Harmonized One Health Trans-Species and Community Surveillance for Tackling Antibacterial Resistance in India: Protocol for a Mixed Methods Study

HOTSTAR-India Study Group; Manoja Kumar Das, MBBS, MD; Ashoka Mahapatra, MBBS, MD; Basanti Pathi, MBBS, MD; Rajashree Panigrahy, MBBS, MD; Swetalona Pattanaik, MBBS, MD; Sudhansu Shekhar Mishra, PhD; Samarendra Mahapatro, MBBS, MD; Priyabrat Swain, PhD; Jayakrushna Das, MVSc, PhD; Shikha Dixit, PhD; Satya Narayan Sahoo, PhD; Rakesh N Pillai, PhD

1The INCLEN Trust International, New Delhi, India
2Department of Public Health, The INCLEN Trust International, New Delhi, India
3Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India
4Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India
5Department of Microbiology, Institute of Medical Sciences and SUM Hospital, Bhubaneswar, Odisha, India
6Department of Microbiology, Hi-Tech Medical College, Bhubaneswar, Odisha, India
7Fish Health Management Division, Central Institute of Freshwater Aquaculture (ICAR), Bhubaneswar, Odisha, India
8Department of Pediatrics, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India
9Department of Veterinary Surgery, College of Veterinary Science and Animal Husbandry (OUAT), Bhubaneswar, Odisha, India
10Department of Environmental Health, The INCLEN Trust International, New Delhi, India

Corresponding Author:
Manoja Kumar Das, MBBS, MD
Department of Public Health
The INCLEN Trust International
F 1/5, 2nd Floor
Okhla Industrial Area, Phase 1
New Delhi, 110020
India
Phone: 91 1147730000
Email: manoj@inclentrust.org

Abstract

Background: India has the largest burden of drug-resistant organisms compared with other countries around the world, including multiresistant and extremely drug-resistant tuberculosis and resistant Gram-negative and Gram-positive bacteria. Antibiotic resistant bacteria are found in all living hosts and in the environment and move between hosts and ecosystems. An intricate interplay of infections, exposure to antibiotics, and disinfectants at individual and community levels among humans, animals, birds, and fishes triggers evolution and spread of resistance. The One Health framework proposes addressing antibiotic resistance as a complex multidisciplinary problem. However, the evidence base in the Indian context is limited.

Objective: This multisectoral, trans-species surveillance project aims to document the infection and resistance patterns of 7 resistant-priority bacteria and the risk factors for resistance following the One Health framework and geospatial epidemiology.

Methods: This hospital- and community-based surveillance adopts a cross-sectional design with mixed methodology (quantitative, qualitative, and spatial) data collection. This study is being conducted at 6 microbiology laboratories and communities in Khurda district, Odisha, India. The laboratory surveillance collects data on bacteria isolates from different hosts and their resistance patterns. The hosts for infection surveillance include humans, animals (livestock, food chain, and pet animals), birds, and fishes triggers evolution and spread of resistance. The One Health framework proposes addressing antibiotic resistance as a complex multidisciplinary problem. However, the evidence base in the Indian context is limited.

Methods: This hospital- and community-based surveillance adopts a cross-sectional design with mixed methodology (quantitative, qualitative, and spatial) data collection. This study is being conducted at 6 microbiology laboratories and communities in Khurda district, Odisha, India. The laboratory surveillance collects data on bacteria isolates from different hosts and their resistance patterns. The hosts for infection surveillance include humans, animals (livestock, food chain, and pet animals), birds, and fishes triggers evolution and spread of resistance. The One Health framework proposes addressing antibiotic resistance as a complex multidisciplinary problem. However, the evidence base in the Indian context is limited.

Methods: This hospital- and community-based surveillance adopts a cross-sectional design with mixed methodology (quantitative, qualitative, and spatial) data collection. This study is being conducted at 6 microbiology laboratories and communities in Khurda district, Odisha, India. The laboratory surveillance collects data on bacteria isolates from different hosts and their resistance patterns. The hosts for infection surveillance include humans, animals (livestock, food chain, and pet animals), birds, and fishes triggers evolution and spread of resistance. The One Health framework proposes addressing antibiotic resistance as a complex multidisciplinary problem. However, the evidence base in the Indian context is limited.
to antibiotic use. The data analysis will follow quantitative (descriptive and analytical), qualitative, and geospatial epidemiology principles.

**Results:** The study was funded in May 2019 and approved by Institute Ethics Committees in March 2019. The data collection started in September 2019 and shall continue till March 2021. As of June 2020, data for 56 humans, 30 animals and birds, and fishes from 10 ponds have been collected. Data analysis is yet to be done.

