

# **Indo-Dutch collaboration on AMR One Health Project Integrated AMR Surveillance in Krishna District in Andhra Pradesh Report**

## **Background**

Antimicrobial resistance is increasing worldwide and is mainly driven by inappropriate use of antibiotics and poor infection prevention in both human health care and food animal production. The Hon'ble Union Minister of Health & Family Welfare, Govt. of India and the Netherlands Minister of Health Welfare and Sports signed a Memorandum of Understanding (MoU) in 2014. Antimicrobial resistance (AMR) has been identified as a priority area for collaboration.

Various discussions were held during Indo-Dutch meetings during the years 2016-2018. Detailed knowledge about the occurrence and spread of AMR between humans, food animals and the environment were discussed to be essential for planning effective AMR control measures. Harmonizing methods and integrating information across the different domains allows integrated analysis of AMR from a One Health perspective. Based on these discussions the following areas of collaboration with a One Health approach were identified:

- Integrated surveillance on AMR/Antimicrobials in humans, food, animals and environment
- Strengthening Infection prevention control (IPC) in health care settings

Based on the preassessment carried out in 3 districts in 3 states, it was agreed to develop a pilot project to be carried out in Krishna district, Andhra Pradesh. After successful implementation and review, the project model can be extended to other districts/states.

Indo-Dutch Pilot project in Krishna district was finalized through a meeting in Dec 2018 at Krishna District with participation from implementing Institutions, NCDC and team from Netherland. Letter sent from MOHFW to Secretary (H), Andhra Pradesh in May 2019 for provision of budget for the project under NHM.

## **Objectives of the project**

The main objectives of the pilot project on AMR are:

1. To gain the insights needed for targeted control of AMR in humans, food animals and the environment by conducting a One Health surveillance project in Krishna district.
2. To improve infection prevention and control in participating healthcare institution/s.
3. To build a sustainable collaborative network between professionals and organizations in the fields of human, food animal and environmental public health.
4. To support implementation of the National/State Action Plan for containment of AMR.

## **Implementing Institutions in Andhra Pradesh and Principal Investigators**

1. Dept. of Veterinary Microbiology, NTR College of Veterinary Science, Sri Venkateshwara Veterinary University, Gannavaram (Veterinary / Animal Health and Environment sectors): Dr. P. Anand Kumar, Principal Investigator

2. Dept. of Microbiology, Siddhartha Medical College, Vijayawada (Human health sector):  
Dr. Ratna Kumari, Principal Investigator and Dr. M Sudha Rani, Co-investigator
3. DG, DCA, Govt. of Andhra Pradesh identified by the state as the state Coordinator for this project

### **Activities planned**

1. To isolate the *Escherichia coli* from following samples in Krishna District:
  - a. Humans: Urine samples from UTI patients
  - b. Animals: Cloacal swabs of chickens from poultry farms & slaughter units
  - c. Environment: Water samples near poultry farms and slaughter units
2. To characterize all the isolated *E. coli* strains by cultural and biochemical tests
3. Surveillance of specific AMR in *E. coli* isolates in selective chicken and environmental samples by culturing the samples on selective bacteriological medium: Extended spectrum beta lactam resistance
4. Develop standard operating procedures (SoPs) for testing of samples, testing all *E. coli* isolates against a harmonized panel of antibiotics, panel of antibiotics tested is at Annexure 1
5. Use existing SoPs for broth microdilution (BMD) testing and Internal Quality control (IQC) testing available under the National AMR Surveillance network (NARS-Net) at NCDC website
6. Provide trainings
7. To conduct antimicrobial susceptibility testing of the characterized *E. coli* isolates as per the standardized SoP
  - Kirby Bauer disc diffusion test for all antibiotics except colistin
  - Broth microdilution test for colistin
8. To enter the AST data and analyze the antibiotic sensitivity profile of *E. coli* isolates using WHONET software
9. To strengthen infection prevention and control through tailored trainings in hygiene practices at the involved hospitals

### **Duration of the project**

1 Year from February 2020 to February 2021, got delayed due to pandemic; lab testing ended in September 2021

### **Funding**

Funds were provided to the implementing agencies (for manpower, small equipment and consumables) from NHM training budget as per approval from GoI

### **Technical support provided**

- The SOPs for the project finalized by experts from NCDC, New Delhi and RIVM, Netherlands and WHO-India office through periodic online meetings with the Principal Investigators of the implementing institutions

