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Supplemental Material

The Prevalence of Extended-Spectrum Beta-Lactamase–Producing Multidrug-Resistant *Escherichia coli* in Poultry Chickens and Variation according to Farming Practices in Punjab, India

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Survey. Survey administered to farmers during structured interview regarding farm managerial and antimicrobial use practices.

Supplemental Material, Table S1: Zone diameter interpretive standards (i.e., zone diameter breakpoints) for Enterobacteriaceae as per CLSI (2013) disk diffusion guidelines.

Antimicrobial Agent	Antimicrobial Class	Disk Content	Zone Diameter Breakpoints (nearest whole mm)		
			<i>S</i>	<i>I</i>	<i>R</i>
Ampicillin (AMP)	β -lactam / Penicillin	10 μ g	≥ 17	14-16	≤ 13
Aztreonam (AT)	Monobactam	30 μ g	≥ 21	18-20	≤ 17
Ceftriaxone (CTR)	Cephalosporin / Cephem (3 rd Generation)	30 μ g	≥ 23	20-22	≤ 19
Cefuroxime (CXM)	Cephalosporin / Cephem (2 nd Generation)	30 μ g	≥ 18	15-17	≤ 14
Ciprofloxacin (CIP)	Fluoroquinolone	5 μ g	≥ 21	16-20	≤ 15
Chloramphenicol (C)	Amphenicol	30 μ g	≥ 18	13-17	≤ 12
Gentamicin (GEN)	Aminoglycoside	10 μ g	≥ 15	13-14	≤ 12
Imipenem (IPM)	Carbapenem	10 μ g	≥ 23	20-22	≤ 19
Nalidixic Acid (NA)	Quinolone	30 μ g	≥ 19	14-18	≤ 13
Nitrofurantoin (NIT)	Nitrofurantoin	300 μ g	≥ 17	15-16	≤ 14
Tetracycline (TE)	Tetracycline	30 μ g	≥ 15	12-14	≤ 11
Co-Trimoxazole (COT)	Folate Pathway Inhibitor / Sulfonamide	1.25 / 23.75 μ g	≥ 16	11-15	≤ 10

Supplemental Material, Table S2: Farm characterization, antimicrobial usage practices and awareness of hygiene and sanitation measures for all 18 poultry farms included in the study based on questionnaire data. Please note that two farms did not complete the entire questionnaire and their responses were recorded as “No Response” where applicable. Also note that all survey data on use of specific antimicrobials was self-reported and thus relied on farmer knowledge or records of antimicrobial use.

Question	Category	Number of Responses (%)	
Farm Characterization			
<i>Type of Farm / Facility Type</i>	Broiler	9 (50.0)	
	Contracted	3 (33.3)	
	Independent	6 (66.6)	
	Layer	9 (50.0)	
	Contracted	3 (33.3)	
	Independent	6 (66.6)	
Antimicrobial Usage			
<i>Antimicrobial Use Reported</i>	Yes	16 (88.9)	
	No	0 (0.0)	
	No Response	2 (11.1)	
<i>Purpose of Antimicrobial Use</i>	Growth Promotion	Yes	12 (66.7)
		No	4 (22.2)
		No Response	2 (11.2)
	Disease Treatment	Yes	16 (88.9)
		No	0 (0.0)
		No Response	2 (11.2)
	Disease Prevention	Yes	16 (88.9)
		No	0 (0.0)
		No Response	2 (11.2)
<i>Awareness of AGPs in Feed Purchased from Feed Mills</i>	Yes	6 (33.3)	
	No	10 (55.6)	
	No Response	2 (11.2)	
<i>Farms Reporting Use of Specific Antimicrobials</i>			
Tetracyclines (Oxytetracycline, Doxycycline)		9 (50.0)	
Fluoroquinolones (Ciprofloxacin, Enrofloxacin or Levofloxacin)		9 (50.0)	
Aminoglycosides (Neomycin)		8 (44.4)	
Trimethoprim / Sulphadiazine		2 (11.2)	

Colistin		1 (5.6)
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Hygiene & Infection Control Practices

<i>Formal Training on Diagnosis of Disease</i>	Yes	0 (0.0)
	No	14 (77.8)
	No Response	4 (22.2)
<i>Isolate or Quarantine Sick Animals</i>	Yes	15 (83.3)
	No	1 (5.6)
	No Response	2 (11.2)
<i>Farms Reporting Precautions Taken When Entering Sheds</i>		
No Precautions are Taken		12 (66.7)
Foot dips / rubs		4 (22.2)
No Response		2 (11.2)

Supplemental Material, Table S3: Purpose of antimicrobial use across all farms and disaggregated by farm (broiler / layer) and facility (independent / contracted) type for the 16 farms that completed the entire questionnaire. Percentages shown in parentheses.

