ANTIBIOTIC RESISTANCE IN POULTRY ENVIRONMENT

Spread of Resistance from Poultry Farm to Agricultural Field

Based on CSE's Pollution Monitoring Laboratory Study: Antibiotic Resistance in Poultry Environment

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Contents

Foreword

Antibiotics are becoming increasingly ineffective. Antimicrobial Resistance (AMR)—Antibiotic Resistance (ABR) in particular—is a huge public-health threat globally, more so in a country like India. It is linked with high disease and economic burden on people and nations. It can also impact food safety, nutrition security, livelihood and attainment of Sustainable Development Goals. ABR is truly a 'One Health' issue which recognizes connections between humans, animals and the environment, both as a cause and solution.

Following the adoption of the Global Action Plan on AMR in 2015 at the World Health Assembly, countries are getting ready with their multi-year National Action Plans (NAPs) to address AMR in line with their nature of problem and extent of technical and financial resources at hand. Many countries have developed and shared their NAP by May 2017. Meanwhile, the World Health Organization (WHO), Food and Agricultural Organization of the United Nations (FAO) and World Organization for Animal Health (OIE) continue to support NAPs. The issue has also received global political support at the United Nations General Assembly in 2016.

While ABR is recognized as a 'One Health' issue, and a lot has been happening across the globe, the misuse of antibiotics in the food-animal production system and environmental spread of ABR continue to receive limited attention as compared to human health. For a country like India, which not only has a poor state of environment and waste management but is also among the top global producers of fish, poultry and dairy, the animal–environment contribution to ABR could be significant. Possibly much more than we could anticipate, if we take into account that the total volume of antibiotic use is likely much higher in animals than in humans.

In 2014, when CSE found antibiotic residues in chicken meat due to rampant non-therapeutic antibiotic use, there were questions raised about its linkages with ABR. We were told that residues do not mean resistance. Now, in order to understand the extent of resistance in poultry environment and its spread outside, our Pollution Monitoring Laboratory has tested for ABR in several poultry farms (broiler) across four states in north India. We have tested litter, poultry farm soil and agriculture soil where litter is used as manure. Our aim was to first understand the extent of ABR in the poultry environment and then establish if the resistance bacteria is moving out of poultry farms into the environment through waste disposal. What we have found indicates that urgent action is required to stop the misuse of antibiotics in poultry. In addition, waste management in the poultry industry must be improved significantly. Disposing of litter in the environment without destroying resistant bacteria is fraught with danger.

We believe that this study will add to the growing scientific evidence and reinforce the need for urgent action on ABR.

Chandra Bhushan

1. CSE study: Antibiotic resistance in poultry environment

1.1. Sampling and methodology

1.1.1 Sample collection

A total of 47 samples were collected from four north Indian states, i.e. Uttar Pradesh, Rajasthan, Haryana and Punjab in 2016-2017. Thirty-five samples were collected from 12 broiler poultry farms in nine districts across these states. All farms were located in different clusters, i.e. villages with at least three to four broiler farms. In six districts out of nine, samples were collected from only one cluster. In the remaining three, samples were collected from two clusters each. The number of birds in farms was 3,000–21,000. Antibiotics were used in all the farms but the exact package of practice was not disclosed.

From each farm, three types of samples were collected. One sample of litter was from inside the shed, one sample of soil was from outside the shed and one soil sample was from nearby agricultural land outside the farm, where reportedly litter was being used as manure. Additionally, for control, 12 more samples were collected from soil (from a nearby road) about 10–20 km from the respective farms. There were no apparent poultry farms nearby and reportedly litter was not thrown (see *Table 1: Sample collection*).

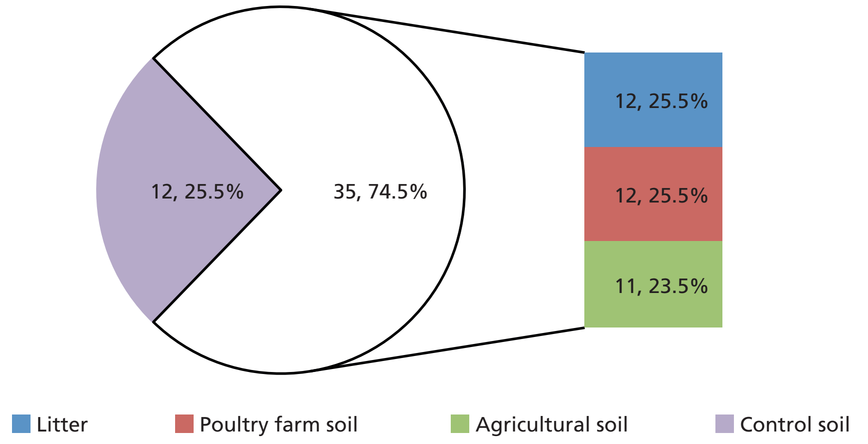
Collectively, a uniform break up of all the samples was attained as, for example, 12 samples each of litter and poultry farm soil, 11 samples of agricultural soil and 12 used as control (see *Fig 1: Sample types*). Only in the case of a farm in Jaipur, agricultural soil sample was not collected.

Table 1: Sample collection

	Total	Uttar Pradesh	Rajasthan	Haryana	Punjab
Samples	**47**	**17**	**8**	**15**	**7**
Samples from poultry farms	***35***	*12*	*5*	*12*	*6*
Control samples	***12***	*5*	*3*	*3*	*1*
Poultry farms	**12**	4	2	4	2
Clusters*	**12**	4	2	4	2
Districts	**9**	3 (Meerut, Bulandshahr, Ghaziabad)	2 (Alwar, Jaipur)	3 (Jind, Panipat, Gurugram)	1 (Ludhiana)

*A cluster refers to a village which has at least three to four broiler farms

Fig 1: Sample types



1.1.2 Isolation and characterization

One gram each of litter and soil samples was aseptically added separately into different sterile vials containing 9 ml of sterile normal saline. Further, they were subjected to ten-fold serial dilution. Samples collected were subjected to their microbial analysis for the isolation of *Escherichia coli*, *Klebsiella* sp. and *Staphylococcus* sp. These bacteria were selected due to their relevance to public health. The samples were also subjected to microbial analysis for Total Viable Count of bacteria. Different standard methodologies were used for the isolation of different bacteria:

- *Escherichia coli:* IS 5887 (Part I)—1976 (Reaffirmed 2005)
- *Klebsiella* sp.: Klebsiella Selective Agar Media (HiMedia)
- *Staphylococcus* sp.: IS 5887 (Part 8/Sec 1): 2002

Isolated cultures from all the samples were characterized and identified by using a combination of colony characteristics, morphology and different biochemical tests. Identity of about 10 per cent isolates, selected on the basis of geographical spread and frequency distribution, was confirmed by 16 S rDNA sequence analysis. The 16 S rDNA sequence analysis of the shortlisted cultures was done by an external laboratory, i.e. Chromous Biotech Pvt. Ltd., Bangalore. **The three identified bacteria were *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae)* and *Staphylococcus lentus (S. lentus).***

1.1.3 Antibiotic susceptibility tests (AST)

The antibiotic susceptibility pattern of all the isolated bacteria from each farm was determined using the disc diffusion method according to the Bauer-Kirby technique. The zones of inhibition obtained for each bacterium was compared with the standards of the Clinical and Laboratory Standards Institute (CLSI). The standards of European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used where CLSI standards were not available.

Sixteen antibiotics from 13 classes were selected based on use in poultry and importance to human health. These included 10 antibiotics from seven critically important classes as per the World Health Organization (WHO). In the case of *S. lentus,* susceptibility was tested against all antibiotics. However, for *E. coli* and *K. pneumoniae,* 13 antibiotics were used. Clindamycin, linezolid and azithromycin were not tested for *E. coli* and *K. pneumoniae* due to non-availability of standards (see *Table 2: Antibiotics against which susceptibility was tested).*

Table 2: Antibiotics against which susceptibility was tested

Antibiotics	**Antibiotic class**
Doxycycline hydrochloride	Tetracyclines
Amoxyclav	**Penicillins**
Nitrofurantoin	Nitrofurans
Levofloxacin	**Fluoroquinolones**
Ciprofloxacin	
Chloramphenicol	Amphenicols
Cefuroxime	Cephalosporins—first and second generation
Cefotaxime	**Cephalosporins—third, fourth and fifth generation**
Ceftriaxone	
Amikacin	**Aminoglycosides**
Gentamicin	
Co-trimoxazole	Sulfonamides, dihydrofolatereductase inhibitors and combinations
Meropenem	**Carbapenems**
Clindamycin	Lincosamides
Linezolid	**Oxazolidinones**
Azithromycin	**Macrolides and ketolides**

Note: Antibiotics in bold denote critically important; classified as per Critically Important Antimicrobials for human medicine—5th revision, Geneva: World Health Organization; 2017

1.2. Results and analysis

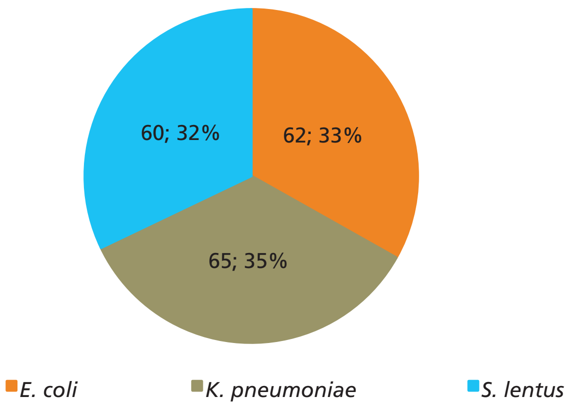
1.2.1 Isolation of bacteria

Overall 217 isolates of bacteria were isolated from all samples collected from poultry environment and for control. A total of 187 isolates of three bacteria, i.e. *E. coli*, *K. pneumoniae* and *S. lentus*, were derived in similar proportion from poultry environment comprising of samples from poultry litter, poultry farm soil and nearby agricultural soil (see *Fig 2: Bacteria isolated from poultry farm environment*).

