AMR at human animal interface: PGIMER research updates

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Chandigarh
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2000 bed tertiary care referral centre in Chandigarh, North India

Caters to patients from seven adjoining States (Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, western parts of Uttar Pradesh, Uttaranchal, and some parts of Rajasthan) thus representing a large geographical area

2,42,3501 outpatients and 87973 admission in 2016
Enteric Lab

Diagnostic (conventional as well as molecular)
- Urine and Stool samples
- Load - 45000 /year
- NABL accreditation

Surveillance and referral services investigating and managing outbreaks of cholera, and food poisoning in this geographic area.

Research in the field of diarrhea, food borne infections and urinary tract infections with special focus on epidemiology and drug resistance and pathogenesis
Antimicrobial resistance in Enteric and Uro pathogens is being studied at community and hospital level by both phenotypic and molecular assays
Conventional Culture for Salmonella, Shigella, Vibrio cholerae, Campylobacter, STEC and Yersinia, C. difficile
Antigen detection for STEC, Campylobacter, C. difficile
Molecular tests
- Multiplex PCR for diarrhoeagenic *E. coli*
- *Shigella* PCR
- STEC PCR
- Campylobacter PCR
Rapid dipstick for cholera and dysentery
Food borne Pathogens

Vibrios, Bacillus cereus, Shigella, Aeromonas, Pleisomonas, Yersinia, Campylobacter, Staphyloccocus aureus, Listeria, testing of milk samples by total plate counts, methylene blue test, turbidity test, coliform tests for milk, full water testing for coliforms, E coli, Enterococci, V. cholerae, testing of water directly for diarrhoeagenic E.coli using in house protocols (PCR).

Molecular assays to detect directly from food samples

Special food (RUTF) which was developed to treat malnutrition was tested by us for component analysis and shelf life of the prepared food.

Receive request from Government labs for testing commercial food and water samples

2017
Capacity for molecular epidemiology

- AFLP
- PFGE
- MLVA
- Ribotyping
- Plasmid typing
- Rep-PCR and RAPD
- MLST
- WGS
Public Health Contribution

Public health role and surveillance
Investigated 25 outbreaks of cholera and gastroenteritis.

Visit the area and collect water samples, stool samples and study the factors responsible for cholera.

Due to fast identification of source of water contamination, we could control the cholera outbreaks in a week.

We are also studying the molecular epidemiology of *V. cholerae* causing the recent epidemics in our region by AFLP, ribotyping and PFGE.

2017
WHO APW project and DFC

Strengthening district public health laboratories for laboratory surveillance of communicable diseases of outbreak potential

To assist in capacity building of the district laboratories.
- Punjab-Moga, Bhatinda, Hoshiarpur, Sangrur
- Haryana-Ambala, Faridabad, Bhiwani, Panchkula
- Uttarakhand-Dehradun, Haridwar, Tehri Garhwal, Pauri

To assist in technical operations & provision of resources needed to ensure required quality of laboratory procedures.

To formulate a framework for establishment of quality control & quality assessment schemes in these laboratories.

To periodically visit the laboratories at district level & monitor the progress
WHO APW project and DFC-Phase 2

**Stool referral system**: A total of 336 stool samples were collected and submitted from the following centers - Moga (N=58), Ambala (N=38), Sangrur (N=17), Panchkula (N=72) and Pauri (N=32) DPHLs. These samples were collected from PHC and DPHL level by active surveillance.

Most of the samples (231, 68%) were sent by local couriers and 95% were received in proper conditions, though some were not sent in double packaging.

**Outbreaks of cholera** were reported from Ambala, Moga and Panchkula, samples were also submitted from suspected cases from Sangrur, Uttarakhand and Chandigarh for confirmation.

Fourteen outbreaks of diarrhoea / food poisoning occurred in 2010 and 2011 followed by 4 outbreaks each of dengue, chickenpox, measles and viral fever, 3 of jaundice, 2 of typhoid one each of viral encephalitis and rubella.

Tool for assessment of biosafety in laboratory

The culture facilities were established at the following centres - Ambala, Bhiwani, Haridwar, Pauri.

Quality assurance panels were sent and responded.
Food borne illnesses

Foodborne illness are of serious concern to public health worldwide

90% bacterial infections followed by parasitic and viral

Major bacterial pathogens: *Campylobacter, Salmonella*, Diarrhoeagenic *E. coli, Listeria, Yersinia, Shigella, Vibrio*, etc.