Farm	Facility	Farms	Reported Purpose of Antimicrobial Use		
			N	Growth Promotion	Disease Treatment
Broiler	Independent	6	5 (83.3)	6 (100.0)	6 (100.0)
	Contracted	2	2 (100.0)	2 (100.0)	2 (100.0)
	Overall	8	7 (87.5)	8 (100.0)	8 (100.0)
Layer	Independent	6	5 (83.3)	6 (100.0)	6 (100.0)
	Contracted	2	0 (0.0)	2 (100.0)	2 (100.0)
	Overall	8	5 (62.5)	8 (100.0)	8 (100.0)
Overall		16	12 (75.0)	16 (100.0)	16 (100.0)

Supplemental Material, Table S4: Prevalence of *E. coli* resistant to 11 antimicrobials across all farms (overall), disaggregated by type of farm (broiler / layer) and type of facility (contracted, CF or independent, IU) for the 18 farms included in the study ($n = 1556$ isolates). Resistance prevalence (%) is shown in parentheses.

Antimicrobial	Overall	Broiler			Layer		
		Overall	CF	IU	Overall	CF	IU
Ampicillin (AMP)	682 (43.8)	412 (51.5)	136 (51.7)	276 (51.4)	270 (35.7)	74 (32.6)	196 (37.1)
Chloramphenicol (C)	111 (7.1)	95 (11.9)	14 (5.3)	81 (15.1)	16 (2.1)	0 (0.0)	16 (3.0)
Ciprofloxacin (CIP)	613 (39.4)	463 (57.9)	138 (52.5)	325 (60.5)	150 (19.8)	31 (13.7)	119 (22.5)
Cotrimoxazole (COT)	656 (42.2)	413 (51.6)	151 (57.4)	262 (48.8)	243 (32.1)	35 (15.4)	208 (39.3)
Ceftriaxone (CTR)	62 (4.0)	53 (6.6)	8 (3.0)	45 (8.4)	9 (1.2)	1 (0.4)	8 (1.5)
Cefuroxime (CXM)	60 (3.9)	52 (6.5)	8 (3.0)	44 (8.2)	8 (1.1)	0 (0.0)	8 (1.5)
Gentamicin (GEN)	200 (12.9)	165 (20.6)	50 (19.0)	115 (21.4)	35 (4.6)	6 (2.6)	29 (5.5)
Imipenem (IPM)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nalidixic Acid (NX)	1340 (86.1)	770 (96.2)	246 (93.5)	524 (97.6)	570 (75.4)	134 (59.0)	436 (82.4)
Nitrofurantoin (NIT)	286 (18.4)	188 (23.5)	51 (19.4)	137 (25.5)	98 (13.0)	16 (7.0)	82 (15.5)
Tetracycline (TE)	731 (47.0)	470 (58.8)	155 (58.9)	315 (58.7)	261 (34.5)	56 (24.7)	205 (38.8)

