Media Briefing
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CSE’s Pollution Monitoring Laboratory (PML)

• Set up in 2000, with state of the art equipment for monitoring air pollution, water pollution and food contamination

• Tests for trace organics (pesticide, antibiotics etc.), heavy metals and conducts microbiological studies

• Investigates issues of public health concern and responds to community requests

• Puts out independent information in public domain for ecological security

Tested for antibiotics residues in honey (2010) and in chicken meat (2014)
What is ABR and how it develops?

Antimicrobial resistance (AMR) – antibiotic resistance (ABR) – in particular arises when bacteria survive exposure to an antibiotic that would normally kill them or stop their growth

- It is a natural process but **accelerates by antibiotic misuse and overuse** in both humans and animals; spreads through food, contact and environment
- Low dose of antibiotics for longer durations favour emergence of resistant bacteria (such as growth promoter at sub-therapeutic levels in food animal production settings)
- At a cellular level, resistance is acquired through mutations or transfer of genetic material (such as resistance genes) from other bacteria through horizontal gene transfer (HGT)

Resistance in one bacterium can be passed on to other kinds of bacteria, for one or for multiple antibiotics
Antibiotic resistance (ABR): a global threat of an unprecedented scale!

- Since 1980s, no new class of antibiotics have been discovered
- ABR leads 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
- Can cause huge health and economic impact on individuals and nations
- Can also impact food safety, nutrition security, livelihood and growth

By 2050, estimated to lead to 10 million deaths and lost output worth US $100 trillion globally – *post-antibiotics world*
Huge global momentum to address the issue of ABR

- **Global Action Plan on Antimicrobial Resistance** endorsed in 2015 by the tripartite alliance of:
  - World Health Organization (WHO)
  - Food and Agricultural Organization of the United Nations (FAO)
  - World Organization for Animal Health (OIE) in 2015

- Received **global political support at United Nations General Assembly** of 2016. Fourth health issue after HIV, NCDs, Ebola to be discussed at that level. Interagency Task Force involving UN organizations established

- Several countries have developed National Action Plans. **Indian Plan released in April 2017 along with Delhi Declaration**

Globally and more so in developing world, antibiotic misuse in humans has been the focus so far; animal and environmental aspect of the problem is slowly gaining attention

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Why this study?

- CSE study of 2014 on chicken meat had found residues of multiple antibiotics:
  - Fluoroquinolones (enrofloxacin and ciprofloxacin)
  - Tetracyclines (oxytetracycline, chlortetracycline, doxycycline)
- Rampant use of antibiotics for non-therapeutic use in poultry highlighted
- Raised public awareness about antibiotic use in food production systems in the country

However, there were questions raised about linkages of antibiotic residues with ABR. We were told that residues do not mean resistance.
Two objectives of the latest study

- **OBJECTIVE 1:**
  - Understand the extent of ABR in the poultry environment

- **OBJECTIVE 2:**
  - Understand the spread of resistant bacteria from poultry farms to the environment
Scope and Sampling
12 broiler farms from 12 clusters in 9 districts of 4 states

- Study conducted in 2016-2017 across four north Indian states which collectively contribute to 40% of total poultry meat production in India.
- One farm per cluster/village was randomly selected. Number of birds in farms was 3000–21000. Antibiotics were used in all farms.

9 districts covered

<table>
<thead>
<tr>
<th></th>
<th>Uttar Pradesh</th>
<th>Rajasthan</th>
<th>Haryana</th>
<th>Punjab</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples collected</td>
<td>17</td>
<td>8</td>
<td>15</td>
<td>7</td>
<td>47</td>
</tr>
<tr>
<td>Samples from poultry farms</td>
<td>12</td>
<td>5</td>
<td>12</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>Control samples</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Poultry farms visited for samples</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

Clusters

Note: Agricultural soil could not be collected from one farm in Jaipur.
Uniform break-up maintained for all samples

- From each farm, three types of samples were collected:
  - **Litter sample** (inside the shed)
  - **Soil sample** (outside the shed)
  - **Soil sample** (nearby agricultural land outside the farm, where reportedly litter was used as manure)

- **Control soil samples** were collected 10-20 km away from farms, where reportedly litter was not thrown
Methodology
Methodology followed

Samples were collected from 12 poultry farms distributed within 4 different states and brought to lab in cold conditions

Sample preparation was carried out using the serial dilution technique

Isolation of intended isolate was carried out using different selective media

Identification of the isolated bacteria was carried out via morphological and biochemical characterization at PML

The identified cultures were then sent to private commercial laboratory for 16S rDNA sequence analysis.