**Conclusions:** This study will inform about the bacterial infection and resistance epidemiology among different hosts, the risk factors for infection, and resistance transmission. In addition, it will identify the potential triggers and levers for further exploration and action.

**International Registered Report Identifier (IRRID):** DERR1-10.2196/23241

(JMI Res Protoc 2020;9(10):e23241) doi: 10.2196/23241

**KEYWORDS**

bacterial infection; antibiotics resistance; sentinel surveillance; drug prescriptions; One Health; India

**Introduction**

The last century has witnessed a significant reduction in infectious disease mortality and morbidity with the use of antimicrobials. Antimicrobial resistance (AMR), especially antibiotic resistance (ABR), poses a major threat to clinical medicine and public health. The development of new antimicrobials and antibiotics is becoming increasingly difficult and is unable to match the pace of emergence of resistance. It is estimated that AMR-attributable deaths shall rise from 700,000 in 2014 to 10 million by 2050, with US $100 trillion lost output [1]. India is the top contributor towards global morbidity and mortality. India also carries the largest burden of drug-resistant organisms worldwide, including multiresistant and extremely drug-resistant mycobacteria and resistant Gram-negative and Gram-positive bacteria. In India, approximately 60,000 newborns die from resistant bacterial infections [2]. It is projected that over 2 million Indians will die because of AMR by 2050 [3]. Infection with methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive S aureus (MSSA) increased the risk of death by 5.6 times and 2.7 times, respectively [4]. The attributable risk of death with MRSA was double that of MSSA by 90 days [4]. Infection with resistant Escherichia coli and S aureus increased mortality risk by 1.8 to 2.5 times at 30 days [5]. Antimicrobial usage has enhanced animal and fish production globally, paralleling the demand.

AMR is a major threat to food safety, food security, and socioeconomics of millions of farming communities. Resistant bacteria are found in humans, animals, birds, aquatics, plants, and the environment (water, soil, and air), and they move between hosts and ecosystems [6]. In India, >70% of Acinetobacter baumannii, E coli, and Klebsiella pneumoniae and >50% of Pseudomonas aeruginosa were resistant to broad-spectrum antibiotics (fluoroquinolones and third-generation cephalosporins) [7]. Extended-spectrum beta-lactamase (ESBL)–producing E coli strains from chickens and multidrug-resistant Salmonellae species have been reported in India [8-12]. In New Delhi, metallo-β-lactamases (NDM-1, superbug), ESBL-producing Gram-negative bacteria, and vancomycin-resistant S aureus (VRSA) have been reported in milk from cows with mastitis [13,14]. ESBL-producing Enterobacteriaceae in tilapia fishes has been reported from urban water bodies and resistant Vibrios from shellfishes has been reported in the market [15,16]. Resistant bacteria and genes have also been isolated from hospital wastewater, sewage, rivers, surface water, and groundwater in India [17-20].

Although ABR and AMR emerges naturally, antibiotic consumption or usage in humans, animals, and agriculture, environmental waste contamination, sanitation, and infection control practices are the potential drivers for increase in ABR and AMR [1,7]. India’s antibiotic consumption (absolute and percentage increase) is highest globally. Between 2000 and 2015, India’s gross antibiotic consumption increased by 103% (3.2-6.5 billion defined daily doses [DDDs]) and antibiotic consumption rate increased by 63% (8.2-13.6 DDDs per 1000 inhabitants) [21]. The prescription behavior, fixed dose combinations, social pressures, and market influences are some of the factors [22]. In 2010, India was the fifth largest consumer of antibiotics in food animals (poultry, pigs, and cattle) and will become the fourth largest consumer of antibiotics in food animals by 2030 [23]. Approximately four-fifth of the antibiotics used in animals are growth promoters [24]. Approximately 40% of the chicken samples in India had high concentrations of antibiotics [25]. Antibiotic residues have been documented in animal milk [24,26]. India is a hot spot for antibiotic use in food animals, with a use of 30 kg per km², which will grow by 312% by 2030 [23]. The global consumption of antibiotics in animals is estimated to be twice that of humans [27].

