply concerning.

254 Inappropriate (and haphazard) use of antibiotics in farms can develop AMR in bacteria, making them more
255 difficult to treat. Such usage not only leads to altered composition and diversity of gut microbiome in poultry
256 [35,36] but also result in high levels of resistance to several classes of antibiotics, including widely used
257 antibiotics such as Colistin, Fluoroquinolones, and Beta-Lactams [37]. Further study needs to be carried out to
258 assess presence of AMR genes in the bacterial pathogens detected in the farms.

259 The World Health Organization (WHO) has developed a classification system for antibiotics called Access,
260 Watch, and Reserve (AWaRe) to guide the appropriate use of antibiotics and combat antimicrobial resistance.
261 The Access group contains antibiotics that should be widely available and affordable, including first-line
262 treatments for common infections. The Watch group contains antibiotics that should be used with caution and
263 reserved for specific indications to prevent the development of resistance. The Reserve group contains last-
264 resort antibiotics, which should be used sparingly and only when all other options have been exhausted [38].
265 Apart from tetracycline, the other two antibiotics that are classified as 'Access' by the WHO are used by farmers
266 sparingly (Pleuromutilin – 7% and Penicillin – 5%). Antibiotics under 'Watch', such as Quinolone (12%),

267 Aminoglycoside (12%) and Macrolide (9%) are widely used by poultry farmers. Alarmingly, Colistin (19%)
268 which is listed as a 'Reserve' by the WHO is the second most abundantly used antibiotics.

269 A complete disregard to AMR stewardship combined with lack of knowledge among poultry farmers can have
270 devastating impact on propagation of AMR. Widespread use of strong antibiotics and indifference to
271 implementing withdrawal period not only aggravates the AMR situation in Nepal but also pose serious food-
272 safety risks. Withdrawal period is a withhold time prior to market distribution of poultry products after antibiotic
273 usage in production; this is to ensure antibiotics have been degraded sufficiently and rendered inactive [39]. A
274 survey conducted in Kathmandu of more than 200 farmers revealed only few poultry farmers knew about
275 withdrawal periods [7] or were aware of the importance of adhering to withdrawal periods after antibiotic use
276 to prevent the development of AMR [14,15]. All of these indicate the need of strict monitoring and control of
277 antibiotic use by concerned government agencies.

278 Farmers in this study mentioned consulting either veterinarians or veterinary technicians regarding antibiotics
279 usage (

280 Table ***3***), however, either they did not fully comprehend the gravity of the looming AMR threat, or they
281 disregarded the information they received. Majority of the farmers (88%) claimed to have received trusted
282 information on AMR from various experts, but almost half of the farmers were unclear about AMR and majority
283 (80%) of them were not willing to observe withdrawal period for their products.

284 In order to enhance biosecurity and reduce the risk of disease outbreaks in the poultry industry, it is necessary
285 to raise awareness among poultry farmers about the usage of antibiotics along with significance of biosecurity
286 measures [15,40]. This can be done through extensive practical training amongst network of poultry farmers.
287 Proper biosecurity measures, such as the frequent disinfection of farm premises and equipment and the provision
288 of protective clothing and footwear for workers can greatly help in prevention and containment of farm borne
289 diseases. Additionally, reducing the use of antibiotics in poultry production and promoting alternative methods
290 of disease prevention, such as immunization, use of probiotics and immune modulators can play a crucial role
291 in improving poultry health and reducing disease risks.

292

Acknowledgement

This study was made possible by the funding support from the Regional Environment, Science, Technology and Health (ESTH) Office for South Asia- the US State Department. We would like to thank Mr. Patrick Gan, Mr. Jay Pal Shrestha, and Ms. Sulakchana Rai of ESTH office for all their support. We would also like to show our gratitude to the Department of Livestock Services. Finally, we would also like to thank laboratory associates of BIOVAC Nepal and CMDN for their help in analyzing the samples.

299 # References

300 1. FAO. Poultry Sector nepal. 2014.