Maximum isolates were derived from litter samples (125; 66.8 per cent). Collectively, over three-fourth of these were *E. coli* (46; 36.8 per cent) and *K. pneumoniae* (49; 39.2 per cent). From soil samples of poultry farms, least number of isolates were attained (24; 12.8 per cent) and most of these were of *S. lentus* (16; 66.7 per cent). There were 38 isolates (20 per cent) from soil samples from the agricultural land. Similar numbers of isolates of three bacteria were obtained from these.

In addition to the 187 isolates, 30 isolates were derived from control soil samples wherein litter was reportedly not used as manure. *S. lentus* was most prominent in these. *E. coli* could not be isolated from these samples. Only nine *K. pneumoniae* were isolated and almost all of those were from samples of Rajasthan (8/9).

Fig 2: Bacteria isolated from poultry farm environment



Isolates of the three bacteria obtained from litter samples and agricultural soil where litter were reportedly used as manure was similar in proportion. This was not the case with isolates from other sample types. For example, isolates of *S. lentus* were by far the most prominent in poultry farm soil and control soil samples and there were no *E. coli* isolates in control samples (see *Fig 3: Proportion of isolates in different samples*).

Multiple isolates were obtained from all farm locations. At least 10 isolates were derived from all 12 farms and average isolates per farm was 15. The *E. coli* and *K. pneumoniae* were isolated from litter samples of almost all the farms but only a few from poultry farm soil samples. In comparison to this, *S. lentus* was isolated from soil samples of more farms (see *Table 3: Isolates from poultry environment*).

Fig 3: Proportion of isolates in different samples (%)

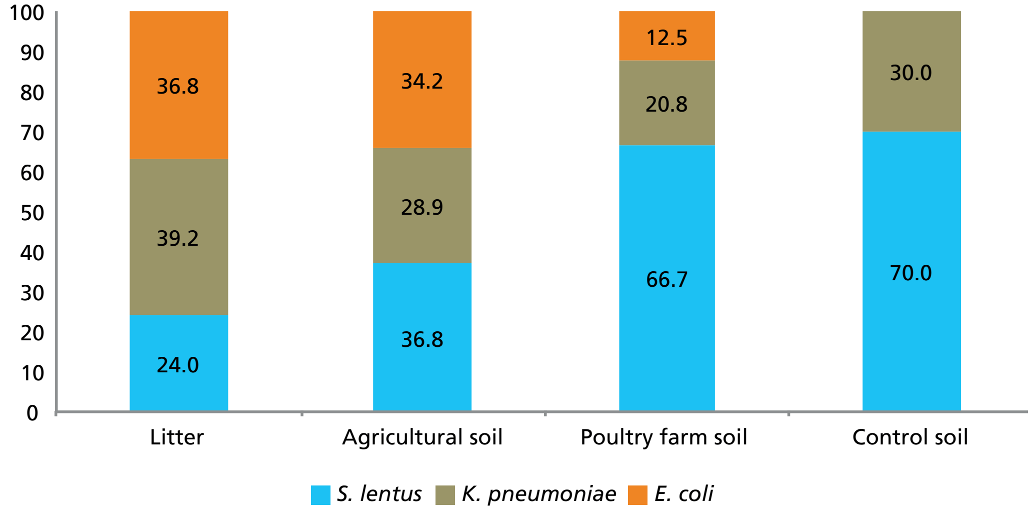


Table 3: Isolates from poultry environment

	E. coli			*K. pneumoniae*			*S. lentus*			Total
	Litter	Poultry farm soil	Agri-cultural soil	Litter	Poultry farm soil	Agri-cultural soil	Litter	Poultry farm soil	Agri-cultural soil	
Safidon town, Jind, Haryana	5		-	5		-	1		-	**11**
Kawi village, Panipat, Haryana (Farm 1)	5	-	2	6	-	-	4	-	2	**19**
Ahmadpur Majra village, Panipat, Haryana (Farm 2)	1	1	1	1	2	1	2	1	1	**11**
Sanpka village, Gurugram, Haryana	2	1	1	2	-	2	1	1	1	**11**
Mamepur village, Meerut, Uttar Pradesh	5	-	-	16	1	1	6	1	-	**30**
Bhaipur village, Bulandshahr, Uttar Pradesh (Farm 1)	2	-	1	-	-	3	3	2	3	**14**
Ranapur village, Bulandshahr, Uttar Pradesh (Farm 2)	3	-	1	3	-	-	4	3	2	**16**
Kushalya village, Ghaziabad, Uttar Pradesh	2	1	2	2	2	-	2	-	1	**12**
Rangala village, Alwar, Rajasthan	10	-	-	4	-	4	-	-	-	**18**
Morija village, Jaipur, Rajasthan	3	-	NA	2	-	-	2	3	NA	**10**
Kotla Shamshapur village, Ludhiana, Punjab (Farm 1)	4	-	3	4	-	-	3	2	2	**18**
Sangatpura village, Ludhiana, Punjab (Farm 2)	4	-	2	4	-	-	2	3	2	**17**
Total	**46**	**3**	**13**	**49**	**5**	**11**	**30**	**16**	**14**	**187**

*NA: Not applicable as sample could not be collected

1.2.2 High multidrug resistance in poultry environment

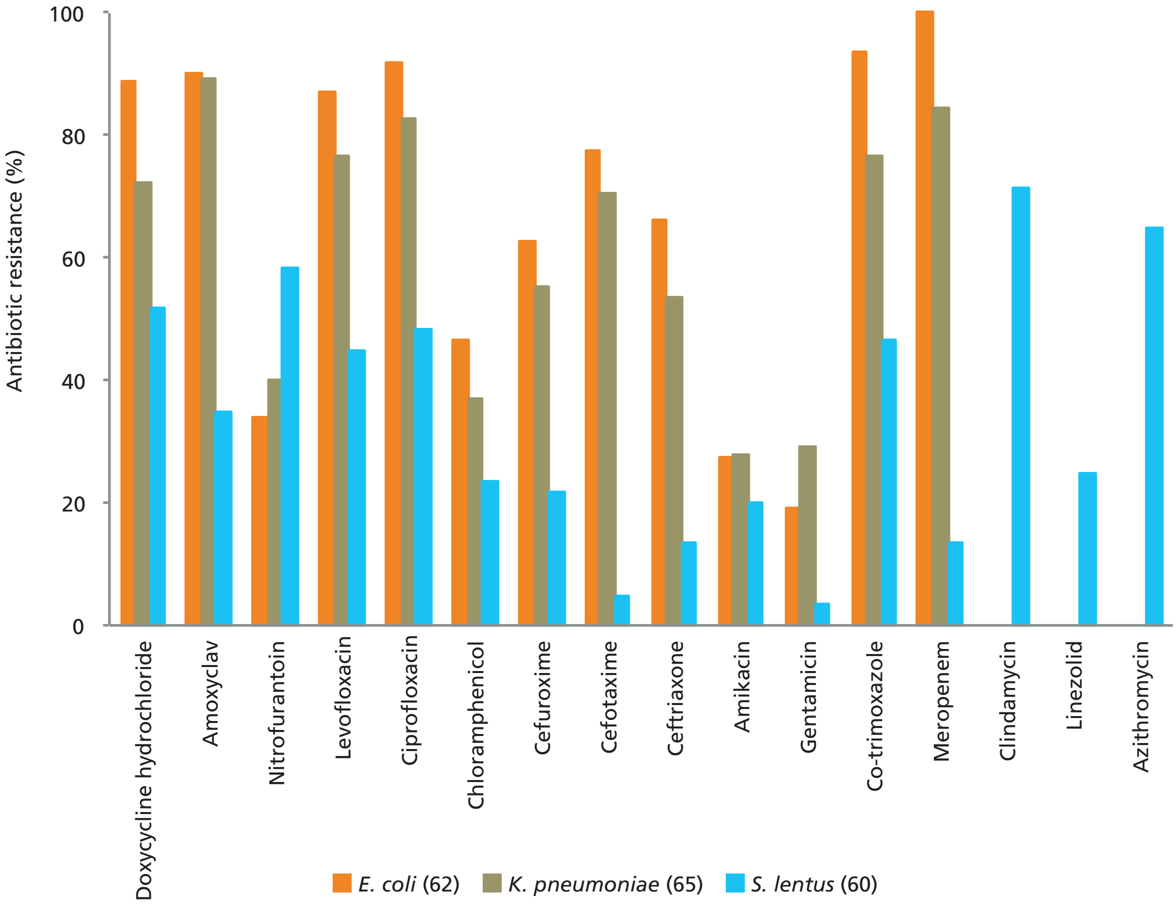
It was found that poultry farm environment comprising poultry litter, poultry farm soil and nearby agricultural land soil was a hotbed for multidrug resistant bacteria (see *Fig 4: Antibiotic resistance in poultry farm environment*). A large number of isolates belonging to all three bacteria were resistant against most antibiotics. Overall, the highest resistance was found in *E. coli*, followed by *K. pneumoniae* and *S. lentus*.