The antibiotic susceptibility assay was performed in triplicates, using the disc diffusion method according to Bauer Kirby technique (Bauer et al., 1966)

The average zones of inhibition obtained were compared with the standards of Clinical and Laboratory Standards Institute (CLSI) and where CLSI standards was not available, European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards were used.
Target bacteria & antibiotics

- Study focussed on *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus* sp.
- These bacteria were selected due to their importance to public health. Several infections are caused by them in humans and high resistance is reported.
- Resistance was tested against 16 antibiotics from 13 classes. These antibiotics were selected based on their use in the poultry industry and importance to human health.
- Of these, 10 antibiotics are classified as critically important for humans by WHO.
One gram of each of litter and soil samples were aseptically added separately into different sterile vials containing 9 mL of sterile normal saline. Further, they were subjected to 10 fold serial dilution.
Isolation of bacteria from collected samples their characterization and identification

Samples collected from poultry farms were subjected to their microbial analysis for the isolation of *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus* sp. These samples were also subjected to microbial analysis for Total Viable Count of Bacteria. Standard methodologies were used for the isolation of different bacteria which are listed below:

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

Isolated cultures from all the samples were characterized and identified using a combination of colony characteristics, morphology, and different biochemical tests using biochemical identification kits of HiMedia.
Identity of over 10% of the isolated bacteria (selected on the basis of geographical and frequency distribution) was confirmed by 16S rDNA sequence analysis. The 16S rDNA sequence analysis of the shortlisted cultures was done by a third party i.e. Chromous Biotech Pvt. Ltd., Bengaluru, Karnataka.

During the analysis, the PCR product (~1500bp) was sequenced using ABI PRISM Big Dye Terminators v 3.1 cycle sequencing kit (Applied Biosystems Foster city, CA, USA) according to the manufacturer’s instruction employing 16S rDNA universal primers. The comparison of the nucleotide sequences of the fragment with the sequences available in the GenBank database was carried out using the NCBI BLAST program.
The antibiotic susceptibility pattern of all the isolated bacteria from each farm as well as from control samples was determined using the disk diffusion method according to the Bauer - Kirby technique (Bauer et al., 1966).

Pure cultures were grown in nutrient broth separately. Further, to grow a homogeneous mat of the bacterium on Muller Hinton Agar plate, pure cultures were swabbed onto the plates using sterile swabs. Discs of different antibiotics were placed aseptically on swabbed plates (3 discs on 1 plate) and incubated at 37°C for 24 hours. All the three targeted bacteria were subjected to antibiotic susceptibility tests against different antibiotics in triplicates.
## Antibiotics used against the three targeted bacteria

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antibiotic and its concentration</th>
<th>Antibiotic Class</th>
<th>E. coli</th>
<th>Klebsiella sp.</th>
<th>Staphylococcus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Doxycycline Hydrochloride (DO – 30 µg)</td>
<td>Tetracyclines</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2.</td>
<td>Amoxyclav (AMC – 30 µg)</td>
<td>Penicillins</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>3.</td>
<td>Nitrofurantoin (NIT – 100 µg)</td>
<td>Nitrofurans</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>4.</td>
<td>Levofloxacin (LE – 5 µg)</td>
<td>Quinolones</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>5.</td>
<td>Ciprofloxacin (CIP – 5 µg)</td>
<td>Quinolones</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>6.</td>
<td>Chloramphenicol (C – 30 µg)</td>
<td>Amphenicols</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>7.</td>
<td>Cefuroxime (CXM – 30 µg)</td>
<td>Cephalosporins - 1st and 2nd generation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>8.</td>
<td>Cefotaxime (CTX – 30 µg)</td>
<td>Cephalosporins - 3rd, 4th and 5th generation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>9.</td>
<td>Ceftriaxone (CTR – 30 µg)</td>
<td>Cephalosporins - 3rd, 4th and 5th generation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>10.</td>
<td>Amikacin (AK – 30 µg)</td>
<td>Aminoglycosides</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>11.</td>
<td>Gentamicin (GEN – 10 µg)</td>
<td>Aminoglycosides</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>12.</td>
<td>Co-trimoxazole (COT – 25 µg)</td>
<td>Sulfonamides, dihydrofolatereductase inhibitors and combinations</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>13.</td>
<td>Meropenem (MRP – 10 µg)</td>
<td>Carbapenems</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>14.</td>
<td>Clindamycin (CD – 2 µg)*</td>
<td>Lincosamides</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>15.</td>
<td>Linezolid (LZ – 30 µg)*</td>
<td>Oxazolidinones</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>16.</td>
<td>Azithromycin (AZM – 15 µg)*</td>
<td>Macrolides and ketolides</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>