301 2. Sharma B. Poultry Production, Management and Bio-Security Measures. J Agric Environ. 2010;11:
302 120–125. doi:10.3126/AEJ.V11I0.3659

303 3. Osti R, Bhattarai D, Zhou D. Climatic Variation: Effects on Stress Levels, Feed Intake, and
304 Bodyweight of Broilers. Brazilian J Poult Sci. 2017;19: 489–496. doi:10.1590/1806-9061-2017-0494

305 4. Gompo TR, Pokhrel U, Shah BR, Bhatta DD. Epidemiology of Important Poultry Diseases in Nepal.
306 Nepal Vet J. 2019;36: 8–14. doi:10.3126/NVJ.V36I0.27746

307 5. CBS. Nepal - Nepal Commercial Poultry Survey 2014-2015. 2015 [cited 26 Mar 2023]. Available:
308 https://microdata.cbs.gov.np/index.php/catalog/72

309 6. Nelson AJ, Manandhar S, Ruzante JM, Gywali A, Dhakal B, Dulal S, et al. Antimicrobial drug
310 resistant non-typhoidal Salmonella enterica in commercial poultry value chain in Chitwan, Nepal. One
311 Heal Outlook. 2020;2.

312 7. Dhakal S, Gompo TR, Dhakal ; S. Assessment of Farmer's Knowledge of Antimicrobial Resistance,
313 Their Practice of Antimicrobial Usage and Biosecurity Status of Poultry Farms in Kathmandu Valley
314 and Chitwan District, Nepal. Int J Appl Sci Biotechnol. 2022;10: 50–59.
315 doi:10.3126/IJASBT.V10I1.41675

316 8. Maduka C V., Igbokwe IO, Atsanda NN. Appraisal of Chicken Production with Associated
317 Biosecurity Practices in Commercial Poultry Farms Located in Jos, Nigeria. Scientifica (Cairo).
318 2016;2016. doi:10.1155/2016/1914692

319 9. Karki S, Lupiani B, Budke CM, Karki N, Rushton J, Ivanek R. Cost-benefit analysis of avian influenza
320 control in Nepal. Rev Sci Tech. 2015; 813–827.

321 10. Conan A, Goutard FL, Sorn S, Vong S. Biosecurity measures for backyard poultry in developing
322 countries: A systematic review. BMC Vet Res. 2012;8: 1–10. doi:10.1186/1746-6148-8-

240/FIGURES/1

11. Veterinary Standards and Drugs Admnistration Office (VSDAO). Biosecurity Manual for Commercial Poultry Production. 2013.

12. Paudel M, Acharya B, Adhikari M. Social determinants that lead to poor knowledge about, and inappropriate precautionary practices towards, avian influenza among butchers in Kathmandu, Nepal. Infect Dis Poverty. 2013;2: 1–10. doi:10.1186/2049-9957-2-10/TABLES/10

13. Pradhan AK, Karanth S. Zoonoses from animal meat and milk. Present Knowl Food Saf A Risk-Based Approach through Food Chain. 2023; 394–411. doi:10.1016/B978-0-12-819470-6.00029-9

14. Koju P, Shrestha R, Shrestha A, Tamrakar S, Rai A, Shrestha P, et al. Antimicrobial Resistance in E. coli Isolated from Chicken Cecum Samples and Factors Contributing to Antimicrobial Resistance in Nepal. Trop Med Infect Dis 2022, Vol 7, Page 249. 2022;7: 249. doi:10.3390/TROPICALMED7090249

15. Koirala A, Bhandari P, Shewade HD, Tao W, Thapa B, Terry R, et al. Antibiotic use in broiler poultry farms in kathmandu valley of nepal: Which antibiotics and why? Trop Med Infect Dis. 2021;6: 1211. doi:10.3390/TROPICALMED6020047/S1

16. Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, et al. Antibiotic resistance pattern and virulence genes content in avian pathogenic Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal. BMC Vet Res. 2018;14: 1–6. doi:10.1186/S12917-018-1442-Z/TABLES/2

17. The Kathmandu Post. Nepal’s population is 29,192,480. 2022. Available: https://tkpo.st/3IDg6cD

18. QGIS. 2023 [cited 28 Mar 2023]. Available: https://www.qgis.org/en/site/

19. Napit R, Poudel A, Pradhan SM, Manandhar P, Ghaju S, Sharma AN, et al. Newcastle disease burden in Nepal and efficacy of Tablet I2 vaccine in commercial and backyard poultry production. PLoS One. 2022;18.

20. Ferguson-Noel N, Armour NK, Noormohammadi AH, El-Gazzar M, Bradbury JM. Mycoplasmosis.

347 Dis Poult. 2019; 907–965. doi:10.1002/9781119371199.CH21

348 21. Saif Y, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE. Diseases of poultry. Dis
349 poultry. 2003.