All 62 *E. coli* isolates were resistant to meropenem antibiotic. Meropenem belongs to carbapenems, a last-resort antibiotic class used in hospitals, and is classified as a 'high priority', critically important (CI) antibiotic class by the WHO. Very high resistance (>70 per cent) was found against co-trimoxazole and antibiotics of other CI classes such as penicillins, fluoroquinolones and one of the third- and fourth-generation cephalosporins (cefotaxime). For cefuroxime and ceftriaxone, high resistance (50–70 per cent) was observed. *E. coli* had low resistance (< 30 per cent) to tested aminoglycosides such as amikacin and gentamicin. Overall, 100 percent *E. coli* were multidrug resistant (MDR), i.e. resistant to antibiotics of three of more classes. About 40 per cent of all *E. coli* isolates were resistant to 10 or more antibiotics, and one in six isolates was resistant to at least 12 of the 13 antibiotics. Two isolates were resistant to all the 13 antibiotics tested.

In the case of *K. pneumoniae* isolates, very high resistance was found against antibiotics belonging to CI classes such as penicillins, fluoroquinolones, carbapenems and one of the third- and fourth-generation cephalosporins (cefotaxime). High resistance was observed against cefuroxime and ceftriaxone. As in *E. coli*, low resistance was found against both aminoglycosides tested. Overall, multidrug resistance was shown by 92.3 per cent of all isolates. Over 30 per cent of isolates were resistant to 10 or more antibiotics and 10 per cent were resistant to all tested antibiotics.

For *S. lentus*, high resistance was found in clindamycin, a lincosamide and azithromycin, a CI macrolide, while in case of co-trimoxazole and antibiotics belonging to other CI classes such of penicillins and fluoroquinolones, resistance observed was in the range of 30-50 per cent. The bacteria had low resistance to chloramphenicol and tested CI antibiotics belonging to carbapenems, oxazolidinones, aminoglycosides and third-, fourth- and fifth-generation cephalosporins. Overall, about 78 per cent of all *S. lentus* isolates were multidrug resistant. About one-fourth isolates of *S. lentus* were resistant to at least eight antibiotics.

Fig 4: Antibiotic resistance in poultry farm environment (%)



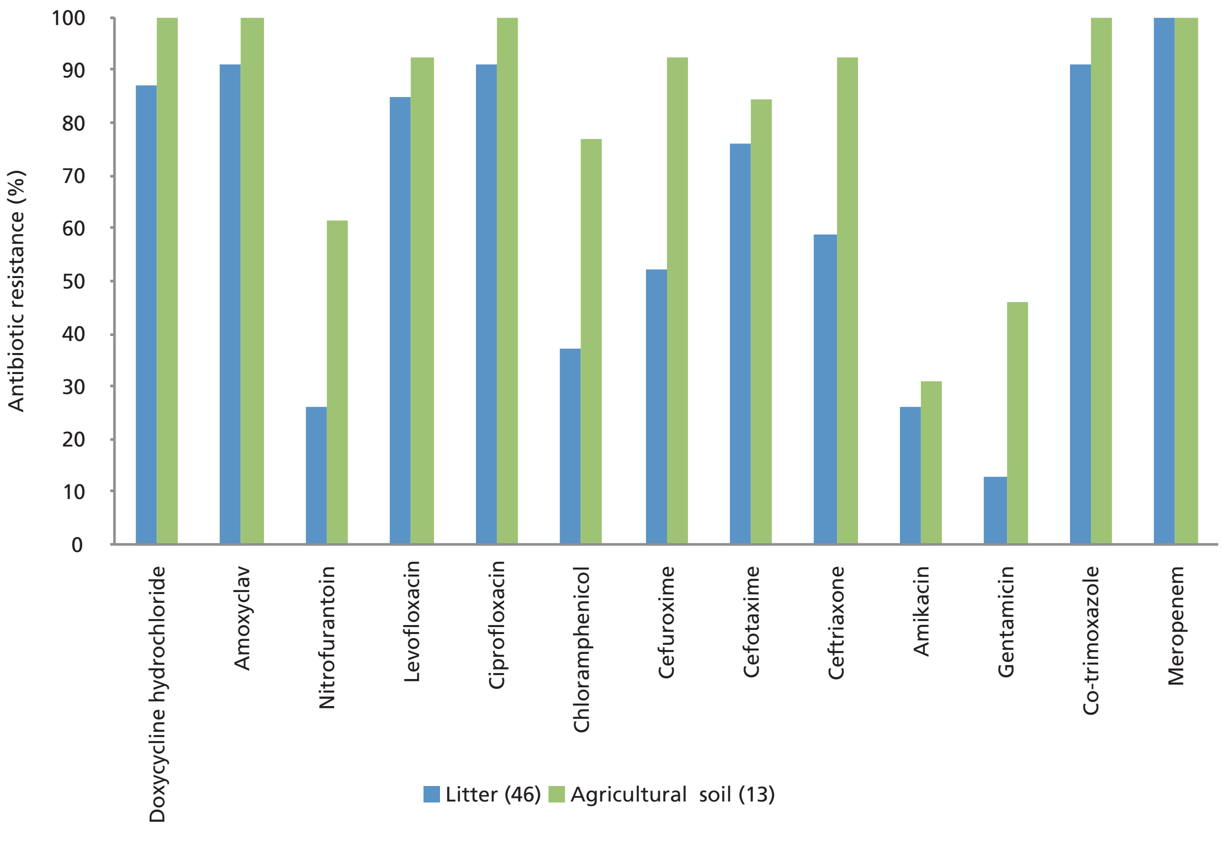
1.2.3 Spread of resistance from farm to field

Antibiotic resistance in isolates from litter and agricultural soil

In order to understand the relationship between resistance pattern inside and outside the farm, the resistance of samples isolated from litter (inside the farm) and agricultural soil (outside the farm where litter was thrown) were compared in case of each bacteria.

Out of the 13 antibiotics tested for *E. coli*, very high (>70 per cent) and similar (in the range of 10-15 per cent) resistance against 7 antibiotics was observed in samples from both litter and agricultural soil (see *Fig 5: Antibiotic resistance in E. coli from litter and agricultural soil samples*). These included doxycycline, amoxyclav, levofloxacin, ciprofloxacin, cefotaxime, co-trimoxazole and meropenem. Resistance against amikacin was also found to be similar but low. To correlate the resistance trends of *E. coli* in litter and agricultural soil, statistical analysis using tools like Pearson correlations and T-test was carried out. A very strong statistical correlation (p value of 0.08 and Pearson's correlation coefficient r = 0.88) was found. Moreover, there were just three isolates recovered from poultry farm soil. These two findings suggest that untreated poultry litter is directly used as manure in the agricultural field, which is causing resistant bacteria to move from farm to the field.

Fig 5: Antibiotic resistance in *E. coli* from litter and agricultural soil samples (%)