Surveillance is an essential tool to document and monitor the ABR and risk factors and appropriately inform policies, infection control, and prevention responses at local, national, and global levels. The Global AMR Surveillance System by the World Health Organization targets 8 bacterial species (Acinetobacter spp., E coli, K pneumoniae, Salmonella, S aureus, Streptococcus pneumoniae, Shigella spp., and Neisseria gonorhoeae) [6]. The Antimicrobial Resistance Surveillance Network coordinated by the Indian Council of Medical Research includes 9 types of bacteria (E coli, K pneumoniae, Enterobacter spp., A baumannii, P aeruginosa, Salmonella spp., S pneumoniae, S aureus, and Enterococcus spp.) [28]. These surveillance efforts are primarily targeted at human infections. Studies involving animals and environments have targeted isolation of resistant bacteria, resistance genes, and molecular characterization without crosslinking the hosts and their ecosystems [7].
The One Health approach attempts to address complex multidisciplinary problems through designing and implementing programs, framing policies and legislation, and conducting research where multiple sectors converge and collaborate for achieving better outcomes in public health, animal or bird or aquatics health, and environmental settings. The One Health approach has been advocated in infectious diseases including zoonoses, food safety, and AMR or ABR, considering the interdependence of human, animal, and environmental factors and determinants for emergence of resistance [29]. There is paucity of data from India on One Health surveillance. An integrated surveillance system considering data from humans, animals, food, and the environment and antibiotic usage or consumption for humans and animals appears to be critical.

As part of the Grand Challenge India on Antimicrobial Resistance program, this district-based ecological surveillance in India attempts to document the resistance pattern of 7 index bacteria isolated from multiple hosts, including humans, animals (livestock, food chain, and pets), birds (food chain birds such as chicken), and freshwater fishes, correlate with their exposure to antibiotics, disinfectants, and other risk factors at individual, household or farm, and community levels, and analyze the data applying geospatial epidemiology.

An intricate interplay of infections, exposure to antibiotics, and disinfectants at individual and community levels among humans, animals, birds, and fishes triggers the evolution and spread of ABR. The directions of ABR transmission across these species are unclear and probably multidirectional. We hypothesize that concurrent surveillance for index bacterial infections and ABR patterns, exposure to antibiotics and disinfectants, and relevant risk factors for different hosts and environments will improve the knowledge base. The application of multidimensional geospatial epidemiology analysis will inform about the interlinkages between exposures and ecosystems. This multi-sectoral, trans-species surveillance follows the One Health approach and includes 7 priority antibiotic-resistant bacteria: A. baumannii, P. aeruginosa, Enterobacteriaceae, E. coli, K. pneumoniae, S. aureus, Enterococcus faecium, and Salmonellae spp [30]. Figure 1 shows the conceptual model for the current surveillance.

Methods

Objectives

This surveillance aims to (1) document the pattern of infections because of the 7 index bacteria in humans, animals (including livestock, food chain, and pets), birds (food chain birds such as chicken), and freshwater fishes sharing the same environment and their resistance patterns, (2) document the risk factors for ABR at the individual and community level related to health, antibiotic consumption, and antibiotic usage in food animal breeding and agriculture, and (3) apply geospatial epidemiology analytical methodology to improve the understanding of bacterial infections and ABR.

This study aims to (1) conduct surveillance for infection with the index bacteria under study and ABR patterns in humans, animals (including livestock, food chain, and pets), birds (food chain birds such as chicken), and freshwater fishes over 1 year; (2) document the potential factors at the individual, household, or habitation or farm level related to illnesses, care seeking, antibiotic usage, and disinfection that influence resistance among the index bacteria under study, (3) document antibiotic prescription and antibiotic sales patterns for humans (therapeutic and nontherapeutic antibiotic usage in animals including livestock, food chain, and pets), birds (food chain birds such as chicken), and freshwater fishes and agriculture and food processing, and (4) apply multidimensional geospatial epidemiology analysis to generate epidemiological patterns of
bacterial infections and ABR and the linkages with the various potential risk factors under study.

**Study Design**

This study combines surveillance and cross-sectional design with mixed methodology (quantitative and qualitative) for data collection.

**Study Setting**

The study is being implemented at 4 medical college hospitals, 1 veterinary college hospital, and the fishery research institute located in Khurda district, including Bhubaneswar city. The fish samples are being collected from the farming sites in Khurda and the neighboring districts (fish supplying zone). The study is recruiting participants from Khurda district for data collection.

**Study Participants**

The study includes 3 categories of participants:

1. **Hosts with infections:** humans, animals (including livestock, food chain, and pets), birds (food chain birds such as chicken), freshwater fishes (excluding crustaceans) with any of the 7 index bacteria isolates.
2. **Patients attending outpatient clinics:** humans and animal or birds attended by doctors for prescription audits.
3. **Stakeholders for in-depth interviews (IDIs).**

The study also involves data collection from 3 types of facilities: (1) microbiology laboratories, (2) chemists and drug distributors for humans and veterinary medicines, and (3) fish farming sites. Table 1 details the categories and numbers of participants.
Table 1. The facilities and study participants for data collection under each category.