350 22. Blagodatski A, Trutneva K, Glazova O, Mityaeva O, Shevkova L, Kegeles E, et al. Avian Influenza in
351 Wild Birds and Poultry: Dissemination Pathways, Monitoring Methods, and Virus Ecology. Pathog
352 2021, Vol 10, Page 630. 2021;10: 630. doi:10.3390/PATHOGENS10050630

353 23. Hill NJ, Bishop MA, Trovão NS, Ineson KM, Schaefer AL, Puryear WB, et al. Ecological divergence
354 of wild birds drives avian influenza spillover and global spread. PLOS Pathog. 2022;18: e1010062.
355 doi:10.1371/JOURNAL.PPAT.1010062

356 24. Tanquilut NC, Espaldon MVO, Eslava DF, Ancog RC, Medina CDR, Paraso MGV, et al. Quantitative
357 assessment of biosecurity in broiler farms using Biocheck.UGent in Central Luzon, Philippines. Poult
358 Sci. 2020;99: 3047–3059. doi:10.1016/J.PSJ.2020.02.004

359 25. Ayim-Akonor M, Krumkamp R, May J, Mertens E. Understanding attitude, practices and knowledge
360 of zoonotic infectious disease risks among poultry farmers in Ghana. Vet Med Sci. 2020;6: 631–638.
361 doi:10.1002/VMS3.257

362 26. Melo AM, Stevens DA, Tell LA, Veríssimo C, Sabino R, Xavier MO. Aspergillosis, Avian Species
363 and the One Health Perspective: The Possible Importance of Birds in Azole Resistance. Microorg
364 2020, Vol 8, Page 2037. 2020;8: 2037. doi:10.3390/MICROORGANISMS8122037

365 27. An HSI Report: Human Health Implications of Intensive Poultry Production and Avian Influenza.

366 28. Collins JD, Wall PG. Food safety and animal production systems: controlling zoonoses at farm level.
367 Rev Sci Tech. 2004;23: 685–700. doi:10.20506/RST.23.2.1510

368 29. Davies R, Wales A. Antimicrobial Resistance on Farms: A Review Including Biosecurity and the
369 Potential Role of Disinfectants in Resistance Selection. Compr Rev Food Sci Food Saf. 2019;18: 753–
370 774. doi:10.1111/1541-4337.12438

371 30. Bagal UR. Pathology and Molecular Diagnosis of Mycoplasma gallisepticum and Mycoplasma
372 synoviae Infections in Broiler Chickens from Western Maharashtra, India. J Anim Res. 2019.

373 31. Meerburg BG, Bonde M, Brom FWA, Endepols S, Jensen AN, Leirs H, et al. Towards sustainable
374 management of rodents in organic animal husbandry. [cited 11 Apr 2023]. doi:10.1016/S1573-
375 5214(04)80014-9

376 32. Rodents in poultry housing: A multi-faceted plan of attack | Stockfarm. [cited 11 Apr 2023]. Available:
377 https://journals.co.za/doi/abs/10.10520/ejc-vp_stock_v11_n5_a25

378 33. Prajapati M, Ranjit E, Shrestha R, Shrestha SP, Adhikari SK, Khanal DR. Status of Antibiotic
379 Residues in Poultry Meat of Nepal. Nepal Vet J. 2018;35: 55–62. doi:10.3126/NVJ.V35I0.25240

380 34. Rijal KR, Banjara MR, Dhungel B, Kafle S, Gautam K, Ghimire B, et al. Use of antimicrobials and
381 antimicrobial resistance in Nepal: a nationwide survey. Sci Reports 2021 111. 2021;11: 1–14.
382 doi:10.1038/s41598-021-90812-4

383 35. Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrient
384 utilization, performance, and health of poultry. J Anim Sci Biotechnol. 2019;10: 1–11.
385 doi:10.1186/S40104-018-0310-9/FIGURES/1

386 36. Schokker D, Jansman AJM, Veninga G, de Bruin N, Vastenhouw SA, de Bree FM, et al. Perturbation
387 of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal
388 immune development. BMC Genomics. 2017;18: 1–14. doi:10.1186/S12864-017-3625-6/FIGURES/5

389 37. Dandachi I, Chabou S, Daoud Z, Rolain JM. Prevalence and emergence of extended-spectrum
390 cephalosporin-, carbapenem- and colistin-resistant gram negative bacteria of animal origin in the
391 Mediterranean basin. Front Microbiol. 2018;9: 2299. doi:10.3389/FMICB.2018.02299/BIBTEX

