Existing AMR surveillance programme in livestock in India and suggestions for future framework

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Existing AMR surveillance
Use of antibiotics in animals

- Both in food and pet animals antibiotics are used.
- Concern regarding use of antibiotics specially in poultry feed.
- Reserve antibiotics are reportedly used in poultry rearing.
- By 2030 there is an estimated increase of antibiotic consumption by 99% in BRICS countries.
- In many cases owners and farmers can not afford antibiotics although, India accounted for 3% (4th largest) of global consumption of antibiotics in food animals behind China(23%), USA (13%) and Brazil (9%), in comparison to India with largest population of diverse livestock species.
Global average annual consumption of antimicrobials per kilogram of animal produced

Boeckel et al., 2015, PNAS 112(18): 5649–5654, doi: 10.1073/
Global trends in antimicrobial use in food animals

Presumed to increase by 67%

Boeckel et al., 2015, PNAS 112(18): 5649–5654, doi: 10.1073/pnas
No quantifiable data is available for use of antimicrobials in animals.

Proportion of different classes of antibiotics used in animals, WB, India.

Data based on personal communication in few areas of WB, India.
- (INSAR) (2009-2012) network of 20 labs with WHO support
- MoHFW/NCDC: AMR surveillance network (10 labs)
- ICMR: AMR Surveillance Network (4 institutions/6 labs)
- Veterinary/Agriculture sector – no organized AMR surveillance and at its infancy
Bad Bugs in animal

- Extended spectrum $\beta$-lactamase producers
- AmpC type $\beta$-lactamase producers
- Fluroquinolone resistant *enterobacteriaceae*
- Methicillin resistant *S. aureus* and CoNS
- Mettalo-$\beta$-lactamase producing *enterobacteriaceae* (EC & KP)
- Vancomycin resistant and intermediate *S. aureus*
- Colistin resistant *enterobacteriaceae*
Their trends.

MRSA, ESBL and quinolone resistant *enterobacteriaceae* in lactating cattle in eastern and NE India during 2012 to till date, ICAR-IVRI

<table>
<thead>
<tr>
<th>Region</th>
<th>MRSA</th>
<th>ESBL</th>
<th>QRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>WB</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>MZ</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>OD</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>JH</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

TP: Tripura, WB: West Bengal, MZ: Mizoram, OD: Odisha, JH: Jharkhan
The occurrence of MRSA in food animal in India is

<table>
<thead>
<tr>
<th>States</th>
<th>Species</th>
<th>Occurrence mean (%) (range with 95% CI)**</th>
<th>SCCmecc*</th>
<th>SPA*</th>
<th>MLST*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripura</td>
<td>Lactating cattle</td>
<td>2.17 (1.14-3.74)</td>
<td>SCCmec IVa,V and NT</td>
<td>t005, t202, t267, t524, t527, t740, t800, t852, t3626, t4463, t4931, and t6297</td>
<td>ST-63, ST-71, ST-97, ST-2219, ST-1297</td>
</tr>
<tr>
<td>Mizoram</td>
<td>Lactating cattle</td>
<td>1.26 (0.46 – 2.78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haryana</td>
<td>Lactating cattle</td>
<td>5.64 (2.4-10.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Bengal</td>
<td>Lactating cattle and goats</td>
<td>1.66 (1.06 -2.9)</td>
<td>Novel spa type t15798 (caprine)</td>
<td>t16344 (buffalo)</td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td></td>
<td>3.27 (1.5-6.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo</td>
<td></td>
<td>0.84 (0.3-2.01)</td>
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</tr>
</tbody>
</table>

* The data reflects analysis from representative samples ** Fisher Exact test (Clopper-Pearson)
### Enterobacteriaceae (E. coli and K. pneumoniae) in Food