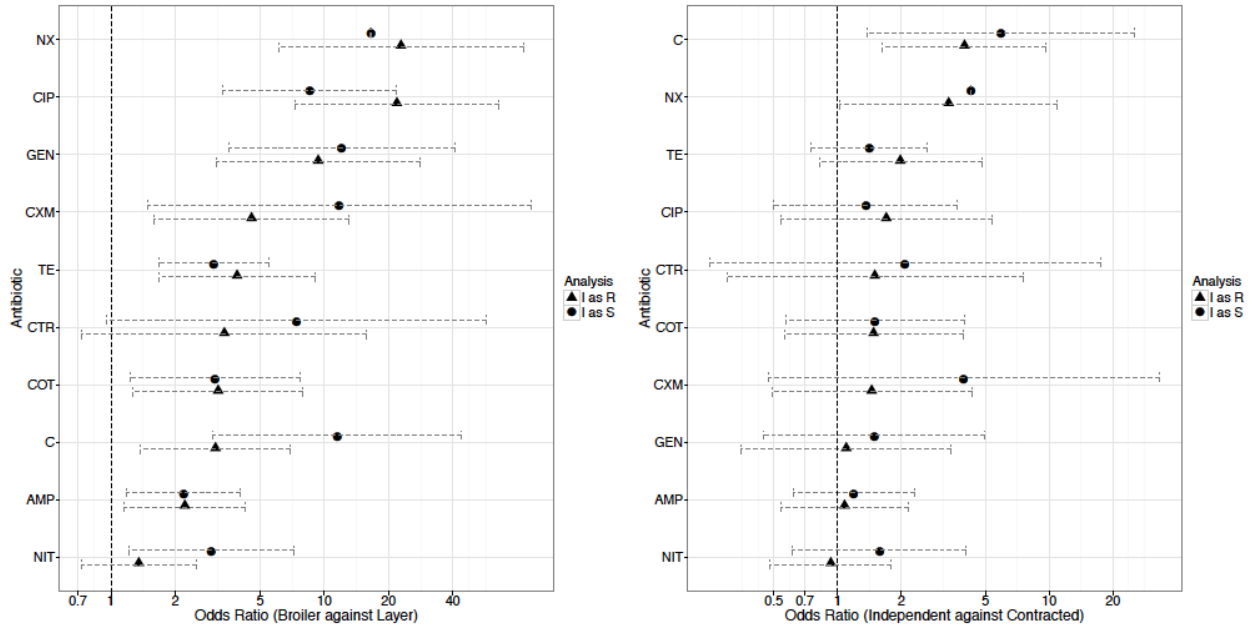
Supplemental Material, Table S5: Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae across all farms (overall) and disaggregated by type of farm (broiler / layer) for the 17 farms included in the ESBL survey ($n = 510$ birds). Resistance prevalence (%) is shown in parentheses.

Farm	Facility	ESBL Status	
		ESBL –	ESBL +
Broiler	Independent	22 (12.2)	158 (87.8)
	Contracted	12 (13.3)	78 (86.7)
	Overall	34 (12.6)	236 (87.4)
Layer	Independent	93 (62.0)	57 (38.0)
	Contracted	46 (51.1)	44 (48.9)
	Overall	139 (57.9)	101 (42.1)
Overall		173 (33.9)	337 (66.1)