- Isolation and identification of clinically significant *E. coli* from community acquired urinary tract infections in humans
- Isolation and identification of *E. coli* and ESBL producing *E. coli* from cloacal swabs of poultry
- Isolation and identification of *E. coli* and ESBL producing *E. coli* from water samples
- Online trainings were provided on:
  - Antimicrobial sensitivity testing
  - Storage and transport of stock cultures
  - Use of WHONET software for AMR surveillance data management
- On-site support visit done by NCDC in September 2021
- The objective on strengthening Infection Prevention and Control required physical trainings and support visits hence could not be initiated as yet due to the pandemic

### **Sample collection and testing**

- A total of 200 chicken cloacal samples/swabs were collected from 100 chickens (two swabs from a bird). Of the two swabs collected from each bird, one was directly cultured on MacConkey agar. Another swab was used on enrichment in tryptic soy broth (TSB), which was subsequently cultured on cefotaxime (1 mg/lit) incorporated MacConkey agar for isolating presumptive Extended spectrum  $\beta$ -lactamase (ESBL) producing *E. coli*
- A total of 100 water samples were collected from poultry farms and chicken shops that maintain birds for slaughter. Water samples include water from troughs used for providing water to birds and water outlets from farms and chicken shops. Out of 100 water samples 93 samples were cultured on Tryptone Bile X-glucuronide (TBX) agar and out of these 93 samples, 12 random samples were cultured on Cefotaxime (1mg/lit) incorporated TBX agar for isolation of presumptive ESBL producing *E. coli*.
- A total of 153 isolates of *E. coli* were isolated from chicken cloacal swabs and water samples.
  - 74 isolates from chicken cloacal swabs directly cultured on MacConkey agar
  - 79 isolates from water samples directly cultured on TBX agar.
- 19 presumptive ESBL producing *E. coli* were isolated on cefotaxime containing media:
  - 14 isolates from chicken cloacal swabs plated on MacConkey agar plates supplemented with 1 mg/lit cefotaxime - presumptive ESBL producing *E. coli*
  - 5 isolates from water samples plated on TBX agar plates supplemented with 1 mg/lit cefotaxime - presumptive ESBL producing *E. coli*
- A total of 100 clinically significant isolates of *E. coli* isolated from urine samples of human patients were included in the study
- All the 253 *E. coli* isolates were characterized by cultural and biochemical tests: 100 from human samples, 74 from cloacal samples (animal) and 79 from water samples (environment)

### **Quality Control**

- Internal quality control for antimicrobial sensitivity testing was conducted using:
  - *Escherichia coli* ATCC 25922 as per the zone diameters in CLSI M-100, 30th Ed. (2020).

- *Klebsiella pneumonia* ATCC 700603 as QC ESBL producing strain as per the zone diameters in CLSI M-100, 30th Ed. (2020)
- For external quality control representative isolates were sent from both the implementing Institutions to NCDC AMR laboratory for confirmation

### **Data entry and analysis**

Data Entry was done in WHONET software by the two participating institutions, both WHONET files submitted to NCDC and data analysis was done at NCDC. Of 253 isolates of *E. coli*, included in this study, 40% are from human urine samples, 31% from water samples and 29% from chicken cloacal samples. Comparison of resistance to all antibiotics tested among the isolates from humans, chicken and water samples shows higher resistance to 3rd and 4th generation cephalosporins in human isolates than in chicken cloacal and water isolates. For carbapenems, 8 isolates from human urine samples and 3 from animal cloacal samples were resistant to imipenem. However, higher resistance is observed in isolates from chicken cloacal swabs than in isolates from human samples to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, and chloramphenicol.

### **Conclusion**

- AMR is an emerging problem not just in human health sector but also in animal health sector
- High rates of AMR are observed in isolates from human samples to antibiotics used frequently in humans namely cephalosporins
- High rates of resistance are observed in isolates from chicken cloacal samples to antibiotics used often in poultry namely tetracycline, ciprofloxacin and chloramphenicol and Cotrimoxazole (trimethoprim-sulfamethoxazole)
- The study has been conducted on small sample size and for a more conclusive outcome requires study on larger number of samples

### **Project outcomes**

- Enhanced communications among AMR stakeholders in various sectors within the state
- Enhanced communications between state and center
- Initiation of development of the draft state action plan on AMR
- Development of harmonized SoPs for integrated AMR Surveillance
- Insight into resistance patterns of *E. coli* across sectors