*Salmonella* is the leading cause of death followed by *Campylobacter* (CA Alert;2008)

Both occur in intestinal tract of sheep, goat, pigs and poultry
*E. coli* is commensal microbiota but some may be pathogenic strains i.e. EPEC, ETEC, EAEC, EIEC, STEC

Transfer of virulence genes can occur through lateral gene transfer

Diarrhoeagenic *E. coli* caused maximum hospitalizations

Contaminated food and water are main sources of infections
Indian scenario

Burden of foodborne diseases is unknown

No surveillance system

No national database of epidemiology of common food borne pathogens

Foodborne infections occur as sporadic cases and also as outbreaks

Underreporting of foodborne outbreaks and cause is rarely established. Foodborne gastroenteritis is clubbed with acute diarrhoea and is not notifiable
In our region too foodborne illnesses are very common but not investigated or reported.

In a study conducted by us, in collaboration with WHO, we found that every year 1,400 to 31,000 cases of suspected food- and-water-borne infections were being reported at the district public health labs (DPHLs) across Punjab, Haryana and Uttarakhand.

Every year one to three outbreaks of food poisoning reportedly occurred at the DPHLs (Unpublished data PGIMER) Almost all of these go uninvestigated.
How antibiotics in livestock affect us

used for prophylactic, therapeutic and growth promotion

suppress gut flora leaving more nutrients to be absorbed by animal leading to greater gain in weight

Most of the antibiotic classes are the ones used for humans

Overuse has lead to emergence of antibiotic resistance

Residues of these antibiotics remain active in animals for certain time and are also excreted in faeces

Consumption of trace levels of these residues in foods may alter human intestinal microflora and cause resistance gene transfer

Low levels of abs released in environment accumulate and affect bacteria and cause transfer of resistance genes

2017
Global antimicrobial consumption in livestock (Van Bockerel; 2015)
What we lack in India

Guidelines on antibiotic use in feed are available, but not implemented.

Antibiotics used for human disease treatment are used in growth promoters.

No monitoring of residue limits in food meat in Indian markets.

We do not know the antibiotic resistance pattern or resistance gene pool in food animals.

We are unaware of the rate of transmission of antibiotic resistance to humans.
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Class / active compound</th>
<th>Human + veterinary</th>
<th>Human (topical) + veterinary</th>
<th>Veterinary</th>
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</thead>
<tbody>
<tr>
<td>Furazolidone</td>
<td>Nitrofuran</td>
<td></td>
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<tr>
<td>Bacitracin</td>
<td>Peptides from B. licheniformis</td>
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<tr>
<td>Neomycin + doxycycline</td>
<td>Aminoglycoside + tetracycline</td>
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<tr>
<td>Colistin</td>
<td>Polymyxin E</td>
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<tr>
<td>Amoxicillin</td>
<td>Penicillin</td>
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<tr>
<td>Levofloxacin</td>
<td>Fluoroquinolone</td>
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<td>Neomycin + doxycycline</td>
<td>Aminoglycoside + tetracycline</td>
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<td></td>
<td></td>
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<tr>
<td>Neomycin + oxytetracycline</td>
<td>Aminoglycoside + tetracycline</td>
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<td></td>
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<tr>
<td>Neomycin + sulphadizamine</td>
<td>Aminoglycoside + sulphonamide</td>
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<tr>
<td>Avilamycin</td>
<td>Orthosomycin</td>
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<tr>
<td>Oxytetracycline</td>
<td>Tetracycline</td>
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<tr>
<td>Halquinol</td>
<td>chloroxine</td>
<td></td>
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<tr>
<td>Tilmicosin</td>
<td>macrolide</td>
<td></td>
<td>veterinary</td>
<td></td>
</tr>
<tr>
<td>Perfloxacin</td>
<td>quinolone</td>
<td>Discontinued in human</td>
<td>Used in Veterinary</td>
<td></td>
</tr>
<tr>
<td>Tiamulin</td>
<td>Pleuromutilin (inhibit protein synthesis by binding to 50S subunit of ribosomes )</td>
<td>Veterinary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxyclozanide</td>
<td>salicylanilide anthelmintic</td>
<td>Veterinary</td>
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<td>Enrofloxacin</td>
<td>Fluroquinolone</td>
<td>Veterinary</td>
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<td></td>
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<td>Chlorotetracycline</td>
<td>Tetracycline</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ceftiofur</td>
<td>Third generation cephalosporin</td>
<td>Veterinary</td>
<td></td>
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<tr>
<td>Amikacin</td>
<td>Aminoglycoside</td>
<td>Veterinary + human</td>
<td></td>
<td></td>
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<tr>
<td>Tylosin</td>
<td>Macrolide</td>
<td>Veterinary</td>
<td></td>
<td></td>
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<tr>
<td>Flumequine</td>
<td>Fluroquinolone</td>
<td>Discontinued in human</td>
<td>Used in Veterinary</td>
<td></td>
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</table>
One Health Approach
WHO-AGISAR Project