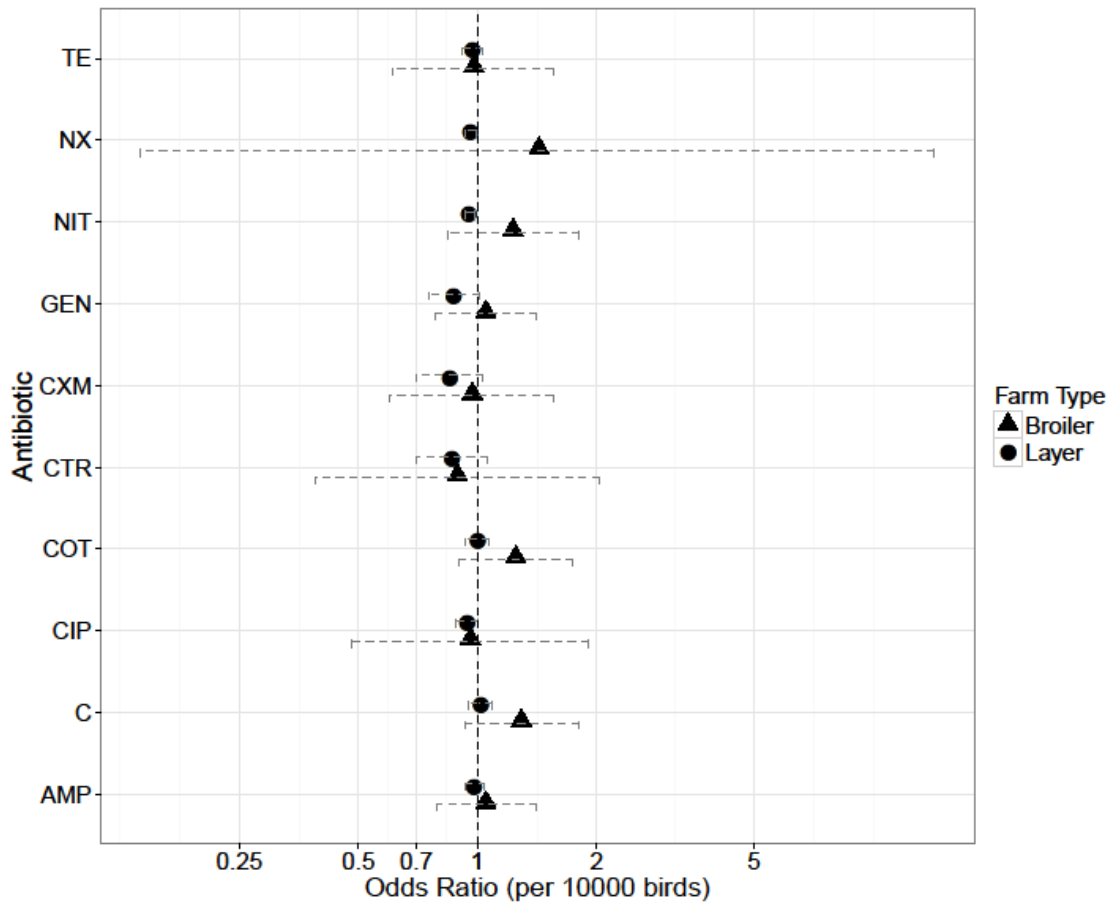
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Farm	Facility	Isolates		Resistance Classification		
		N	Not resistant	Singly-Resistant (1)	Moderate MDR (2-4)	Extreme MDR (>4)
Broiler	Independent	537	0 (0.0)	31 (5.8)	311 (57.9)	195 (36.3)
	Contracted	263	2 (0.8)	19 (7.2)	159 (60.5)	83 (31.6)
	Overall	800	2 (0.3)	50 (6.3)	470 (58.8)	278 (34.8)
Layer	Independent	529	16 (3.0)	155 (29.3)	292 (55.2)	66 (12.5)
	Contracted	227	31 (13.7)	101 (44.5)	89 (39.2)	6 (2.6)
	Overall	756	47 (6.2)	256 (33.9)	381 (50.4)	72 (9.5)
Overall		1556	49 (3.1)	306 (19.7)	851 (54.7)	350 (22.5)

Supplemental Material, Figure S1: Sensitivity analysis to compare the effects of treating “intermediate” isolates as “susceptible” (“I as S”) compared to “resistant” (“I as R”), using logistic regression of resistance profiles on farm and facility type. The figures shows odds ratios for resistance against each antimicrobial and associated 95% confidence intervals for effects of farm type (left panel) and facility type (right panel).



Supplemental Material, Figure S2: Odds ratios of increased resistance prevalence to all antimicrobials for an increase in farm size by 10,000 birds, stratified by farm type (broiler and layer) and adjusted for facility type. The x-axis represents odds ratios in a log scale. The horizontal lines represent 95% confidence intervals for the estimated odds ratios.



Supplemental Material, Survey: Survey administered to farmers during structured interview regarding farm managerial and antimicrobial use practices. Adapted from a survey used in another Public Health Foundation of India study (Kakkar and Rogawski 2013). Please note that both branded facilities (BF) and unbranded facilities (UF) were treated as contracted facilities (CF) in our analysis given these facilities both produce poultry and poultry products under a contractual arrangement with large-scale poultry producers.