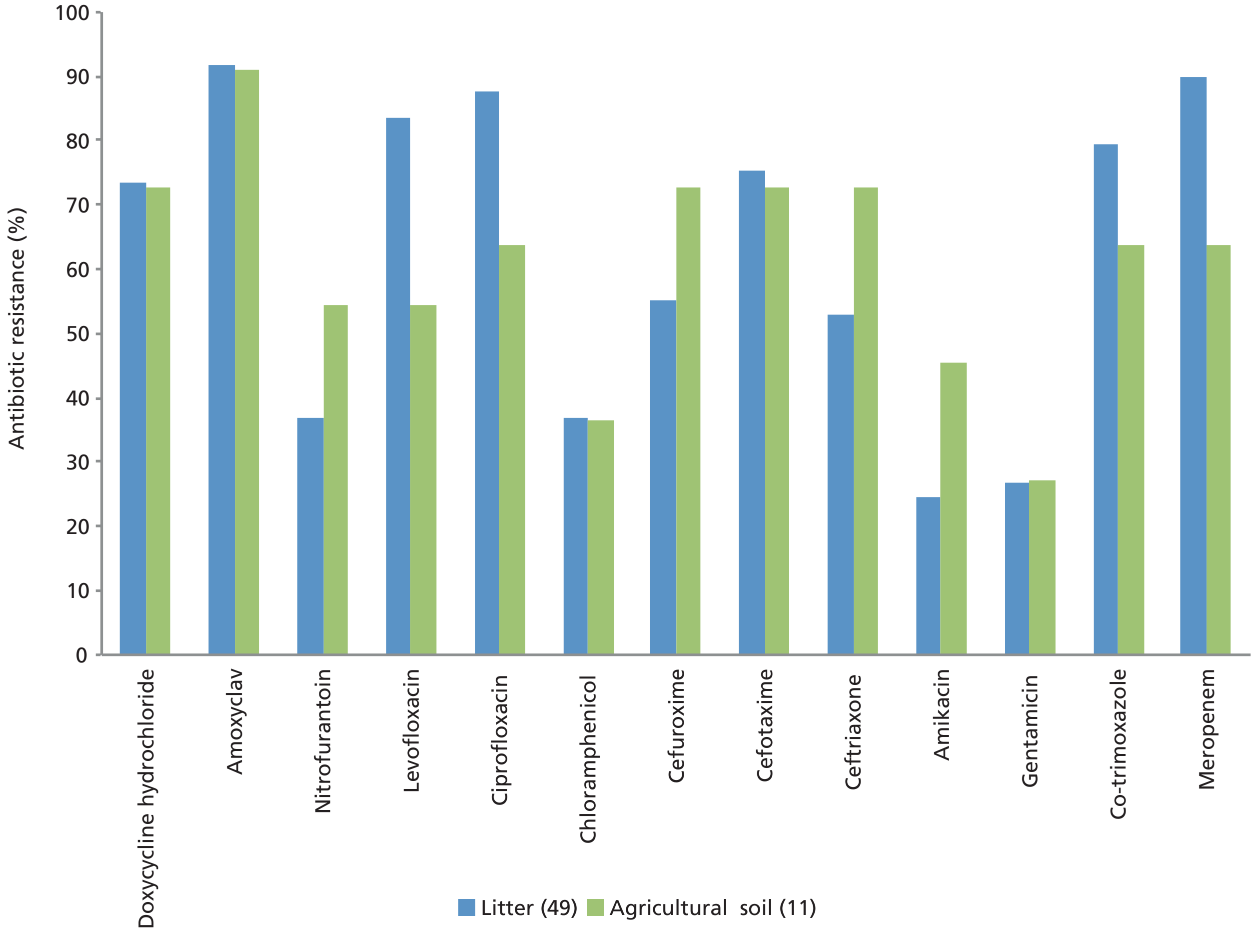


Statistical correlation for *E. coli*

	p value	Pearson coefficient (r)
Litter vs agricultural soil	0.08	0.88

In *K. pneumoniae*, isolates from both litter and agricultural soil showed a very high resistance of 90 per cent against amoxyclav. The isolates also had very high and similar resistance to three out of the 13 antibiotics tested (see *Fig 6: Antibiotic resistance in K. pneumoniae from litter and agricultural soil samples*). These were doxycycline, amoxyclav and cefotaxime. Resistance against chloramphenicol was similar and against gentamicin was similar but low. A strong statistical correlation between resistance patterns of *K. pneumoniae* in litter and agricultural soil could not be obtained. A low isolate recovery from poultry farm soil (five isolates) also indicated the possible direct use of litter in agricultural lands as manure.

Fig 6: Antibiotic resistance in *K. pneumoniae* from litter and agricultural soil samples (%)

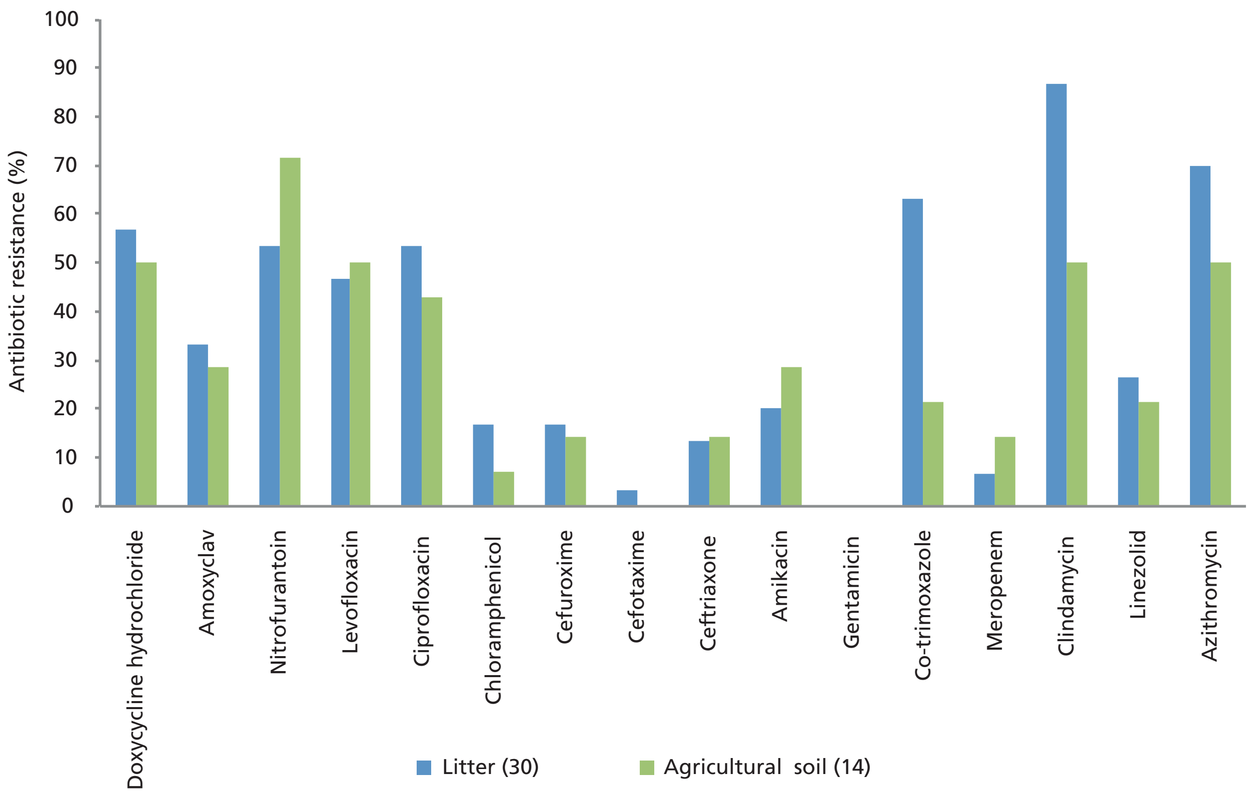


Statistical correlation for *K. pneumoniae*

	p value	Pearson's correlation coefficient (r)
Litter vs agricultural soil	0.83	0.70

In *S. lentus* isolates from litter and agricultural soil, similar resistance was observed for four out of the 16 antibiotics tested. These were doxycycline, amoxyclav, levofloxacin and ciprofloxacin against which resistance was observed in the range of 30-50 per cent (see *Fig 7: Antibiotic resistance in S. lentus from litter and agricultural soil samples*). Similar, but low resistance was also observed in case of cefuroxime, ceftriaxone, chloramphenicol, amikacin, meropenem and linezolid. No resistance was found against gentamicin in isolates from both sources. However, the resistance pattern of the litter and agricultural soil isolates in *S. lentus* were not statistically comparable.

Fig 7: Antibiotic resistance in *S. lentus* from litter and agricultural soil samples (%)



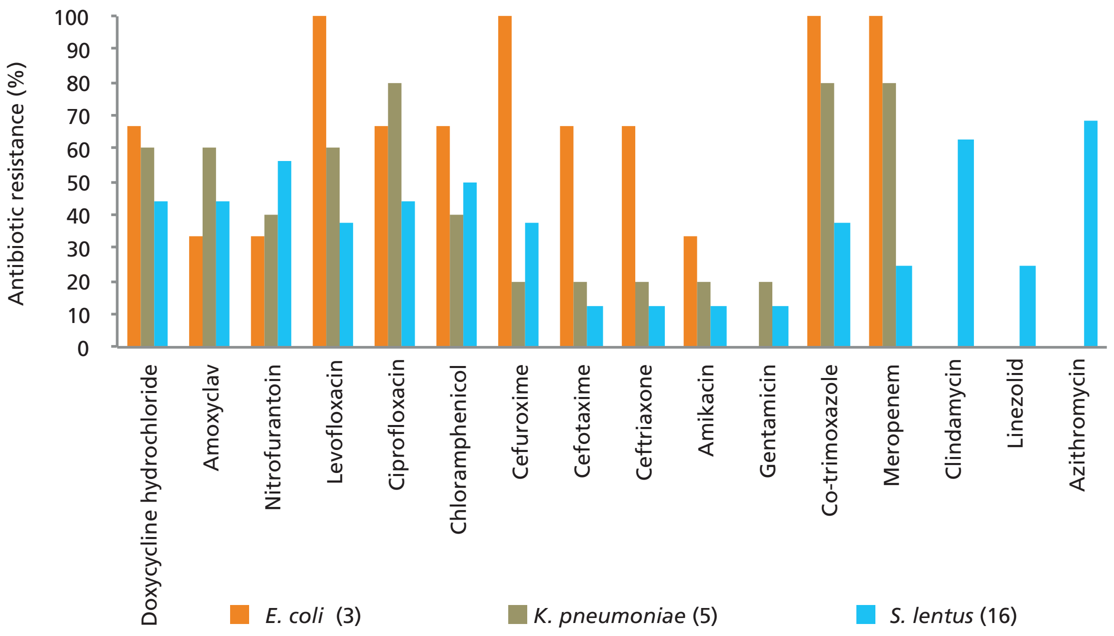
Statistical correlation for *S. lentus*

	p value	Pearson coefficient (r)
Litter vs agricultural soil	0.45	0.81

Antibiotic resistance in isolates from poultry farm soil

In the poultry farm soil sample, very few *E. coli* (three isolates) and *K. pneumoniae* (five isolates) were obtained, while the number of isolates of *S. lentus* was 16. In case of *S. lentus*, when compared statistically with isolates from litter, the resistance pattern was different (see *Fig 8: Antibiotic resistance in isolates from poultry farm soil*).