392 38. WHO. 2021 AWaRe classification. [cited 27 Mar 2023]. Available:
393 https://www.who.int/publications/i/item/2021-aware-classification

394 39. Ahmed S, Hasan A, Khatun R, Howlader AJ, Islam N, Alam K, et al. Validation of the Declared
395 Withdrawal Periods of Antibiotics Evaluation of Disinfectants towards Pathogens View project Razia

Khatun Validation of the Declared Withdrawal Periods of Antibiotics. Univers J Public Heal. 2018;6: 14–22. doi:10.13189/ujph.2018.060103

40. Dhakal S, Gompo TR. Farmer's Knowledge on Anti-Microbial Resistance, Their Practise Of Antimicrobials Usage and Biosecurity Status of Poultry Farms in Kathmandu Valley and Chitwan District. Malaysian Anim Husb J. 2021;1: 26–32. doi:10.26480/mahj.01.2021.26.32

Figure Captions

Figure 4: Selected poultry farms in the districts (Kavre, Sindhupalchowk, Ramechhap and Nuwakot) surrounding Kathmandu valley. The top map shows location of the districts in Nepal (shown in red border) and the bottom map shows their location in respect with Kathmandu Valley (shown just for reference, not a sampling site). The map was generated using QGIS software, Version 3.30.0

Figure 5: Graphical representation of BBCM scores received by all 16 farms for various biosafety & biosecurity parameters. Scores (percentage) received by all farms assessed in this study using BBCM score. Numbers in parenthesis in the legend refers to total number of activities assessed within each criterion.

Figure 6: Various types of antibiotics used (in %) in the surveyed poultry farms.

Supplementary Figure Captions

Figure S 1 (A and B): Bacterial and viral diseases detected in poultry farms of Nuwakot District. The four farms were numbered from N1 to N4. Each sample represents pooled oral and cloacal samples. The gel was run with ladder in the first well and positive and negative controls in the last two well respectively. The two images show results of PCR for Mg (left, A) and IBD (right, B).

Figure S 2: IAV detected in poultry farms of Ramechhap District. The four farms were numbered from R1 to R4. Each sample represents pooled oral and cloacal samples. The gel was run with ladder in the first well and positive and negative controls in the last two well respectively.

Figure S 3: Mg detected in poultry farms of Sindhupalchowk District. The four farms were numbered from S1 to S4. Each sample represents pooled oral and cloacal samples. The gel was run with ladder in the first well and positive and negative controls in the last two well respectively.

Figure S 4 (A and B): Bacterial diseases detected in poultry farms of Ramechhap District. The four farms were numbered from R1 to R4. Each sample represents pooled oral and cloacal samples. The gel was run with ladder in the first well and positive and negative controls in the last two well respectively. The two images show results of PCR for Mg (left, A) and Ms (right, B).

Figure S 5: Mg detected in poultry farms of Kavre District. The four farms were numbered from K1 to K4. Each sample represents pooled oral and cloacal samples. The gel was run with ladder in the first well and positive and negative controls in the last two well respectively.

435 **Table Captions**

436

437 Table 4: PCR primers for each of the pathogen designed using PrimerBlast®

438 Table 5: Total number and percentage of BBCM criteria met by the farms as per our assessment checklist.
439 Overall average BBCM score was calculated by dividing the sum of all BBCM fulfilled criteria (n) and
440 calculating the percentage. The highest BBCM score (Green cell- Sindhupalchowk Farm 4) was 67%, and the
441 lowest BBCM score was 12% (Orange cell- Kavre Farm 3). The total number of activities assessed in each
442 criterion are listed within parenthesis. Details of the activities assessed are shown in Supplementary Table 1.

443 Mg – Mycoplasma gallisepticum, Ms – Mycoplasma synoviae, IBD – Infectious Bursal Disease, IAV –
444 Influenza A Virus

445 Table 6: Knowledge and perception of AMR among poultry farmers. Responses (in percentage) obtained from
446 farmers on AMR-related questions.

447

448 **Supplementary Table Captions**

449 Supplementary Table 1: Biosafety and biosecurity checklist used to assess the farms

450 Supplementary Table 2: Antibiotic stewardship survey used to assess farm owners' knowledge on antibiotics
451 and their usage.