SECTION A: PERSONAL/GENERAL INFORMATION

Name of Farmer/Respondent: _____ Sex: Male Female

Farmer's Telephone No. _____

Name of farm (if applicable): _____

District: _____ Town/Village: _____

Poultry Operation: Broiler [B] Layer [L]

Farm type: Branded facility [BF] Unbranded facility [UF] Independent Unit [IU]

Type(s) of animal associated with owner: (select all that apply)

Chicken Duck Other, including pet animals (canine, bird) or ruminants (cattle, goat, sheep): _____

EXAMPLE SAMPLE ID:

Poultry (Cloacal Swab) Sample 1 ID: __ - __ __ - __ __ - 01

<p style="text-align: center;">Poultry Sample ID Generation: Poultry Operation + Interview Serial No. + Facility type + Sample No. Ex: B-01-BF-01 Ex: L-04-IU-02</p>

SECTION B: HYGIENE AND DISEASE CONTROL PRACTICES

1. What precautions do you and others in your farm take when entering a chicken shed?
 No precautions Gloves Mask Foot dips/rubs Other: _____

2. Once you have sold a flock, do you wait before introducing a new flock to the same shed?
 Yes No
 - a. If YES, why? _____
 - a. What is the average time between flocks? _____ days
 - b. If NO, why not? _____

3. I will now ask you a few questions about how you maintain hygiene in the chicken sheds.
[For each of the following procedures, ask the following questions]
 - a. How often is this activity conducted?
 - b. What methods or products are used? Examples:
 - a. Lime wash
 - b. Disinfectant liquid (quaternary ammonium compounds)
 - c. Fumigation
 - d. Flame burning
 - e. Combination
 - f. Other
 - c. Who is in charge of this activity?

Method of sanitation & hygiene	a. Frequency	b. Method(s) used	c. Who is responsible?
General cleaning of poultry sheds			
Removal of waste/droppings from poultry sheds			
Disinfection of poultry sheds			
Cleaning of feed containers			
Cleaning of water applicators			
Removal of carcasses			
Other (specify)			

4. Have your poultry received any vaccinations? If yes, please list, to the best of your knowledge all diseases for which they have been vaccinated:
- Ranikhet disease (Newcastle disease)
 - Infectious Bursal Disease (Gumboro disease)
 - Avian Infectious Bronchitis
 - Marek's disease
 - Fowl Pox
 - Others, specify: _____
5. What do you do when you notice that one of your chickens is sick?
- I treat the animal myself without consulting anyone else
 - I contact the contract company for information
 - I go to seek help from a chemist/pharmacist
 - I call a disease investigation officer or state department veterinarian/para-veterinarian
 - I take a sample to _____
 - Other (specify) _____
6. Do you isolate or quarantine sick animals? Yes No
7. When do you decide to use an antibiotic for your chickens?
- When a veterinarian prescribes or recommends it
 - When I see symptoms or a condition that I have treated successfully with antibiotics in the past or know it can be treated with antibiotics
 - When friends or family recommend that I do
 - Other (specify) _____
- a. Do you have any formal training on the diagnosis of disease in animals?
- Yes No

SECTION C: USE OF ANTIBIOTICS IN POULTRY PRODUCTION

8. We may have already discussed this, but could you please tell me again whether you give your flocks any antibiotics? Yes No
9. For what general purposes do you give them antibiotics?
- Promote growth of chickens Treat diseases in chickens
 - Prevent diseases in chickens Other (specify) _____
 - Not sure
10. What company do you get your feed from? _____
11. Are you aware of antibiotics (AGPs) included in the feed? Yes No

12. What different stages of feed are administered, when are they given, and for how long are they given?

Stage of feed	Source of Feed (Brand or Name)	Number of days given?	Physical Form
Starter 1 (Pre-starter)		_____ days	<input type="checkbox"/> Mash <input type="checkbox"/> Crumble <input type="checkbox"/> Pellets
Starter 2		_____ days	<input type="checkbox"/> Mash <input type="checkbox"/> Crumble <input type="checkbox"/> Pellets
Finisher 1 (Pre-finisher)		_____ days	<input type="checkbox"/> Mash <input type="checkbox"/> Crumble <input type="checkbox"/> Pellets
Finisher 2		_____ days	<input type="checkbox"/> Mash <input type="checkbox"/> Crumble <input type="checkbox"/> Pellets

13. List all types of feed (brand names) used on the farm and their antibiotic content (types and quantities). *[Ask to see the feed label and take a picture/record all antibiotics]*

Feed/Brand Name	Antibiotics (AGPs)	Quantity of Antibiotics

14. Do you have any control over the antibiotics (AGPs) included in the feed? Yes No

a. If YES, do you prefer specific AGPs in the feed? Why?

b. If NO, do you prefer to add additional AGPs? Why?