<table>
<thead>
<tr>
<th>SL No</th>
<th>States</th>
<th>Species</th>
<th>Occurrence mean (%) (range with 95% CI)*</th>
<th>PMQR</th>
<th>Efflux pumps</th>
<th>QRDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tripura</td>
<td>Lactating cattle</td>
<td>9.56 (6.03-14.8)a</td>
<td>qnrA, qnrB, qnrS, aac(6′)-Ib-cr AcrAB</td>
<td>QepA AcrAB</td>
<td>Mutation in gyrA and ParC</td>
</tr>
<tr>
<td>2</td>
<td>Mizoram</td>
<td>Lactating cattle</td>
<td>14.1 (8.06-23.5)a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>West Bengal</td>
<td>Lactating cattle</td>
<td>11.3 (7.28-17.1)a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poultry</td>
<td>19.56 (15.2-24.7)b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buffalo</td>
<td>2.3 (1.29-4.09)a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Jharkhand</td>
<td>Lactating cattle</td>
<td>12.8 (6.6-22.7)a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poultry</td>
<td>20.9 (12.9-32) b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Among ESBL producers only; b on basis of Phenotypic Valutest Score (Wilson) with Fleiss Quadratic Correction. 

Note: SI = Species Occurrence; PMQR = Plasmid-Mediated Quinolone Resistance; QRDR = Quinolone Resistance Determining Regions.
## L and MBL enterobacteriaceae (E. coli and K. pneumoniae) in food

<table>
<thead>
<tr>
<th>SI No</th>
<th>States</th>
<th>Species</th>
<th>Occurrence mean (%) (range with 95% CI)*</th>
<th>ESBL</th>
<th>ACBL</th>
<th>MBL</th>
<th>Resistance determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tripura</td>
<td>Lactating cattle</td>
<td>10.1 (6.6-15)</td>
<td>6.06 (3.5-10.3)</td>
<td>NDM-5 (2)</td>
<td>blaCTXM-15, blaNDM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>blaAmpC, blaMOX, int1</td>
</tr>
<tr>
<td>2</td>
<td>Mizoram</td>
<td>Lactating cattle</td>
<td>5.8 (2.4-12.7)</td>
<td>4.8 (1.8-11.5)</td>
<td>blaNDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>was reported in dairy animals (Ghatak et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactating cattle</td>
<td>4.4 (1.9-9.2)</td>
<td>4.4 (1.9-9.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buffalo</td>
<td>2.3 (1.29-4.09)</td>
<td>1.47 (0.6-3.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poultry</td>
<td>14.5 (9.5-21.1)</td>
<td>20.1 (14.6-27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pigs</td>
<td>6 (3.2-10.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>West Bengal</td>
<td>Lactating cattle</td>
<td>12.8 (6.6-22.7)</td>
<td>10.2 (4.8-19.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Score (Wilson) with Fleiss Quadratic Correction
Characteristics of ESBL producers in food animals from India

- The isolates were detected among dairy animals, organized poultry and piggery sector.

- Most of the isolates carried multiple ESBL genes.

- The genes \(\text{bla} \text{CTX-M-15}, \text{bla} \text{CTX-M-9}, \text{bla} \text{SHV-12} \) and non ESBL gene \(\text{bla} \text{TEM-1} \) were more common.

- Presence of multiple resistance gene cassettes (\(\text{dfrA} \) and \(\text{aadA} \)) leads to MDR phenomenon.
of resistance pattern of MRSA isolates from lactating cattle of eastern and N (2012- till date, ICAR-IVRI)

Relatively sensitive to aminoglycosides, fluoroquinolones and glycopeptides

Resistant to beta-lactam or potentiated beta-lactam and macrolides
of resistance pattern of ESBL producers from lactating cattle of eastern and (2012- till date, ICAR-IVRI)

Relatively sensitive to carbapenem, colistin and chloramphenicol

to beta-lactam or potentiated beta-lactam, tetracycline, aminoglycosides and fluoroc
o-infection of . \ . and . producers are frequent cause of therapeutic failure in animals
Table 1. Characteristics of Vancomycin-Resistant and Intermediate Staphylococcus aureus Isolated from Milk of Bovine and Caprine Clinical and Subclinical Mastitis