Note*: Not tested against *E. coli* and *Klebsiella sp.* due to the unavailability of the standards.
All the isolates of *Staphylococcus* sp. were analyzed for antibiotic susceptibility test against a total of 16 antibiotics. However, the isolates of *E. coli* and *Klebsiella* sp. were tested for their antibiotic susceptibility test against 13 antibiotics i.e. all mentioned above except for CD, LZ and AZM (due to the unavailability of standards).

The zones of inhibition obtained (in mm) for each bacterium were compared with the standards of Clinical and Laboratory Standards Institute (CLSI) and where CLSI standard was not available, European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards were used.

These antibiotics were selected based on their use in poultry, extent and importance of use in human health. These included 10 antibiotics from 7 critically important antibiotic (CIA) classes.
Antibiotic Susceptibility Test plate

*E. coli*
Antibiotic Susceptibility Test plate

*K. pneumoniae*
Results

Isolation of bacteria
Similar number of three bacteria isolated from poultry environment

- Overall, **217 isolates** of three bacteria were isolated from all samples from poultry environment (poultry litter, poultry farm soil and nearby agricultural soil) and control
  - **187 isolates** were derived in similar proportion from poultry environment
  - **30 isolates** were derived from control soil samples
Similar proportion of isolates from litter and agricultural soil

- In the poultry environment
  - Maximum isolates from litter (125; 66.8%).
  - 38 isolates (20%) from agricultural soil
  - Least number of isolates from poultry farm soil samples (24; 12.8%)

- In control, S. lentus most prominent (21). No E. coli. Few K. pneumoniae (9)
High number of isolates from litter and agricultural fields

<table>
<thead>
<tr>
<th></th>
<th><strong>E. coli</strong></th>
<th><strong>K. pneumoniae</strong></th>
<th><strong>S. lentus</strong></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Litter</td>
<td>Poultry farm soil</td>
<td>Agricultural soil</td>
<td>Litter</td>
</tr>
<tr>
<td>Satidon town, Jind, Haryana</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Kawli village, Panipat, Haryana (Farm 1)</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Ahmadpur Majra village, Panipat, Haryana (Farm 2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sannka village, Gurugram, Haryana</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mamepur village, Meerut, Uttar Pradesh</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Bhiapour village, Bulandshahr, Uttar Pradesh (Farm 1)</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ranapur village, Bulandshahr, Uttar Pradesh (Farm 2)</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Kushaluya village, Ghaziabad, Uttar Pradesh</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Rangala village, Alwar, Rajasthan</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Moria village, Jaipur, Rajasthan</td>
<td>3</td>
<td>-</td>
<td>NA</td>
<td>2</td>
</tr>
<tr>
<td>Kotla Shamshapur village, Ludhiana, Punjab (Farm 1)</td>
<td>4</td>
<td>-</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sangatpura village, Ludhiana, Punjab (Farm 2)</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>5</td>
<td>13</td>
<td>49</td>
</tr>
</tbody>
</table>

At least 10 isolates were derived from each farm and near by; average isolates were 15
Results

Antibiotic resistance in poultry farm environment
Poultry farm environment: a hotbed of multi-drug resistant (MDR) bacteria

- Highest resistance was found in *E. coli*, followed by *K. pneumoniae* and *S. lentus*
All E. coli isolates were multi-drug resistant; high resistance against CIAs

- 100% E. coli showed MDR, i.e. resistant to antibiotics of 3 or more classes
- About 40% E. coli isolates resistant to at least 10 antibiotics; one in six isolates resistant to at least 12 of the 13 antibiotics; Two isolates resistant to all the 13 antibiotics tested
- Very high resistance (>70% isolates) against co-trimoxazole and tested antibiotics of CI classes such as penicillins, fluoroquinolones, carbapenems and one of the third- and fourth-generation cephalosporins (cefotaxime)
- High resistance (50–70%) against cefuroxime (second gen CS) and ceftriaxone (third- and fourth-gen CS)