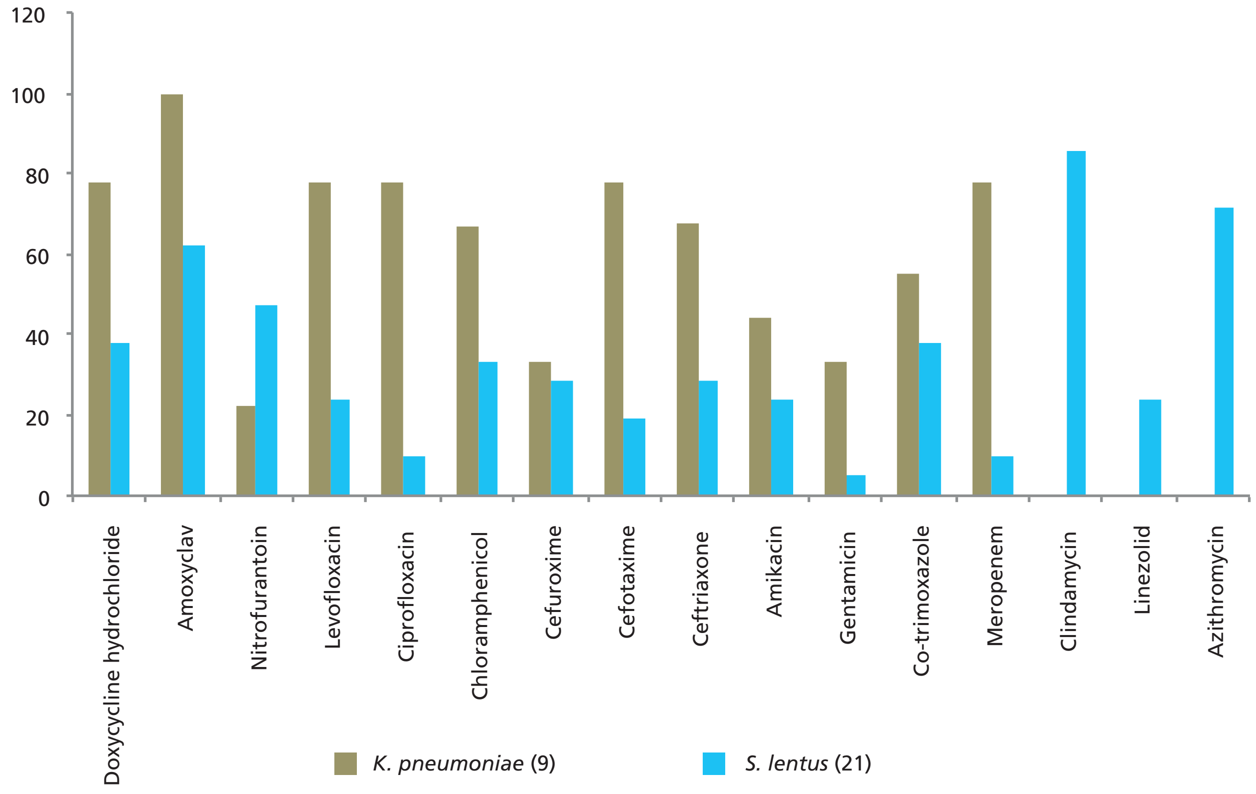
Fig 8: Antibiotic resistance in isolates from poultry farm soil (%)



Antibiotic resistance in isolates from control soil

In the control soil samples, no isolates of *E. coli* was found. Only a few isolates of *K. pneumoniae* could be isolated (nine isolates) and *S. lentus* (21 isolates) was most common. In both, the overall resistance levels observed were high but of statistically different pattern in comparison to what was found in agricultural soil where litter was not thrown (see *Fig 9: Antibiotic resistance in isolates from control soil*).

Fig 9: Antibiotic resistance in isolates from control soil (%)



1.3 Conclusion

- High multidrug resistance found in poultry environment (poultry litter, poultry farm soil and nearby agricultural soil). Overall, the highest resistance was found in *E. coli*, followed by *K. pneumoniae* and *S. lentus*.
- Multidrug resistance is moving from farms to agricultural fields in the case of *E. coli*. This is seen through presence of similar proportion of isolates, similar pattern of resistance and strong statistical correlation in *E. coli* resistance in both litter and agricultural soil.
- A statistical correlation could not be observed for resistance of *K. pneumoniae* and *S. lentus* in litter and agricultural soil. More studies are required to understand their behaviour in view of different sources of bacteria such as other animals and synthetic fertilizer and pesticides in the agricultural fields.

2. Antibiotic misuse in poultry and spread of resistance

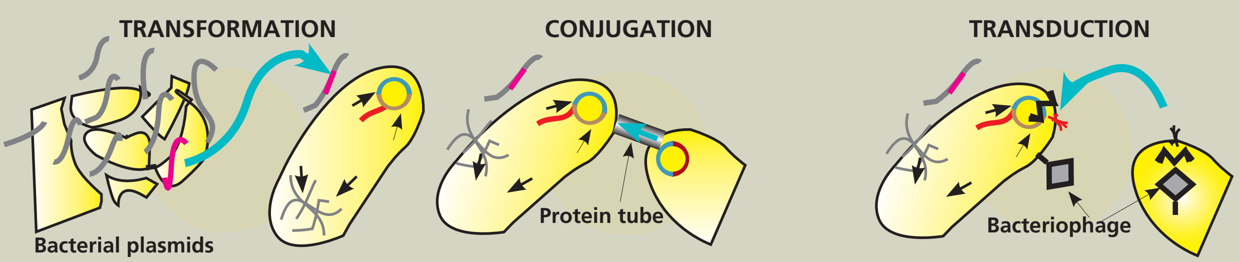
2.1 Science of antibiotic resistance

Low doses of antibiotics used routinely in food-producing animals (such as meat, egg or milk-producing animals) favour emergence of resistant bacteria in animals.[1] Sub-optimum doses help step-wise selection of resistance. Such non-therapeutic use for reasons such as growth promotion and mass disease prevention is rampant across India in intensive farming of food-producing animals such as poultry and fish.

Resistant bacteria proliferate and can make resistant other bacteria that are present in animals. Resistant bacteria can also be transferred to humans through several routes such as direct contact of handlers, live animals and

HOW ANTIBIOTIC RESISTANCE DEVELOPS

The emergence of resistance is a natural process. However, it accelerates and spreads by antibiotic misuse and overuse. While some bacteria are naturally resistant, antibiotic use exerts greater selection pressure on bacteria, causing susceptible populations to die and resistant ones to survive. At a cellular level, resistance is acquired through mutations in bacteria or transfer of genetic material (such as resistance genes) from other bacteria through horizontal gene transfer (HGT). This means that resistance in one bacterium can be passed on to other kinds of bacteria, even for multiple antibiotics.