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Isolates</th>
<th>Districts</th>
<th>Host</th>
<th>mecA</th>
<th>mecC</th>
<th>mecB</th>
<th>vanA</th>
<th>vanB</th>
<th>Resistant to</th>
<th>MIC\textsubscript{VISA} (µg/ml)</th>
<th>MIC\textsubscript{VRSA} (µg/ml)</th>
<th>MIC\textsubscript{MIC} (µg/ml)</th>
<th>SCCmec</th>
<th>ERIC profile</th>
<th>Ridomil type variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VRSA1</td>
<td>Nadia</td>
<td>Cow</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>AK, AMC,</td>
<td>8.0</td>
<td>64</td>
<td>16</td>
<td>NT</td>
<td>B</td>
<td>NTU\textsuperscript{a}</td>
</tr>
<tr>
<td>2</td>
<td>VRSA2</td>
<td>Malda</td>
<td>Goat</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>&gt;256</td>
<td>64</td>
<td>64</td>
<td>V</td>
<td>A</td>
<td>A\textsuperscript{b}</td>
</tr>
<tr>
<td>3</td>
<td>VRSA3</td>
<td>Kolkata</td>
<td>Cow</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>AK, AMC,</td>
<td>16.0</td>
<td>64</td>
<td>64</td>
<td>V</td>
<td>B</td>
<td>D\textsuperscript{c}</td>
</tr>
<tr>
<td>4</td>
<td>VRSA4</td>
<td>Kolkata</td>
<td>Cow</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>Co, PIT,</td>
<td>8.0</td>
<td>32.0</td>
<td>16.0</td>
<td>V</td>
<td>B</td>
<td>D\textsuperscript{c}</td>
</tr>
<tr>
<td>5</td>
<td>VRSA5</td>
<td>North 24</td>
<td>Cow</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>CTR, Gd</td>
<td>8.0</td>
<td>32.0</td>
<td>32.0</td>
<td>NT</td>
<td>C</td>
<td>D\textsuperscript{c}</td>
</tr>
<tr>
<td>6</td>
<td>VRSA6</td>
<td>Nadia</td>
<td>Cow</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>AMC, Co,</td>
<td>8.0</td>
<td>&gt;256</td>
<td>16.0</td>
<td>NT</td>
<td>D</td>
<td>D\textsuperscript{c}</td>
</tr>
<tr>
<td>7</td>
<td>VRSA7</td>
<td>Hooghly</td>
<td>Goat</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>AMC, Co,</td>
<td>8.0</td>
<td>32</td>
<td>32</td>
<td>IV</td>
<td>A</td>
<td>A\textsuperscript{d}</td>
</tr>
<tr>
<td>8</td>
<td>S. aureus ATCC 29213</td>
<td>Cow</td>
<td></td>
<td>N</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>Control</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All the isolates were sensitive to gatifloxacin and levofloxacin, but resistant to ampicillin, cefepime, and cefazolin.

\textsuperscript{b} A sequence that is in a cross-over. Not does not exist in database.

\textsuperscript{c} Intermediate for CLEI guidelines: MIC\textsubscript{VISA} for vancomycin; MIC\textsubscript{MIC} for oxacillin; MIC\textsubscript{MIC} for teicoplanin; MIC\textsubscript{MIC} for oxacillin; MIC\textsubscript{MIC} for teicoplanin; NT, non-typeable; ERIC, enterococcal repetitive intergenic consensus; \textsuperscript{d} ID, positive; ID, negative; MIC, minimum inhibitory concentration; VRSA, vancomycin-resistant S. aureus.
Adopted pathway map of antimicrobial resistance (AMR) dissemination within agriculture, the environment, and the food processing industry. Movement of antimicrobial agents and AMR is indicated by overlapping circles and arrows, respectively; different colors define different groups of reservoirs. Thanner et al., mBio 7(2):e02227-15
Suggestions for future frame
What is the target population?
1. Dairy and poultry sector should be given major emphasis for systematic surveillance
2. Others include pigs, sheep and goat
In case of meat producing animals, both on-farm and slaughter house sampling are required.