All 62 isolates were resistant to meropenem. Meropenem belongs to a last-resort antibiotic class of carbapenem used in hospitals, and also categorized as ‘high priority’ CIA
Almost all *K. pneumoniae* showed multi-drug resistance; high resistance against CIAs

- Multi-drug resistance was shown by **92.3%** isolates
- Over 30% isolates were resistant to at least 10 antibiotics and **10 %** were resistant to all tested antibiotics
- Very high resistance (>70%) against co-trimoxazole and tested antibiotics of CI classes such as *penicillins*, *fluoroquinolones*, *carbapenems* and one of the *third- and fourth-generation cephalosporins* (cefotaxime)
- High resistance (50–70%) against cefuroxime (second gen CS) and ceftriaxone (third- and fourth-gen CS)
78% S. lentus isolates were MDR

- About **one-fourth isolates** of S. lentus were resistant to **at least eight antibiotics**

- High resistance against clindamycin, a lincosamide and **azithromycin**, a critically important macrolide

- In case of co-trimoxazole and antibiotics tested of CI classes such of **penicillins** and **fluoroquinolones**, resistance observed was in the range of 30-50%
Results
Spread of ABR from poultry farm to agricultural field
Similar resistance pattern in *E. coli* from litter & agricultural soil where litter disposed

- Very high (>70 per cent) and similar (in the range of 10-15 per cent) resistance against seven antibiotics
- **Strong statistical correlation in resistance pattern** (T-Test p value=0.08; Pearson’s correlation r=0.88)
- Resistant bacteria from litter is getting into the environment.

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ABR in *K. pneumoniae* from litter and agricultural soil

- The isolates had very high and similar resistance to three out of the 13 antibiotics tested
- **No strong statistical correlation in resistance pattern** (p value=0.83; r=0.70)

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ABR in *S. lentus* from litter and agricultural soil

- Similar resistance observed for four out of the 16 antibiotics tested
- **The resistance pattern was not statistically comparable** (p value=0.45; r=0.81)

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No *E. coli* in control soil. In *K. pneumoniae* and *S. lentus*, resistance pattern were statistically different between isolates from control soil and agricultural soil. ABR is in the control soil but of different kind than poultry environment.
# Resistance pattern in bacteria from different sample types

## E. coli

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Litter</th>
<th>Poultry Farm Soil</th>
<th>Agricultural Soil</th>
<th>Control Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 19.6% of the isolates &gt; 5-10 antibiotics in 73.9% of the isolates 3-5 antibiotics in 6.5% of the isolates &lt; 3 antibiotics in none of the isolates</td>
<td>Resistance was observed against 12 out of 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 33.3% of the isolates &gt; 5-10 antibiotics in 66.7% of the isolates 3-5 antibiotics in none of the isolates &lt; 3 antibiotics in none of the isolates</td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 61.5% of the isolates &gt; 5-10 antibiotics in 38.5% of the isolates 3-5 antibiotics in none of the isolates &lt; 3 antibiotics in none of the isolates</td>
<td>--</td>
</tr>
</tbody>
</table>

## K. pneumoniae

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Litter</th>
<th>Poultry Farm Soil</th>
<th>Agricultural Soil</th>
<th>Control Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 18.4% of the isolates &gt; 5-10 antibiotics in 67.3% of the isolates 3-5 antibiotics in 12.2% of the isolates &lt; 3 antibiotics in 2% of the isolates</td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in none of the isolates &gt; 5-10 antibiotics in 80% of the isolates 3-5 antibiotics in none of the isolates &lt; 3 antibiotics in 20% of the isolates</td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 54.5% of the isolates &gt; 5-10 antibiotics in 9.1% of the isolates 3-5 antibiotics in 18.2% of the isolates &lt; 3 antibiotics in 18.2% of the isolates</td>
<td>Resistance was observed against all the 13 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 22.2% of the isolates &gt; 5-10 antibiotics in 66.7% of the isolates 3-5 antibiotics in 11.1% of the isolates &lt; 3 antibiotics in none of the isolates</td>
</tr>
</tbody>
</table>