15. Do you keep records of drugs used on your farm? Yes No

a. If YES, may we see them? [TAKE PICTURE OF RECORDS]

[Record information from records in its original format; use back of this page if necessary]

16. What antibiotics and other medicines do you routinely give your chickens? [For each drug ask the following questions and record in the table below]:

- a. What is the name of the drug? Is it known by more than one name? If so, please tell me all names you use for this drug.
- b. At how many weeks do you administer this drug? [If administered multiple times, record each time on a separate line]
- c. What dose do you administer and for how many days?
- d. Why do you give this drug?
- e. How do you administer this drug?

	a. Drug/ Vaccine	b. Interval/ Frequency	c. Dosage amount (ml/mg) and duration	d. Purpose	e. Route of administration
1			Amount: _____ Duration: _____	<input type="checkbox"/> Growth promotion <input type="checkbox"/> Disease treatment <input type="checkbox"/> Prevention of disease <input type="checkbox"/> Not sure <input type="checkbox"/> Other: _____	<input type="checkbox"/> Feed <input type="checkbox"/> Water <input type="checkbox"/> Other (specify):
2			Amount: _____ Duration: _____	<input type="checkbox"/> Growth promotion <input type="checkbox"/> Disease treatment <input type="checkbox"/> Prevention of disease <input type="checkbox"/> Not sure <input type="checkbox"/> Other: _____	<input type="checkbox"/> Feed <input type="checkbox"/> Water <input type="checkbox"/> Other (specify):
3			Amount: _____ Duration: _____	<input type="checkbox"/> Growth promotion <input type="checkbox"/> Disease treatment <input type="checkbox"/> Prevention of disease <input type="checkbox"/> Not sure <input type="checkbox"/> Other: _____	<input type="checkbox"/> Feed <input type="checkbox"/> Water <input type="checkbox"/> Other (specify):
4			Amount: _____ Duration: _____	<input type="checkbox"/> Growth promotion <input type="checkbox"/> Disease treatment <input type="checkbox"/> Prevention of disease <input type="checkbox"/> Not sure <input type="checkbox"/> Other: _____	<input type="checkbox"/> Feed <input type="checkbox"/> Water <input type="checkbox"/> Other (specify):
5			Amount: _____ Duration: _____	<input type="checkbox"/> Growth promotion <input type="checkbox"/> Disease treatment <input type="checkbox"/> Prevention of disease <input type="checkbox"/> Not sure <input type="checkbox"/> Other: _____	<input type="checkbox"/> Feed <input type="checkbox"/> Water <input type="checkbox"/> Other (specify):

17. Who usually administers all these drugs to the chickens? (Check all that apply)

- Myself
- Farm Manager (if different from self)
- My children [*specify whether son or daughter*] _____
- My farmworker
- A veterinarian
- Para-veterinarian
- Other (specify) _____

18. Do you have any training on the use of antibiotics? Yes No
 a. IF YES, what kind of training?

19. Do you usually consult anybody about the use of antibiotics? Yes No
 a. If YES, whom do you consult and how times have you consulted them in the last one year?

Service provider consulted	Tick as appropriate	No. of times consulted in past year
Veterinary doctor	<input type="checkbox"/>	
Para-veterinary provider	<input type="checkbox"/>	
Chemist/Pharmacist	<input type="checkbox"/>	
Local traditional animal health practitioner	<input type="checkbox"/>	
Contracting company	<input type="checkbox"/>	
Other (Specify) _____	<input type="checkbox"/>	

20. Where does your farm purchase the antibiotics used in your poultry? (Check all that apply)

- Veterinary pharmacy
- Human pharmacy
- Individual veterinarian
- Supplied by contractor (for contract farmers)
- Other (Specify): _____
- Don't know

21. Does the seller provide any information about the drug? Yes No Don't know
 IF YES:

- a. What kind of information do they provide?
 - About the dosage
 - About drug administration, e.g., the route of administration
 - About the withdrawal period
 - Other (specify) _____
- b. Does the seller **voluntarily** provide the information about the drug?
 - Yes No

[Ask whether the buyer has to request for the information]

22. When was the last time that a round of antibiotics was given to one of your flocks?
 days ago weeks ago months ago not given

For what purpose were the antibiotics given?