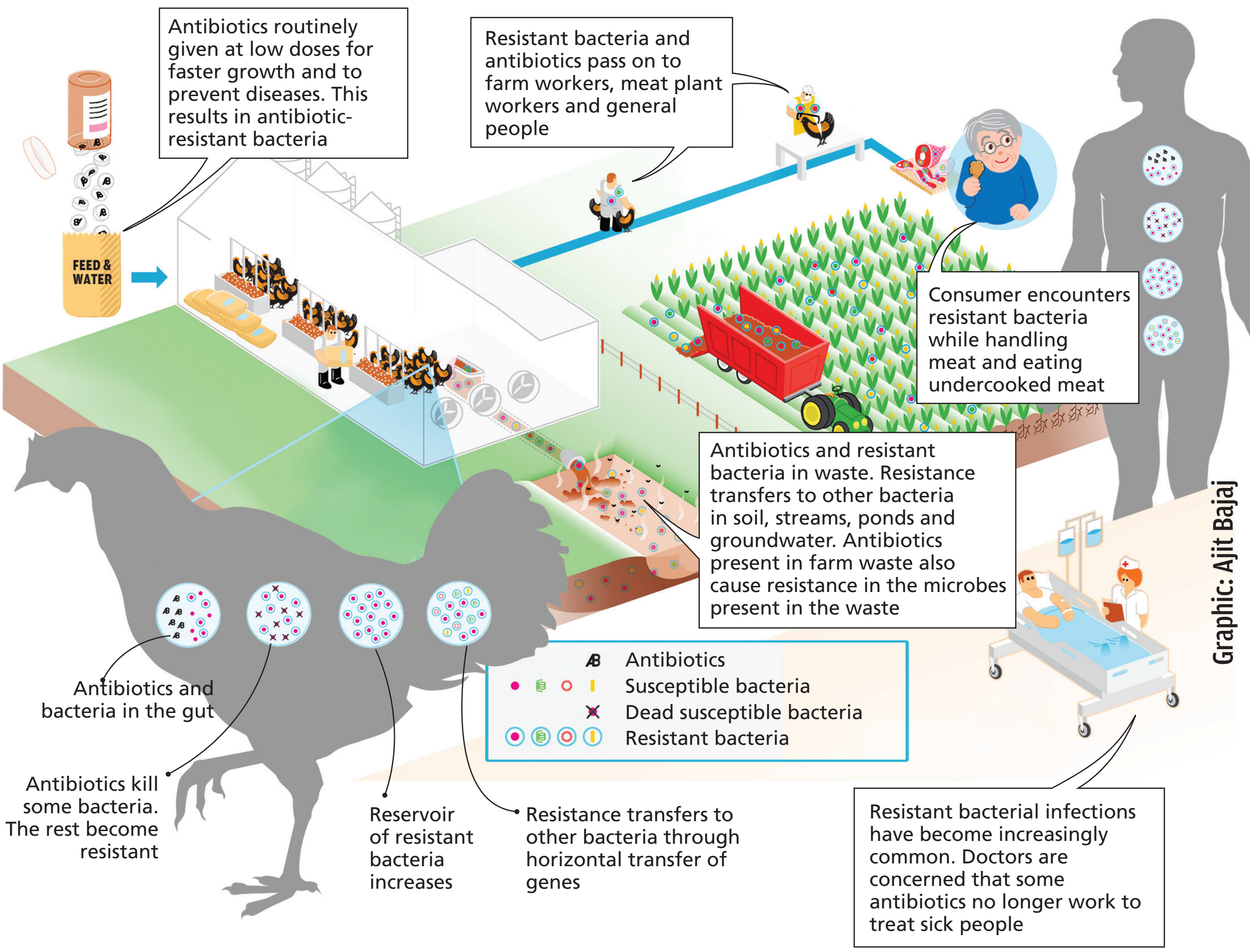
Bacteria acquire new genetic material from the environment

It is the process of direct transfer of DNA from one bacterium to another through a protein tube

Bacteriophage, viruses that infect bacteria pick up genetic material in the process and pass it on to other bacteria

Subsequently, the bacteria undergo structural and chemical alterations that render the antibiotic ineffective. These changes may include one or more of the following: reduced membrane permeability to the drug, alteration of the drug-binding site at the cell wall, enzymatic degradation of the drug and normal function of bacteria bypassing the drug-affected enzyme or pathway.

Fig 10: Smart moves of a deadly microbe



carcasses at poultry farms and slaughterhouses; human consumption of meat, eggs and milk with resistant bacteria; and environmental contamination of soil, water and air through animal excreta and farm waste (see *Fig 10: Smart moves of a deadly microbe*). Besides resistant bacteria, antibiotic residues in environment and those entering into humans through consumption of food may also create selective pressure in bacteria.

2.2 Evidence on transmission: farm to field

Several papers in India clearly establish the growing incidence of ABR and the role of antibiotic misuse in rearing food animals[2] and its further linkages to the spread of ABR in the environment. WHO recognizes the non-therapeutic use of antibiotics in feed and water in food-producing animals to contribute to the development of antimicrobial resistance (AMR) in humans.[3]

CSE STUDY ON ANTIBIOTIC RESIDUES IN CHICKEN MEAT

In 2014, CSE found residues of multiple antibiotics such as fluoroquinolones (enrofloxacin and ciprofloxacin) and tetracyclines (oxytetracycline, chlortetracycline, doxycycline) in chicken meat samples from Delhi NCR.[4] The study also highlighted the practice of rampant use of antibiotics for non-therapeutic use in poultry. Medically important antibiotics were being used as growth promoters through feed, or for routine prophylactic administration. Antibiotics were used throughout lifecycle of the bird and in parent stock, with no withdrawal periods followed.

A report, published in 2016 by Bloomberg highlighted the rampant use of CI antibiotics for non-therapeutic purposes in poultry farms in Hyderabad region of India.[5] The report investigated the logbooks and receipts of poultry farms which were on contract with some of the biggest chicken companies in the country. In a recent study published in *Environmental Health Perspectives* in July 2017, antibiotic use in 18 broiler and layer poultry farms in Punjab was linked to emergence of ABR.[6] A high prevalence of multidrug resistant *E. coli* strains from cloacal swab samples of birds in broiler farms was reported in this study. Also, broiler farms were reported to be more likely to harbour resistant *E. coli* and multidrug resistance as compared to layer farms. In another study published in *Microbiology Research* in 2017, researchers at Bhabha Atomic Research Centre, Mumbai, found the presence of drug-resistant *Salmonella* in ready-to-cook (RTC) poultry products in India.[7] About 81.4 per cent of samples were resistant to five or more antibiotics. The study links such observations to indiscriminate antibiotic use during poultry farming and improper food-processing practices.

The transmission of resistant bacteria from animal farms into the surrounding and larger environment has also been studied. Poultry litter or manure have also been indicated as a source of resistant bacteria bearing linkages to the transmission of ABR into external environment. Studies have reported isolation of some of the common drug-resistant *Staphylococcus, E. coli* and *Salmonella* from samples from poultry litter, nearby surface- and groundwater samples, boot swabs, exhaust air samples from poultry farms etc. (see *Table 4: Select evidence on environmental transmission of resistance from poultry farms).* In 2016, a study published in the journal *PLOS ONE* highlighted the role of manure application from commercial swine farms in North Carolina and Iowa in the dissemination and persistence of antimicrobial resistant *Salmonella* in the environment.[8] A continuation study by the same research group published in *Applied and Environmental Microbiology* showed strong evidence of dissemination AMR determinant-carrying plasmids of *Salmonella* in the environment after manure application.[9]

Table 4: Select evidence on environmental transmission of resistance from poultry farms

Environmental sample	Country	Year	Key findings
Litter from poultry farm[10]	India	2009	• Transformation and conjugation could be an important mechanism for HGT between bacteria in poultry litter
Surface- and groundwater samples near poultry farms, litter samples from poultry farms[11]	Canada	2013	• *E. faecium* and *E. faecalis* isolates from litter and environmental samples shared same resistance patterns. Resistances may have resulted from cross-resistance to antibiotics used in poultry production • Multiple antibiotic resistant indices suggested an increased presence of antibiotics in surface water, likely from poultry sources as there were no other wastewater contributions in the area
Slurry samples, boot swabs, exhaust air samples from broiler chicken farms[12]	Germany	2014	• Highest detection of ESBL/AmpC-*E. coli* in slurry and faecal emission; found also on surfaces in the vicinity, barn air, ambient air • Possible spread to surroundings via air or different vectors
Chicken faeces, upstream and downstream waters around chicken farms[13]	China	2014	• ESBL-producing *E. coli* from faecal and downstream water isolates had a higher resistance rate than those from upstream water • > 90 per cent similarity in some ESBL-producing *E. coli* from downstream water and faecal isolates • Study suggests effluent from animal farms could contribute to the spread of resistance genes
Poultry environment and poultry byproduct meal[14]	Brazil	2015	• Isolates from poultry environment were significantly more resistant to antimicrobials than isolates from other sources

* ESBL= extended spectrum beta-lactamase, AmpC = AmpC beta-lactamases

3. Public health linkages

ABR is known to lead to greater spread of infectious diseases, difficulty in treating common infections, uncertainty in success of high-end procedures, longer hospital stays and more expensive treatments. It can put a huge burden on health and economics of individuals and nations. It can also impact food safety, nutrition security, livelihood and attainment of Sustainable Development Goals. It is estimated that by 2050, antimicrobial resistance (AMR; includes resistance to antibiotics as well as antifungals, antivirals, etc.) can lead to 10 million deaths per year and lost outputs worth US $100 trillion globally.[15]

3.1 Global developments

Recognizing the public health impact, the Global Action Plan on Antimicrobial Resistance was endorsed by the tripartite alliance of the WHO, Food and Agricultural Organization of the United Nations (FAO) and World Organization for Animal Health (OIE) in 2015.[16] The issue also received global political support at the United Nations General Assembly in 2016. Countries are now getting ready with their multi-year National Action Plans (NAPs). In 2017, the WHO published its first ever list of antibiotic-resistant 'priority pathogens' in need for research on development of newer and effective antibiotic treatments.[17] The WHO Model List of Essential Medicines also categorizes antibiotics under 'Access', 'Watch' and 'Reserve' category to preserve their effectiveness.[18] Recognizing the gravity of AMR transmission through food-borne pathogens, the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) came out with a Guidance document on integrated surveillance of antimicrobial use and AMR in food-borne bacteria.[19]