## S. lentus

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Litter</th>
<th>Poultry Farm Soil</th>
<th>Agricultural Soil</th>
<th>Control Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance was observed against 15 out of 16 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in none of the isolates &gt; 5-10 antibiotics in 53.3% of the isolates 3-5 antibiotics in 36.7% of the isolates &lt; 3 antibiotics in 10% of the isolates</td>
<td>Resistance was observed against all the 16 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 12.5% of the isolates &gt; 5-10 antibiotics in 43.8% of the isolates 3-5 antibiotics in 18.8% of the isolates &lt; 3 antibiotics in 25% of the isolates</td>
<td>Resistance was observed against 14 out of 16 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in none of the isolates &gt; 5-10 antibiotics in 50% of the isolates 3-5 antibiotics in 14.3% of the isolates &lt; 3 antibiotics in 35.7% of the isolates</td>
<td>Resistance was observed against all the 16 antibiotics tested. Of which resistance was found against: &gt;10 antibiotics in 4.8% of the isolates &gt; 5-10 antibiotics in 42.9% of the isolates 3-5 antibiotics in 42.9% of the isolates &lt; 3 antibiotics in 9.5% of the isolates</td>
</tr>
</tbody>
</table>
Conclusion of results

- **High multidrug resistance found in poultry environment.** Overall, the highest resistance was found in *E. coli*, followed by *K. pneumonialae* and *S. lentus*

- **Multidrug resistance is moving from farms to agricultural fields in the case of *E. coli*.** No such trend could be established for *K. pneumonialae* and *S. lentus*

- **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**
Spread of ABR from farms to the environment

*Routes and evidences from international studies*
Environment: a key route of ABR spread from poultry farms

The common practice of using untreated poultry litter as manure in agricultural land is transferring bacteria that are resistant to multiple antibiotics.
• **Samples:** chicken faeces, upstream and downstream waters around chicken farms

• **Result:**
  – 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

• **Conclusion:** *Animal farm effluent could contribute to the spread of resistance genes*

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Samples: surface- and groundwater samples near poultry farms, litter samples from poultry farms

Result: *E. faecium* and *E. faecalis* isolates from litter and environmental samples shared same resistance patterns. Resistances may have resulted from cross-resistance.

Conclusion: Multiple antibiotic resistant indices suggest increased presence of antibiotics in surface water, likely from poultry sources.
Study highlights role of manure application from commercial swine farms in the dissemination and persistence of antimicrobial resistant Salmonella in the environment.

A continuation study by the same research group showed strong evidence of dissemination AMR determinant-carrying plasmids of Salmonella in the environment after manure application.
Public health linkages
Linkages with bacteria tested (1)

- *E. coli* and *K. pneumoniae* cause several infections in the community and hospitals across age groups

  - *E. coli* accounts for 85% of community-acquired urinary tract infections (UTIs) and 50% of hospital acquired UTIs; Pathogenic *E. coli* can cause bloody diarrhoea, neonatal meningitis, gastrointestinal infections and pneumonia

  - Patients with weaker immune systems at high risk of *K. pneumoniae* infections; *K. pneumoniae* can cause UTIs, RTIs and bloodstream infections in neonates and also contribute to diarrhoea, meningitis, septicemia and nosocomial infections

**E.Coli and K. pneumoniae cause several infections becoming difficult to treat due to antibiotic resistance**
Linkages with bacteria tested (2)

- High resistance in *E. coli* and *K. pneumoniae* isolated from humans

<table>
<thead>
<tr>
<th></th>
<th><em>E. coli</em></th>
<th><em>Klebsiella sp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrofurantoin</td>
<td>8-48</td>
<td>48-76</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>61-76</td>
<td>66-88</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>59-70</td>
<td>52-66</td>
</tr>
<tr>
<td>Amikacin</td>
<td>19-60</td>
<td>46-61</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>30-58</td>
<td>38-64</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>70-81</td>
<td>69-80</td>
</tr>
<tr>
<td>Meropenem</td>
<td>13-30</td>
<td>28-42</td>
</tr>
</tbody>
</table>

2015-2016 data for AMR trend from 7 hospitals across India involved in AMR Surveillance network. Sourced from National Centre for Disease Control

WHO has prioritized carbapenem-resistant *Enterobacteriaceae* (including *E. coli* and *K. pneumoniae*) as ‘priority pathogens’ having ‘CRITICAL’ priority in need for research on development of newer and effective antibiotic treatments
## Linkages with antibiotics tested