Monitoring the Antimicrobial resistance profile of bacterial food borne pathogens in Humans, food animals and retail meat in India
Human Samples

Sample collection
Total samples collected = 1968
proforma for history was filled
Diarrheal stool samples were collected & transported in Cary and Blair transport medium
Map showing sites of human stool sample collection
2. Sample Processing

- **E. coli**
  - MacConkey agar + Peptone broth
  - 37°C for overnight incubation
  - Subculture from Peptone to MacConkey agar
  - 37°C for overnight incubation

- **Salmonella**
  - MacConkey agar + XLT4 agar + CHROMagar (37°C) + Rappaport broth (42°C) for overnight incubation
  - Subculture from Rappaport broth to MacConkey agar + XLT4
  - 37°C for overnight incubation

- **Campylobacter**
  - Campy-Cefex agar + CHROMagar + Bolton broth at 42°C under microaerophillic conditions for 48 hours
  - Subculture from Bolton broth to Campy-Cefex agar + CHROMagar

Colonies were identified by MALDI-TOF *
- *Salmonella* identified up to genus level
- Further serotyping to be done
Animal samples

Samples collected: 839
chickens: 487
pigs: 352
Map Showing sites ofimal sample collection
Animal samples

Animal (goat, sheep, pig) meat and stool samples were collected from a slaughter house, Chandigarh which receives animals from Chandigarh and nearby areas of Panchkula, Manimajra, Mohali and Punjab.

Poultry stool samples were collected from farms in and around Chandigarh.

These samples were processed for *Campylobacter*, Non-Typhoidal *Salmonella* and *E. coli* detection by culture methods.
Animal sample processing

Sheep/Goat, Pig, Poultry

Stool

Direct inoculation on respective agar + Enrichment broth for *E. coli*, *Salmonella*, *Campylobacter*

Meat

Approx. 10 g of meat in 990 ml Buffered peptone water + Rappaport broth + Bolton broth

Subcultured on respective agar for *E. coli*, *Salmonella*, *Campylobacter*
<table>
<thead>
<tr>
<th>Source</th>
<th>Sample (n)</th>
<th>Campylobacter (%)</th>
<th>Salmonella (%)</th>
<th>E. coli (%)</th>
<th>Multiplex PCR identified pathotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>Stool (90)</td>
<td></td>
<td>7 (7.78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meat (191)</td>
<td></td>
<td>3 (1.57)</td>
<td></td>
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<tr>
<td>Pig</td>
<td>Stool (57)</td>
<td></td>
<td>22 (38.6)</td>
<td></td>
<td>EAEC (1%)</td>
</tr>
<tr>
<td></td>
<td>Meat (151)</td>
<td></td>
<td>29 (19.21)</td>
<td></td>
<td>EPEC (8%)</td>
</tr>
<tr>
<td>Chicken</td>
<td>Stool (340)</td>
<td></td>
<td>38 (11.18)</td>
<td></td>
<td>STEC/EHEC (4.5%)</td>
</tr>
<tr>
<td></td>
<td>Meat (10)</td>
<td></td>
<td>0</td>
<td></td>
<td>ETEC (5.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>839</td>
<td>5% samples has both Campylobacter and Salmonella</td>
<td>99 (11.8)</td>
<td></td>
<td></td>
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</table>
Table showing results of human stool samples