The need to address the environmental aspect of AMR has also picked momentum globally. The FAO Action Plan on AMR talks about integrated surveillance systems for antimicrobial use (AMU) and AMR that covers food, agriculture as well as environment.[20] The WHO has also been working towards the development and pilot implementations of a globally harmonized protocol for integrated surveillance of single key indicator bacteria, ESBL-producing *E. coli* in humans, food chain, and environment (WHO-AGISAR ESBL *E. coli* Tricycle Project).[21] The United Nations also established the Inter-Agency Coordination Group on AMR, which also recognizes the role of the environment in the emergence and spread of AMR.[22] India too has framed up its strategic National Action Plan for 2017-2021, wherein it aims to aggressively address environmental spread of AMR.[23]

3.2 Possible high impact in India

In the case of India, with tropical climate, largely unsanitary conditions, limited infection prevention and control, inadequate environmental policies and practices, and suboptimal health systems, the burden of infectious diseases and ABR would likely be much higher than anticipated. The major causes of infectious diseases related deaths in India are respiratory tract infections (RTI), diarrhoea and tuberculosis.[24] A WHO factsheet mentions that multidrug resistant/rifampicin resistant-tuberculosis caused 250,000 deaths in 2015, most of which occurred in Asia.[25] Drug resistant tuberculosis is prevalent in India. Further, considering that India is among the big producers of food from animals such as poultry, dairy and aquaculture as well as antibiotic active pharmaceutical ingredients, the animal and environmental contribution to ABR could be very high. In particular, the public linkages of this study could be assessed with the human relevance of the bacteria and the antibiotics against which the susceptibility is tested.

3.3 Linkages with bacteria tested

Both *E. coli* and *K. pneumoniae* cause several common infections in the community and hospitals across age groups. For example, certain pathogenic *E. coli* strains can cause bloody diarrhoea, neonatal meningitis, gastrointestinal infections, urinary tract infections (UTIs) and respiratory illnesses such as pneumonia. *E. coli* are the most common organism causing UTI and accounts for 85 per cent of community-acquired UTIs and 50 per cent of hospital-acquired UTIs.[26, 27] Patients with weaker immune systems such as the very young or the elderly are at highest risk of being infected by *K. pneumoniae*. It can cause UTIs, RTIs and bloodstream infections in neonates and also contribute to diarrhoea, meningitis, septicaemia and certain nosocomial infections. *S. lentus*, on the other hand, is not linked with infectious disease burden in humans. However, research indicates that in the environment, it could be involved in HGT of antimicrobial resistance determinants to similar pathogens, for example, *Staphylococcus aureus*, which are significant for human health.[28]

As a part of the National Programme on Containment of Antimicrobial Resistance (2012-2017),[29] AMR surveillance in humans was conducted for four common bacterial pathogens of public health importance: *Klebsiella, Escherichia coli, Staphylococcus aureus* and *Enterococcus* species. The surveillance was carried out across network laboratories identified in different geographical regions of the country. Resistance data available from National Centre for Disease Control (NCDC) shows considerable high resistance in *E. coli* and *Klebsiella* sp. against ciprofloxacin, cefotaxime and co-trimoxazole (see *Table 5: Antibiotic resistance in bacteria isolated from humans).*

Table 5: Antibiotic resistance in bacteria isolated from humans (%)*

	E. coli	*Klebsiella* sp.
Nitrofurantoin	8–48	48–76
Ciprofloxacin	61–76	66–88
Cefotaxime	59-70	52-66
Amikacin	19–60	46-61
Gentamicin	30–58	3–64
Co-trimoxazole	70–81	69–80
Meropenem	13–30	28-42

* 2015-2016 data for AMR trend from seven hospitals across India. Sourced from NCDC: http://cseindia.org/userfiles/India_Sunil-Gupta_National-AMR-containment.pdf

3.4 Linkages with antibiotics tested

The study found high degree of resistance to all critically important classes. This is of concern since critical importance of a drug implies that the antiboitic class may be the sole or one of the limited available therapies to treat infections in people or that it is used to treat infections, caused by bacteria (from non-human sources) which can be transmitted to humans or which may have acquired resistance genes. In India, most of these antibiotics are used in the treatment of several infectious diseases or conditions and some are used as last resort antibiotics in hospitals. Moreover, of the CI antibiotics used in the present study, five belonged to the 'highest priority' category and the rest were 'high priority' CIAs[30] (*Table 5: Prioritization of Critically Important Antibiotics*). Very high resistance was found in four out of five highest priority CIAs and two out of four high priority CIAs.

Table 6: Prioritization of Critically Important Antibiotics

Antibiotics used in current study	Class of Critically Important Antibiotics	Prioritization of Critically Important Antibiotics*
Azithromycin	Macrolides and ketolides	Highest priority
Levofloxacin	Quinolones	
Ciprofloxacin		
Cefotaxime	Third-, fourth- and fifth-generation cephalosporins	
Ceftriaxone		
Amikacin	Aminoglycosides	High priority
Gentamicin		
Meropenem	Carbapenems	
Linezolid	Oxazolidinones	
Amoxyclav	Penicillins	

*The WHO uses three prioritization criteria for critically important antimicrobials (CIA): (a) High proportion of use in patients afflicted with infections for which the antimicrobial class is the sole or one of the few alternatives available for treatment; (b) High frequency of use for any indication in human medicine or high proportion of use in patients with serious infections; (c) Used to treat infections in people for whom there is evidence of transmission of resistant bacteria or genes from non-human sources. CIAs that fulfil all three prioritization criteria are categorized as 'highest priority', while CIAs that fulfil any one or two of the prioritization criteria are categorized as 'high priority'.

4. Policy analysis: Poultry waste management

4.1 Indian landscape

The Central Pollution Control Board (CPCB) along with the State PCBs, under the Ministry of Environment, Forests and Climate Change (MoEFCC), is responsible for waste management in the country. However, the poultry sector takes a back seat and does not get adequate focus from the perspective of waste management. The MoEFCC considers the poultry industry to have a lower pollution potential and places it under the 'green' category' of industry categorization.[31] The Environmental Guidelines for Poultry Farm by CPCB broadly suggests composting and biogas-generation approaches for manure management. Although the guideline was circulated to all state pollution control boards in late 2015, only few states such as Punjab,[32] West Bengal[33] and Haryana[34] have so far notified these guidelines officially. A 'Poultry Farm Manual' released by the Department of Animal Husbandry, Dairying and Fisheries (DADF) in 2015 also mentions that manure should be either composted or used to produce biogas.[35] The anaerobic process of biogas generation can also be used to generate electricity and heat energy which can be used in-house. The manual also mentions that anaerobically digested manure, as in biogas generation, leads to greater stabilization of solids and liquids and are therefore safer to be used as a fertilizer or feed supplement.

4.1.1 Key issues with current guidelines on litter/manure management

- The litter/manure management guidelines do not focus on ABR.
- The guidelines are voluntary in nature and do not require to be mandatorily followed.
- Although the guidelines talk about necessary size requirements for manure storage during composting and other parameters, there is no mention of parameters like site approval, process validation or microbial standards.
- There are no instructions on precautions related to land application of litter/manure.

4.2 Global best practices: key points

After review of some international guidelines and regulations on poultry farm waste management from the EU, UK and USA, the following best practices have emerged. These have not been addressed so far in the Indian guidelines but can be considered from point of view of addressing the key issues outlined above.

INDIA'S STRATEGIC NATIONAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

The Strategic NAP on Antimicrobial Resistance was released in April 2017. The Plan focuses on six strategic priority areas: Awareness and understanding through education, communication and training; strengthening knowledge and evidence through surveillance; infection prevention and control; optimized antimicrobial use in health, animals and food; AMR-related research and innovation and strengthened leadership and commitment at international, national and sub-national levels. The Plan highlights the need for tackling AMR across multiple sectors such as human health, animal husbandry, agriculture and environment in consideration of the 'One-Health' approach. The Plan talks about conducting national-level surveillance of antibiotic resistance and residues in environment, including waste from farms, factories making animal feed, processing meat, dairy, fish, veterinary and human health care settings, pharmaceutical industry. The NAP also aims to reduce environmental contamination with resistant pathogens and antimicrobial residues through strengthening of necessary laws and regulations, environment risk assessment; extended producer responsibility for expired/unused antibiotics.

- The UK guidance on 'Handling of manure and slurry to reduce antibiotic resistance' emphasizes on preferable spreading of slurry or manure on arable land and not grazing land.[36] If it is to be spread on grazing land, then grazing or cropping should not be allowed for at least eight weeks.
- The Guidance on using animal byproducts at compost and biogas sites[37] in UK mentions the need for site approval for composting/biogas production, following which validation of processes and systems are needed. At least 12 consecutive validation tests (a test to check if the process is removing bacteria in each batch of compost or digestate) should be passed for each bacteria before each site is validated. Manure can also be burnt to generate power, but manure burning can be done only after approval.
- The Produce Safety Final Rule of Food and Drug Administration (FDA) of USA outlines microbial standards for processes used to treat manure.[38] The standards set limits on detectable amounts of bacteria (for example *E. coli* 0157:H7) in manure treatment processes.