<table>
<thead>
<tr>
<th>Antibiotics used in current study</th>
<th>Class of CIAs</th>
<th>Prioritization of CIAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>Macrolides and Ketolides</td>
<td>Highest priority</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Quinolones</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>3\text{rd}, 4\text{th} and 5\text{th} generation</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Cephalosporins</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>Aminoglycosides</td>
<td>High priority</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>Carbapenems</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>Oxazolidinones</td>
<td></td>
</tr>
<tr>
<td>Amoxyclav</td>
<td>Penicillins</td>
<td></td>
</tr>
</tbody>
</table>

- **The study found high resistance to all seven CI classes**
- **Critical importance implies that**
  - the antibiotic class may be the sole or one of the limited available therapies to treat infections in people or
  - it is used to treat infections caused by bacteria that can be transmitted to humans or that may have acquired resistance genes

> Very high resistance (>70%) was found in four out of five highest priority CIAs and two out of four high priority CIAs

Centre for Science and Environment
High impact of ABR through animal and environment routes in India!

- Burden of infectious diseases and ABR is much higher in India due to:
  - Tropical climate
  - Largely unsanitary conditions
  - Limited infection prevention and control
  - Inadequate environmental policies and practices
  - Sub-optimal health systems

- India is among **the big global producers of poultry, dairy and aquaculture** due to growing demand of protein from animal sources and exports potential

- High animal antibiotic use due to **growing intensification of food production systems** and largely unregulated industry

- Indian, a global hub for **antibiotic active pharmaceutical ingredients**

Centre for Science and Environment
Policy analysis: Poultry waste management
Poultry sector does not get adequate focus from the perspective of waste management

• The MoEFCC places the poultry industry under the ‘green’ category’ i.e. low pollution causing potential

• The ‘Environmental Guidelines for Poultry Farm’ by Central Pollution Control Board (CPCB) and ‘Poultry Farm Manual’ by Department of animal husbandry (DADF) suggests composting and biogas-generation approaches for litter/manure management

• Key issues
  – The litter/manure management guidelines do not focus on ABR
  – The guidelines are voluntary in nature
  – Although the guidelines talk about necessary size requirements for manure storage, 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

Centre for Science and Environment
CSE Recommendations

1. To reduce antibiotic misuse in food-animal production
2. To reduce spread of ABR from poultry farms
To reduce antibiotic use in food-animal production (1)

Central and state departments of animal husbandry, drug control and food safety must take a lead. The government should:

• Prohibit antibiotic use for growth promotion and mass disease prevention; only therapeutic use under vet supervision to be allowed;

• Not allow antibiotics in feed and feed supplements; regulate feed business

• Encourage development, production and use of alternatives such as antibiotic-free growth promoters and vaccination for bacterial disease

• Not allow animal use of antibiotics which are critically important for humans

• Ensure that only 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

• FSSAI to set standards of residues in poultry meat

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To reduce antibiotic use in food-animal production (2)

- Legally enforce **biosecurity guidelines** of Central Poultry Development Organisation on big producer companies; capacity of small farmers must be enhanced.

- Train **veterinarians** on judicious antibiotic use and infection prevention; disallow incentives for prescribing more antibiotics.

- Introduce a **system for labelling** poultry raised without use of antibiotics.

- Develop an **integrated surveillance system** to monitor antibiotic use and resistance trends in humans, animals and food chain; and a **national-level publically available database**.
To reduce spread of ABR from poultry farms (1)

The MoEFCC and CPCB should have a greater leadership role and develop ABR-centric environmental regulations for farms (and factories/industry); CPCB guidelines, ‘Environmental Guidelines for Poultry Farm’ should be strengthened and notified as mandatory regulation and SPCBs should ensure implementation:

- **Pollution causing potential** of the poultry farm sector should be re-prioritized
- **Less risky litter/manure management** approaches such as biogas generation must be preferred over land application. Other options of waste to energy conversion can also be explored
- **Big/integrated poultry farms** must manage waste through in-house biogas generation plants; should become a licensing criteria
- **Small poultry farmers**, particularly those operating in clusters 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 in select states
To reduce spread of ABR from poultry farms (2)

- Land application of untreated litter must be prohibited through necessary laws, awareness and surveillance.
- Proper composting for treatment of litter/manure should be encouraged only under very high level of supervision; laws related to approval of composting sites, validation of treated manure and timing of application of litter/manure should be made.
- Poultry litter must not be allowed to be used as feed for fishes in aquaculture; Central and State fisheries departments must ensure this.
- The ABR research agenda should include,
  - Understanding the impact of litter/manure treatment through composting/biogas generation on resistant bacteria
  - Mechanism and movement of transfer of resistance from farms to environment through waste