<table>
<thead>
<tr>
<th>State</th>
<th>Locations</th>
<th>Number of samples</th>
<th>Campylobacter</th>
<th>Salmonella</th>
<th>DEC E. coli Pathotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EAEC</td>
</tr>
<tr>
<td>Punjab</td>
<td>PGIMER</td>
<td>850</td>
<td>10</td>
<td>13</td>
<td>36</td>
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<tr>
<td></td>
<td>Panchkula</td>
<td>35</td>
<td>4</td>
<td>0</td>
<td>5</td>
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<tr>
<td></td>
<td>Manimajra</td>
<td>745</td>
<td>12</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ambala</td>
<td>29</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Haryana</td>
<td>Kangra</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Shimla</td>
<td>70</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Hamirpur</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Himachal Pradesh</td>
<td>Kangra</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Shimla</td>
<td>70</td>
<td>3</td>
<td>1</td>
<td>7</td>
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<tr>
<td></td>
<td>Hamirpur</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Uttarakhand</td>
<td>Rudrapur</td>
<td>19</td>
<td>3</td>
<td>0</td>
<td>2</td>
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<tr>
<td></td>
<td>Haldwani</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td></td>
<td>Rishikesh</td>
<td>86</td>
<td>4</td>
<td>1</td>
<td>6</td>
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<td></td>
<td>Dehradun</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Haridwar</td>
<td>38</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Rajasthan</td>
<td>Jaipur</td>
<td>24</td>
<td>3</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Punjab</td>
<td>Ludhiana</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1941</td>
<td>45 (2.32%)</td>
<td>24 (1.24%)</td>
<td>98 (5.05%)</td>
</tr>
</tbody>
</table>
Antibiotic usage and residues
Antibiotic susceptibility
Antibiotic resistance profile of ETEC in humans (n=94)

- 52.13% ESBL producers
- 51.06% multi drug resistant

High frequency of resistance to cephalosporins and fluoroquinolones
Antibiotic resistance profile in *Salmonella* from Humans (n=24)

- **72% ESBL producers**

- High frequency of resistance to fluoroquinolones
- Resistance seen against 3rd generation cephalosporins
- No resistance to carbapenems
Low resistance was detected
Antimicrobial susceptibility in commensal *E. coli* from animal stool samples
Antimicrobial susceptibility in commensal E. coli from meat samples

2017
MDR Commensal *E. coli*

- 23%
- 16%

ESBL producing *E. coli*

- 38%
- 32%
- 30%

Legend:
- Chicken
- Goat
- Pig

Year: 2017
Antibiotic resistance genes
One of the ETEC strain belongs to ST131 complex and some new sequence types have been identified. ST131 has emerged worldwide and is linked to \( \text{bla}_{\text{CTX-M-15}} \). Extensive studies investigating the association of the multilocus sequence typing (MLST) clonal complex ST131 and \( \text{bla}_{\text{CTX-M-15}} \) have been reported from Canada, India, Kuwait, France, Switzerland, Portugal, Spain, Korea and Japan; worldwide dissemination of \( \text{bla}_{\text{CTX-M-15}} \) seems to be linked to this clonal complex, which is situated in the phylogenetic group B2. This demonstrates the need for constant surveillance in developing countries to prevent the spread of these multiresistant isolates. Another ETEC strain belong to ST 117 strains isolated worldwide (such as Brazil, USA, Egypt, Denmark, Sri Lanka, and South Korea) have ColV related plasmids, were involved in osteomyelitis and arthritis cases. These STs are commonly shared by APEC and human ExPEC strains. These hypothetical hybrid strains could have the potential to infect humans and birds. Many new STs are found.
Future work

Serotyping of *Salmonella* isolates

Perform MIC testing of *Campylobacter, Salmonella* and *E. coli* pathotypes isolates using sensititre plates

Estimate Antibiotic residue levels in meat samples by LC-MS-MS

Farm enviorns, water sewage using one health approach

Perform Whole Genome Sequencing of isolates to understand the transmission dynamics and determine AMR with more precision
Team Members

Dr. Balvinder Mohan, Dr. Chandra Deo, Harpreet, Naveen, Vinay Modgil, Jaspreet Mahindroo, Vishal, Varun Shahi, Meenakshi, Yousuf, Pinki Shankar, Ritu Verma, Bhaskar Samaui, Dhananjay
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