5. CSE recommendations to contain ABR spread from poultry farms

5.1 Recommendations to reduce antibiotic use in food-animal production

CSE's 2014 study on antibiotic residues in chicken meat highlighted rampant use of antibiotics in chicken. CSE proposed a number of recommendations to regulate and limit the antibiotic misuse in poultry sector. Minimizing antibiotic use in food animal production is the most effective way to address resistance spread from farms. The Central and state animal husbandry departments, drug control departments and food safety departments must take a lead in this. Since no control on antibiotic misuse has been attained so far, we urge concrete action on the following recommendations:

- Non-therapeutic use of antibiotics for growth promotion and mass disease prevention should be prohibited. It should only be used to cure the sick, based on prescription of veterinarians
- Antibiotics should not be allowed in feed and feed supplement. The government should set standards for animal feed, regulate the business
- Antibiotics that are critical for humans should not be allowed for use in animals
- The development, production and use of alternative antibiotic-free growth promoters, such as herbal supplements, should be encouraged
- It should be ensured that licensed antibiotics reach registered users through registered distributors or stockists of veterinary medicines. All animal antibiotics should be traceable from the manufacturing site to user. Stringent control on import of antibiotics and feed supplements should be implemented
- Good farm management practices should be followed to control infection and stress among the flock. Biosecurity guidelines of the Central Poultry Development Organisation should be improved and applied to all farms. Capacity of small farmers must be enhanced so that they can comply with the guidelines. The guidelines should be legally enforced on big companies
- Set standards for antibiotic residues in chicken meat
- Alternatives to antibiotics should be explored and adopted. For example, vaccinations should be promoted against bacterial diseases
- Veterinarians should be trained and educated on judicious use of antibiotics and infection prevention. The government should ensure that veterinarians do not get incentives for prescribing more antibiotics
- There is a need to introduce a labelling system wherein poultry raised without use of antibiotics should be labelled through reliable certified

schemes to facilitate consumer choice. Poultry produced with antibiotics must also be labelled accordingly. This would incentivize the farmer who can charge a premium and provide consumer with a healthy choice

- Lack of data on the use of antibiotics and drug resistance is a major problem in India. It is necessary to create an integrated surveillance system to monitor antibiotics use and antibiotics resistance trends in humans, animals and food chain. A national-level database should be developed and kept in the public domain.

5.2 Recommendations to reduce the spread of ABR from farms

The Indian NAP-AMR aims to address the environmental aspect of antibiotic resistance through necessary laws and environmental surveillance. The implementation of it will be a bigger task and is yet to be seen. Management of waste from farms will therefore require adoption of a new ABR-centric approach and a greater leadership role of environment regulators such as the CPCB and SPCBs and the nodal ministry, i.e. MoEFCC. CSE being a stakeholder in implementation of NAP-AMR recommends that the following recommendations be considered.

- The MoEFCC and CPCB should develop ABR-centric environmental regulations for farms and factories/industry. Additionally, for poultry sector, the existing CPCB guidelines, 'Environmental Guidelines for Poultry Farm' should be modified and strengthened in view of the below mentioned recommendations and notified. The SPCBs should make it mandatory in states and ensure its implementation
  - Pollution causing potential from poultry farm sector should be re-categorized and prioritized to provide the required mandate to develop laws and conduct ABR surveillance by CPCB and SPCBs
  - Manure management approaches in poultry farms which pose lesser risk to the spread of ABR should be preferred than more risky approaches such as land application of manure. For example, biogas generation must be the most preferred approach of managing litter/ manure from farms. Other options of waste to energy conversion can also be explored
  - Big/integrated poultry farms having large volumes of litter/manure must only be allowed to manage waste through in-house biogas generation plants. This should also become a part of criteria for licensing and renewal of farms going forward
  - Small poultry farmers, particularly those operating in a cluster should be encouraged to develop and manage a common biogas generation plant. This should be supported by a national-level programme which starts from key hubs and select poultry producing states
  - Land application of untreated litter must be prohibited through necessary laws, awareness and surveillance. Only application of treated litter/manure should be allowed if the option of biogas generation is not feasible
  - Proper composting for treatment of manure should be encouraged only under very high level of supervision. In this regard, laws in line with global best practices should be framed with reference to approval

of composting sites, validation of treated manure and timing of application of litter/manure and type of land it could be applied to

- In order to prevent resistance spread across food animal production settings, poultry litter must not be allowed to be used as feed for fishes in aquaculture. Central and state Fisheries departments must ensure this through necessary laws, awareness and surveillance
- Finally, the ABR research agenda should include, understanding the impact of litter/manure treatment through composting/biogas generation on resistant bacteria and mechanism and movement of transfer of resistance from farms to environment through waste. This should be led by the scientific community which includes those from the Indian Council of Agricultural Research, State colleges of veterinary sciences and environmental studies etc. with the support from regulatory surveillance.

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Annexure

Table 1: Antibiotic resistance in poultry farm environment (%)

	E. coli (62)	*K. pneumoniae* (65)	*S. lentus* (60)
Doxycycline hydrochloride	88.7	72.3	51.7
Amoxyclav	90.3	89.2	35.0
Nitrofurantoin	33.9	40.0	58.3
Levofloxacin	87.1	76.9	45.0
Ciprofloxacin	91.9	83.0	48.3
Chloramphenicol	46.8	36.9	23.3
Cefuroxime	62.9	55.3	21.7
Cefotaxime	77.4	70.7	5.0
Ceftriaxone	66.1	53.8	13.3
Amikacin	27.4	27.7	20.0
Gentamicin	19.4	29.2	3.3
Co-trimoxazole	93.5	76.9	46.7
Meropenem	100.0	84.6	13.3
Clindamycin	-	-	71.7
Linezolid	-	-	25.0
Azithromycin	-	-	65.0

Note: '-' indicates that resistance was not tested against these antibiotics.

Table 2: Antibiotic resistance in *E. coli* isolates in different samples (%)

	Litter (46)	**Poultry farm soil (3)**	**Agricultural soil (13)**
Doxycycline hydrochloride	87.0	66.7	100.0
Amoxyclav	91.3	33.3	100.0
Nitrofurantoin	26.1	33.3	61.5
Levofloxacin	84.8	100.0	92.3
Ciprofloxacin	91.3	66.7	100.0
Chloramphenicol	37.0	66.7	76.9
Cefuroxime	52.2	100.0	92.3
Cefotaxime	76.1	66.7	84.6
Ceftriaxone	58.7	66.7	92.3
Amikacin	26.1	33.3	30.8
Gentamicin	13.0	0.0	46.2
Co-trimoxazole	91.3	100.0	100.0
Meropenem	100.0	100.0	100.0

Note: No *E. coli* isolates were found in control soil samples.

Table 3: Antibiotic resistance in *K. pnuemoniae* isolates in different samples (%)

	Litter (49)	Poultry farm soil (5)	Agricultural soil (11)	Control soil (9)
Doxycycline hydrochloride	73.5	60.0	72.7	77.7
Amoxyclav	91.8	60.0	90.9	100.0
Nitrofurantoin	36.7	40.0	54.5	22.2
Levofloxacin	83.7	60.0	54.5	77.7
Ciprofloxacin	87.8	80.0	63.6	77.7
Chloramphenicol	36.7	40.0	36.4	66.7
Cefuroxime	55.1	20.0	72.7	33.3
Cefotaxime	75.5	20.0	72.7	77.7
Ceftriaxone	53.1	20.0	72.7	67.7
Amikacin	24.5	20.0	45.5	44.4
Gentamicin	26.8	20.0	27.3	33.3
Co-trimoxazole	79.6	80.0	63.6	55.5
Meropenem	89.8	80.0	63.6	77.7

Table 4: Antibiotic resistance in *S. lentus* isolates in different samples (%)

	Litter (30)	Poultry farm soil (16)	Agricultural soil (14)	Control soil (21)
Doxycycline hydrochloride	56.7	43.8	50	38.1
Amoxyclav	33.3	43.8	28.6	61.9
Nitrofurantoin	53.3	56.3	71.4	47.6
Levofloxacin	46.7	37.5	50.0	23.8
Ciprofloxacin	53.3	43.8	42.9	9.5
Chloramphenicol	16.7	50.0	7.1	33.3
Cefuroxime	16.7	37.5	14.3	28.6
Cefotaxime	3.3	12.5	0.0	19.0
Ceftriaxone	13.3	12.5	14.3	28.6
Amikacin	20.0	12.5	28.6	23.8
Gentamicin	0.0	12.5	0.0	4.8
Co-trimoxazole	63.3	37.5	21.4	38.1
Meropenem	6.7	25.0	14.3	9.5
Clindamycin	86.7	62.5	50.0	85.7
Linezolid	26.7	25.0	21.4	23.8
Azithromycin	70.0	68.8	50.0	71.4

In 2014, CSE and Pollution Monitoring Laboratory, in their report 'Antibiotic Residues in Chicken Meat' highlighted the widespread use of antibiotics in intensive chicken farming in the country, revealing abuse of this 'public-health good'. Subsequently, CSE was asked at several fora, directly or otherwise: What is the connecting link? How is antibiotic use in chickens leading to increase in antibiotic resistant bacteria?

This new study establishes that poultry environment is a reservoir of multidrug resistant bacteria and points towards the role of litter in spreading ABR in the environment.

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