| Serial no. and participant and facility category | Number |
|------------------------------------------------|--------|
| **A: Study participants**                |        |
| **A1: Hosts with infections**               |        |
| A1.1: Humans with positive isolates\(^b\); (newborns \([n=25-30]\); children >1 month to 5 years \([n=25-30]\); and >5 years including adults \([n=50-60]\)) | 100-120 |
| A1.2: Animals (including birds) with positive isolates\(^c\); (animals \([n=30-35]\) and birds \([n=20-25]\)) | 50-60  |
| A1.3: Fishes with positive isolates\(^d\); (fish farms \([n=20]\); 5-6 fishes each weighing >100 grams per farm) | 20     |
| **A2: Patients attending out-patient clinics** |        |
| A2.1: Human patients for prescription audit (for antibiotics) |        |
| Patients (400 per doctor; 100 every quarter) | 6000   |
| Doctors (for human patients) for prescription audit\(^e\); (disciplines: medicine \([n=5]\), pediatrics \([n=5]\), and surgery \([n=5]\)) | 15     |
| A2.2: Veterinary patients for prescription audit (for antibiotics) |        |
| Animals or birds (100 per doctor; 25 every quarter) | 800-1000 |
| Veterinary doctors\(^e\) | 8-10   |
| **A3: Stakeholders for in-depth interview** |        |
| Farmers (food and vegetable) | 20     |
| Agriculture stockists | 5     |
| Food animal breeders | 5     |
| Poultry breeders | 5     |
| Fish breeders or farmer | 5     |
| Animal food processors and distributors | 10     |
| **B: Study facilities**               |        |
| B1: Microbiology laboratories at the participating institutes | 6 |
| Medical microbiology laboratories | 4     |
| Veterinary microbiology laboratory | 1     |
| Fish microbiology laboratory | 1     |
| B2: Chemists and drug distributors for humans and animals | N/A |
| Medical chemists | 12 |
| Medical college pharmacy (n=4) | 4 |
| Other hospital pharmacy (n=4) | 4 |
| General chemist and distributor (n=4) | 4 |
| Veterinary chemists | 4 |
| Near the veterinary college | 1     |
| Other veterinary chemist | 3     |
| B3: Fish farming sites (for antibiotic usage) | 4-5 |
| Quarterly audit (4 per farm; once every quarter) | 16-20  |

\(^a\)N/A: not applicable.

\(^b\)The patients with positive growth for any one of the 7 index bacteria from any of these samples: blood, urine, stool, pus, sputum, and other sterile body fluid such as cerebrospinal fluid, pleural fluid, and peritoneal fluid.

\(^c\)The animal and bird with positive growth for any one of the 7 index bacteria from any of these samples: blood, urine, pus, stool, other body parts, and milk.

\(^d\)Fish with positive growth for any one of the 7 index bacteria from gut and gill samples.

\(^e\)The prescription audit includes consecutive new patients (not follow-up patients) seen by the respective doctor.
Selection of Participants
The various study participants and stakeholders shall be selected following strategy.

**Humans With Positive Isolates**
The eligibility criteria included (1) patients from Khurda district, (2) admitted to inpatient departments of the 4 medical college hospitals, and (3) positive culture growth for any index bacteria from samples collected within 48 hours of hospitalization shall be eligible. Of these eligible patients, we shall randomly select according to the age strata (newborns, >1 month to <5 years, and >5 years), type of bacteria, and departments to obtain representative distribution.

**Animals With Positive Isolates**
The eligibility criteria included (1) animal (including livestock, food chain, and pets) and birds (food chain birds such as chicken) with any infection from Khurda district attending the veterinary college hospital, (2) with positive culture growth for any index bacteria from samples, and (3) inpatient samples collected within 48 hours of hospitalization or outpatient samples collected from fresh patients or fresh samples collected from animals or birds in the farms. Of these eligible patients, we shall randomly select according to the animal or bird type; livestock, pet, or food chain animals and birds; and the specimen types to obtain a representative distribution.

**Fish Farms With Positive Isolates**
The eligibility criteria included (1) freshwater fish farms from Khurda or surrounding districts supplying to Khurda district and (2) with positive culture growth for any index bacteria from samples.

**Doctors (for Human Patients) for Prescription Audits**
The doctors shall be identified from the 4 hospitals (1 per discipline) and from other hospitals or clinics in the Bhubaneswar area. These doctors shall be informed about the activity and consent shall be obtained.

**Veterinary Doctors for Prescription Audit**
The doctors shall be identified from the veterinary hospital and other clinics in the Bhubaneswar area. These doctors will be informed about the activity and consent will be obtained.

**Patients for Prescription Audit**
The patients (humans and animals or birds) attending the selected doctors should be eligible. Patients attending for fresh illness (not follow-up visits) will be approached for consent and data collection.