What should be the target organisms?
1. Indicator: *E. coli*, *S. aureus*
2. Food borne pathogens: *Salmonella*, *Campylobactor*
3. Sentinel organism: *E. coli*, *Enterococcus*
4. Others: *Claustridium*
Surveillance in food animals: role of surveillance

Sampling

1. Many potential sampling points in the production and processing
2. Production/holding/processing slaughter/retail continuum
3. Environmental sampling - manure, water, feed etc
4. Slaughter house: caecal content, floor, slaughter house drainage, hand swab
5. **Sample information:** animal species, time and place of Collection, age and clinical status of the animal and AMU

Cross-sectional, time series, case-control, cohort analysis- what will be appropriate? – a very pertinent question to understand

Prospective/passive/random/systematic

Sentinel surveillance
Sample size
1. Precision
2. CI
3. Target population
4. Expected prevalence

Sampling frequency

1. To understand trend analysis sampling should be continuous or at regular interval
   - Quarterly throughout the year.
2. Based on incidence and seasonality of the bacteria or diseases
Surveillance: how to proceed...

Desired number of animals from every possible representative farms/individual owners from each state/districts/block

For a zone with 40 farms with 1000 birds - for AMR studies with respect to *E. coli*
Sample size: 385 with 95% CI and precision of 0.05 (expected level: 30%)

Samples check points at poultry/broiler production unit samples/farm:

1. Cloaca swab/ freshly voided droop
2. Water
3. Feed samples
4. Farm floor
5. Farm disposal/ drainage/ sewage
Surveillance: Sampling points and steps

**Dairy animals**

**PRODUCTION**
- Dairy animals
- Freshly collected milk from each individual quarter
- Separately collected milk from affected/unaffected quarter (SCM/CM)
- Farm floor
- Milking machine
- Hand swab of milkman
- Animal attendants/handler
- Adjunct samples
- Rectal swab
- Calf pen
- Manure/litter
- Pooled milk
- Feeding/water trough

**PROCESSING**
- Milk processing unit
- Collecting utensils
- Packaging unit
- Spraying machine
- Drainage

**RETAIL**
- Finished products
- Milkman
- Milk-stalls
Surveillance: Sampling points and sources for poultry animals...

**PRODUCTION**
- Poultry
- Cloacae swab/ freshly voided dropping
- Farm floor
- Animal attendants/ handler
- Utensils
- Drainage/ sewage
- Dust, Fluff

**HOLDING**
- Holding pen
- Excreta / litter
- Cage/ crate/vehicle
- Drainage

**POST SLAUGHTER**
- Caecal content
- Carcass rinsate
- Carcass swab
- Lymph nodes
- Meat juice
- Liver
- Floor/ground sample

**RETAIL**
- Finished product processed / unprocessed

**Adjunct samples**
- Feed
- Water
- Dust, Fluff
- Environmental samples (litter/ manure)
What new you may get?

1. Unexpected resistance characteristics….

2. Abrupt variation from what others say

3. AMU/AMR may not be linear here

4. Risk factor analysis – status- reservoir indication

5. Three tier – farm/individual owner/ backyard
Roadmap – implementation and sensitization

Optimization of use of antimicrobials in livestock

DADF, Govt of India

State Animal Husbandry Department

General recommendations

VO/RVP

Industries

Private entrepreneurs

Owner and farmers

Grass-root level workers

Prani-mitra, veterinary field assistant

Control and containment of AMR

ICAR

ICAR laboratories

SAU

Research and Surveillance data

KVKs
Thank you