- Growth promotion
- Disease treatment
- Prevention of disease
- Not sure
- Other, specify: _____

[Ask Q 23 if *CONTRACT farm*]

23. Do you require permission from the company to use antibiotics? Yes No

SECTION D: FARM CHARACTERIZATION

24. Do you own this farm or have you leased it?
 Own independent farm Own commercial farm Leased farm (owner name): _____

25. Do you sell branded or unbranded chicken or eggs from this farm?
 Branded Unbranded

26. What is the size of this farm? _____ *Bigha* Acres

27. How many sheds do you have on this farm? _____ sheds

28. What is the approximate number of birds in each shed? _____ birds per shed

29. What is the total number of birds on this farm? _____ birds

30. What is the approximate size of each shed (in m²)? _____

31. How many flocks of poultry have you had in the last calendar year? _____ flocks

Please answer the following questions with respect to an average flock at your farm.

Characteristics of flocks	Average flock
32. What is the average size of your flocks?	
33. What breed(s)/varieties are in your flocks?	
34. How many days on average do you keep each flock?	
35. How many chicks on average are placed in each flock?	
36. What proportion of birds die?	

SECTION E: OUTREACH SERVICES

37. How far is your farm from the following locations?

	Km		Km
Veterinary pharmacy/chemist		Human health pharmacy/chemist	
Veterinary clinic/hospital		Human health clinic/hospital	
Market		Major town	

38. How many times in the last 12 months has a veterinarian visited the farm? _____ times

39. What kind of information did the veterinarian provide?

- Information about feed
- General information on diseases that could affect your poultry
- Information for preventing disease (vaccines, drugs, hygiene)
- Information for treatment of diseases
- Other, specify: _____

40. Have you attended an animal health-related seminar/field demonstration in the last 12 months?

Yes No

a. If YES, how many animal health-related seminars/field days/field demonstrations have you attended in the last 12 months? _____

41. Are you a member of a livestock-related community-based organization (CBO)?

Yes No

If YES:

a. Which one(s)? (e.g. poultry cooperative society, etc.)

b. Does the CBO provide information on diseases that could affect your poultry?

Yes No

c. Does the CBO provide drug-related information for your poultry?

Yes No

i. IF YES, does it provide information on antibiotics?

Yes No

1. If, YES which specific information about antibiotics does the CBO provide? _____

SECTION F: KNOWLEDGE AND PERCEPTIONS OF ANTIBIOTIC USE

42. I will read to you some statements about antibiotics use and I would like you to tell me whether you strongly agree, just agree, are neutral, disagree, or strongly disagree with each of them.

Statement	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Don't Know
The first line treatment for sickness in my flocks should include antibiotics.						
Based on my experience, I have noted that the use of antibiotics makes my broilers grow faster or layers produce more eggs.						
I use antibiotics in my chickens because I feel competition with other producers.						
If I don't use antibiotics on my farm, it will expose my chickens to an unreasonable amount of risk.						
I have noted that some of the antibiotics I use in my birds do not cure diseases even at high doses.						
Antibiotics are regularly available to purchase for therapeutic use.						

43. In your opinion, what is more affordable in preventing animal disease, antibiotics or hygiene and sanitation measures?

Antibiotics

Hygiene and sanitation measures

SECTION G: DEMOGRAPHICS

Please provide the following information about yourself:

44. How old are you? _____ years

45. What is the highest level of school you have completed? _____ standard

Thank you for your time.

References:

Clinical and Laboratory Standards Institute (CLSI). 2013. *Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement; CLSI document M100-S23*. Clinical and Laboratory Standards Institute: Wayne, PA.

Kakkar M, Rogawski E. 2013. Antibiotic Use and Residues in Chicken Meat and Milk Samples from Karnataka and Punjab, India. Public Health Foundation of India: New Delhi, India.