**Data Collection**
Table 2 shows the data to be collected for different components and study participants. The data collection for different hosts is detailed below and in Table 2.
Table 2. The data components to be collected from various study participants.

| Serial no. and category | Data components to be collected                                                                 | Frequency                  |
|-------------------------|--------------------------------------------------------------------------------------------------|----------------------------|
| A: Study participants   |                                                                                                  |                            |
| A1: Hosts with infections |                                                                                                |                            |
| A1.1 Humans with positive isolates | Sociodemography and occupation • Illnesses, care seeking, and antibiotic usage • Sanitation, waste handling, and disinfection practices and animals or birds exposure • Household location and environmental risk factors (GPS) | Target: 100-120 10-12 per month Once for each participant |
| A1.2 Animals with positive isolates | Demography (types and number of animals or birds, location, and farming period) • Illnesses, care seeking, antibiotic usage, and outcome • Sanitation, waste handling, and disinfection practices • Feeding and nontherapeutic antibiotic usage • Caretaker’s illness and antibiotic usage • Farm location and environmental risk factors (GPS) | Target: 50-60 5 per month Once for each animal or bird |
| A1.3 Fishes with positive isolates | Demography (species, farm address, and farming period) • Any illness, antibiotics used, and outcome • Sanitation, waste handling, and disinfection practices • Feeding and nontherapeutic antibiotic usage, pesticides, and disinfectant usage • Caretaker’s illness and antibiotic usage • Farm (habitation for domesticated or nonfarm animals or birds) location and environmental risk factors (GPS) | Target: 20 farms 5-6 farms per quarter Once for each farm |
| A2: Patients attending outpatient clinics |                                                                                                  |                            |
| A2.1 Human patients for prescription audit | Age, gender, diagnosis, and medicines prescribed • Doctor attended | Once for each patient 8-10 new prescriptions per week for specific doctor |
| A2.2 Veterinary patients for prescription audit | Animal or bird type, diagnosis, and medicines prescribed • Doctor attended | Once for each animal or bird 8 new prescriptions per month for specific doctor |
| A3 Stakeholders for in-depth interview | Perceptions, knowledge, attitude, practices, and barriers related to antibiotic usage in agriculture, animal breeding, food industry and potential influence on resistance | Once for each participant Interviews once during study period |
| B: Study facilities     |                                                                                                  |                            |
| B1 Human and animal or bird microbiology laboratories | Number of samples received, types of samples (body part or fluid), number of samples with positive bacteria growth, number of samples with positive index bacteria growth, and antibiotic sensitivity | Daily screening |
| B2 Fish microbiology laboratory | Number of samples collected and processed, types of samples (body part), number of samples with positive bacteria growth, number of samples with positive index bacteria growth, and antibiotic sensitivity | Periodic, when sample collected or processed |
| B3 Chemists and drug distributors | For humans and animals: • Volume of antibiotics sold | Monthly |
| B4 Fish farming sites    | Antibiotic usage, pesticides, and disinfectant usage on quarterly basis | Quarterly |
Infections in Humans

Surveillance of Bacterial Infections
At the 4 medical college hospitals, daily surveillance will be conducted to identify any positive index bacteria isolates from the samples of the hospitalized patients. For the positive index bacteria isolates, information about antibiotic sensitivity; dates of sample collection and admission; and patient information including diagnosis, antibiotics used, outcome, and basic demography (age and gender) will be documented. Among these patients with positive bacteria isolates, eligible patients (as per the eligibility criteria defined above) will be identified from the admission registers for detailed data collection at the household or farm level.

Detailed Individual Data Collection for the Humans With Positive Bacteria Isolates
Of the eligible patients, approximately 120 patients (10-12 patients every month) will be randomly selected. These selected patients (and family members) will be contacted after the discharge or death of the patient to schedule the home visit. During home visits, informed written consent will be obtained, followed by data collection using Case Record Forms (CRFs).

Infections in Animals and Birds

Surveillance of Bacterial Infections
For the veterinary college hospital, daily surveillance will be conducted to identify any positive index bacteria isolates from the samples of the animal or bird patients attending the hospital or those that were admitted. For the positive index bacteria isolates, information about antibiotic sensitivity, diagnosis, antibiotic use, outcome, and animal or bird type will be documented. Among these animal or bird patients with positive bacteria isolates, eligible animals or birds will be identified from the records for detailed data collection at the household or farm level.

Detailed Individual Data Collection for the Animals and Birds With Positive Bacteria Isolates
Of the eligible animal or bird patients, approximately 60 patients (5 patients every month) were randomly selected. For these selected animals or birds, their owners or caretakers will be contacted for scheduling household or farm visits. During household or farm visits, informed written consent from the owner or caretaker will be obtained, followed by data collection using the CRF.

Fish Farms With Positive Isolates
For the freshwater fish farms with positive isolates from fishes (not crustaceans), the owners or caretakers shall be contacted and farm visits shall be made for informed written consent followed by data collection using the CRF.

Antibiotic Sales
From the identified chemists and drug distributors for humans, animals, and birds at the hospitals and outside, the data on antibiotic procurement or indent and sales shall be collected on a monthly or quarterly basis. The list shall include oral (tablets, capsules, and syrups) and injectable forms for the different types of antibiotics.

Antibiotic Usage at Fish Farming Sites
For the fish farming sites, quarterly visits will be made to collect information on the use of different antibiotics or disinfectants or chemicals or growth promoters (therapeutic or nontherapeutic) and their quantity.

Doctors for Prescription Audits
For the doctors (human and veterinary), the hospital or clinic and specialty will be documented.

Patients for Prescription Audits
The consecutive patients (and their parents) attending the outpatient department of the identified doctors with a new illness will be approached at exit (consultation completed) for participation. For patients who consent, the age, sex, diagnosis, and medications prescribed will be captured. Similarly, for the animal or bird patients attending the veterinary doctors, the owners or caretakers will be approached for consent to participate. For the animals and birds, the species type, diagnosis, and medicines prescribed will be captured.

Geospatial Data Collection
The precise location data (latitude and longitude) for the households or habitations or farms of the recruited human participants, animals and birds, and fish farming sites with positive bacteria isolates shall be collected using a GPS device (Garmin Montana 680, Garmin). For these participants, neighborhood mapping covering a 100-m radius around their locations will capture the potential risk factors for infection (garbage dump, wastewater, animal or poultry farm, egg or meat vending, hospital or clinic, chemist, hotel or food selling, industry, etc.) with their GPS positions. The technologies to be used for geospatial mapping include GPS, geotagging, and geographic information systems. The GPS data points collected shall be mapped on the state and district satellite map.

Stakeholders for IDIs
The stakeholders or key informants shall be identified purposively considering the profession (agriculturist, stockiest, breeder, food processing, and distribution) and geography to suit the objectives. All IDIs shall be conducted at a convenient place for the participant after obtaining informed consent and the conversation shall be digitally recorded with consent. The IDI will focus on exploring the perceptions, knowledge, attitude, practices, and barriers related to antibiotic use in agriculture, animal breeding and food industry and the potential influence on resistance. The IDIs conducted in local language shall be transcribed verbatim and translated to English. Quality checks of transcripts and translation will be performed for 25% of the audio recordings by another member.

The surveillance and data collection workflow are shown in Figure 2.
Data Management

The surveillance and quantitative data shall be collected using customized software installed on tablets (developed using open source platforms: Android; PHP, the PHP Group; and MySQL, Oracle Corporation) and uploaded to the server through a mobile network. The data collection and transmission process shall have encryption and security measures. The qualitative data shall be collected using IDI guides on paper, followed by transcription, translation, and data entry. All electronic data will be stored in a secured server with multilayered security and daily backup. The investigators and authorized research staff have data access.

Data Analysis

The quantitative data are expressed as means (with standard deviations), medians (with interquartile ranges), and proportions using descriptive statistics. The data for different groups will be compared using $t$ tests, chi-square tests, Mann-Whitney test, and Kruskal-Wallis test, as appropriate. The Jonckheere-Terpstra test will be used to assess the monthly trend of bacteria isolates (proportions), the ABR pattern for antibiotics, antibiotic prescription, antibiotic sales, and statistical significance. The Pearson correlation coefficient will be used to examine the relationship between antibiotic prescription and sales and ABR rates. The qualitative data will be analyzed by content analysis as per the domains identified and follow processes: free listing, coding, axial coding, and cross tabulation. The spatial data will be analyzed using geospatial epidemiology principles, including point pattern analysis (clustering and density), kernel density map (hot spots and catchment), hub analysis (common exposures and catchment area), and overlay analysis (exposures or risk factors layering and spatial correlation; Figure 3). The GPS and geospatial data will be analyzed using ArcGIS, QGIS, and Global Mapper 2.0.
Validity and Reliability
Uniform laboratory processing, antibiotic sensitivity testing, and interlaboratory comparison will be practiced. Monthly teleconference and quarterly site visits will focus on protocol adherence and data validation. The data collected will undergo consistency and range checks by the data management team.

Ethical Aspects
The study protocol was reviewed and approved by the INCLEN Ethics Committee, New Delhi (Ref: IIEC-056), Institute Ethics Committee, All India Institute of Medical Sciences, Bhubaneswar (Ref: T/EMF/Micro/18/6); Kalinga Institute of Medical Sciences Medical Research Committee (KIMS/R&D/255/2018), Institute Ethics Committee, Institute of Medical Sciences & SUM Hospital (Ref: DMR/IMS.SH/SOA/180167); Institutional Ethics Committee for Human Research, Hi-Tech Medical College & Hospital (Ref: HMCH/IEC/19-004). The participants are being recruited after obtaining written informed consent. Appropriate data confidentiality, storage, and access authorization procedures are being adopted.

Results
The study was funded in May 2019 and approved by Institute Ethics Committees in March 2019. The data collection started in September 2019 and shall continue till March 2021. As of June 2020, data for 56 humans, 30 animals and birds, and fishes from 10 ponds have been collected. The other data collection is also in progress. Data analysis is yet to be done.

Discussion
Antibiotics Resistance Problem in India
A scoping report on ABR in India reported that resistance to broad-spectrum antibiotics fluoroquinolones and third-generation cephalosporin was >70% in *A baumannii*, *E coli*, and *K pneumoniae* and >50% in *P aeruginosa* [7]. The isolation of ESBL-producing *E coli* from chickens, multidrug-resistant *Salmonella* from chicken meat samples, VRSA from cow milk samples, and ESBL-producing *Enterobacteriaceae* from fishes indicate the spread of ABR across all food animals and in the environment. Relatively unregulated and high antibiotic usage in humans and among animals and aquatics in India is worrisome. The heavy use of antibiotics is based on high infectious disease burden and past experiences of mortality and a limited understanding of the impact across sectors. The implementation of the One Health framework in policy and program has been challenging in view of simultaneous and connected evidence from the Indian context. There is a need to understand the drivers for the emergence of ABR and transmission across the host-environment systems for appropriate action.

Strengths and Limitations
The multihost simultaneous surveillance for the 7 resistant-priority bacteria among humans, animals, birds, and fishes following the One Health framework is a strength of this study. Therapeutic antibiotic usage for therapeutic purposes among these patients and nontherapeutic usage for animal breeding will allow drawing possible linkages with the resistance pattern. The geospatial epidemiology analysis is expected to provide information about the pattern and linkages between the variables. The shorter observation period may not permit inferring any causal association between the various risk factors and antibiotic resistance.

Conclusions
This study will generate evidence on (1) epidemiology of infections with 7 high-priority bacteria among the different hosts (human, animal, birds, and fishes) and their resistance patterns, (2) the potential risk factors for ABR in the infected hosts at individual and immediate environment levels, (3)
therapeutic and nontherapeutic antibiotic usage for humans, animals, fishes, agriculture, and food processing along with the potential triggers, and (4) potential linkages between data from various sources and identification of possible risk factors for ABR across various hosts and their ecosystems. India is critically important for ABR and transmission of multidrug-resistant bacterial species because of high antibiotic consumption, unrestricted therapeutic and nontherapeutic usage across all sectors, and other environmental and behavioral risk triggers. This multisectoral, trans-species surveillance for bacterial infection and ABR using One Health’s perspective and geospatial epidemiology techniques will improve our understanding of the pattern and spread of resistance and potentially inform about the potential levers for actions in public health, animal, bird and aquatics health, and environmental settings.

Acknowledgments

The authors appreciate the support from the administration of the partner institutes for their facilitation and support in implementing this study. They are highly appreciative of the doctors, nurses, and laboratory staff at the hospitals for their support in conducting the study and data collection. They also wish to acknowledge the commitment and dedication of the research staff engaged in the project: Prikanka Malik, Rajendra Barik, Amruta Mohanty, Subham Ravi Nayak, Birasen Behera, Asima Das, Manas Mahanta, Sushree Subhasmita Sahoo, Arabinda Jena, and Sonali Pattanayak.

This study is funded by the INCLEN Trust International by the Biotechnology Industry Research Assistance Council, a Government of India Enterprise under the Grand Challenge India Initiative on Antimicrobial Resistance (Grant Ref. No. BT/AMR0317/05/18). The study protocol was reviewed and approved by the Institute Ethics Committees of the participating institutes.

Authors’ Contributions

This study was conceptualized by MD with inputs from SD, AM, S Mahapatro, BP, R Panigrahy, SP, S Mishra, PS, SS, and R Pillai. AM, S Mahapatro, BP, R Panigrahy, SP, S Mishra, PS, SS, JD, and MD supervised are supervising data collection. MD prepared the first draft of the manuscript. All authors have contributed to this manuscript and reviewed and approved the final version of the manuscript.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Peer review reports from PMU-BIRAC.

[PDF File (Adobe PDF File), 8 KB-Multimedia Appendix 1]

References

1. O’Neill J. Tackling Drug-resistant Infections Globally: Final Report and Recommendations. BioMérieux Connection. 2016. URL: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf [accessed 2020-10-09]
2. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. Lancet Infect Dis 2013 Dec;13(12):1057-1098. [doi: 10.1016/S1473-3099(13)70318-9] [Medline: 24252483]
3. Resistance Map: India. Center for Disease Dynamics Economics Policy. 2019. URL: https://resistancemap.cddep.org/resmap/c/in/India [accessed 2020-04-20]
4. Wolkewitz M, Frank U, Philips G, Schumacher M, Davey P, BURDEN Study Group. Mortality associated with in-hospital bacteraemia caused by Staphylococcus aureus: a multistate analysis with follow-up beyond hospital discharge. J Antimicrob Chemother 2011 Feb;66(2):381-386. [doi: 10.1093/jac/dko424] [Medline: 21098543]
5. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. Mortality and hospital stay associated with resistant Staphylococcus aureus and Escherichia coli bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Med 2011 Oct;8(10):e1001104 [FREE Full text] [doi: 10.1371/journal.pmed.1001104] [Medline: 2202233]
6. Global Antimicrobial Resistance Surveillance System (GLASS) Report Early Implementation 2017-18. World Health Organisation. 2018. URL: https://www.who.int/glass/resources/publications/early-implementation-report-2017-2018/en/ [accessed 2020-10-09]
7. Gandra S, Joshi J, Trett A, Lamkar GS, Laxminarayan R. The Antimicrobial Resistance Situation in India. 2017. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5653737/ [accessed 2020-10-09]
8. Brower CH, Mandal S, Hayer S, Sran M, Zehra A, Patel SJ, et al. The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant in poultry chickens and variation according to farming practices in Punjab, India. Environ Health Perspect 2017 Jul 20;125(7):077015. [doi: 10.1289/EHP292] [Medline: 28749780]
9. Shrivastav A, Sharma RK, Sahni YP, Shrivastav N, Gautam V, Jain S. Study of antimicrobial resistance due to extended spectrum beta-lactamase-producing in healthy broilers of Jabalpur. Vet World 2016 Nov;9(11):1259-1263 [FREE Full text] [doi: 10.14202/vetworld.2016.1259-1263] [Medline: 27956778]
10. Kar D, Bandyopadhyay S, Bhattacharyya D, Samanta I, Mahanti A, Nanda PK, et al. Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing Escherichia coli isolated from poultry and cattle in Odisha, India. Infect Genet Evol 2015 Jan;29:82-90. [doi: 10.1016/j.meegid.2014.11.003] [Medline: 25445661]

11. Kaushik P, Anjay, Kumar S, Bharti SK, Dayal S. Isolation and prevalence of Salmonella from chicken meat and milk collected from local markets of Patna, India. J Vet World 2014 Feb;7(2):62-65 [FREE Full text]

12. Naik VK, Shaqya S, Patyal A, Gade NE, Bhoomika. Isolation and molecular characterization of Salmonella spp from chicken and milk collected from different districts of Chhattisgarh, India. J Vet World 2015 Jun;8(6):702-706 [FREE Full text] [doi: 10.14203/jvetworld.2015.702-706] [Medline: 27065632]

13. Ghatak S, Singha A, Sen A, Guha C, Ahuja A, Bhattacharjee U, et al. Detection of new Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in Escherichia coli isolated from mastitic milk samples. Transbound Emerg Dis 2013 Oct;60(5):385-389. [doi: 10.1111/bed.12119] [Medline: 23870003]

14. Bhattacharyya D, Banerjee J, Bandyopadhyay S, Mondal B, Nanda PK, Samanta I, et al. First report on vancomycin-resistant staphylococcus aureus in bovine and caprine milk. Microb Drug Resist 2016 Dec;22(8):675-681. [doi: 10.1089/mdr.2015.0330] [Medline: 26990514]

15. Marathe NP, Gaikwad SS, Vaishampayan AA, Rasane MH, Shouche YS, Gade WN. Mossambicus tilapia (Oreochromis mossambicus) collected from water bodies impacted by urban waste carries extended-spectrum beta-lactamases and integron-bearing gut bacteria. J Biosci 2016 Sep;41(3):341-346 [FREE Full text] [doi: 10.1007/s12038-016-9620-2] [Medline: 27581926]

16. Sudha S, Mridula C, Silvester R, Hatha A. Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market. Indian J Geo-Mar Sci 2014 Mar;43(5):815-824 [FREE Full text]

17. Akiba M, Senba H, Otagiri H, Prabhansankar VP, Taniyasu S, Yamashita N, et al. Impact of wastewater from different sources on the prevalence of antimicrobial-resistant Escherichia coli in sewage treatment plants in South India. Ecotoxicol Environ Saf 2015 May;115:203-208. [doi: 10.1016/j.ecoenv.2015.02.018] [Medline: 25704279]

18. Akiba M, Sekizuka T, Yamashita A, Kuroda M, Fujiy Y, Murata M, et al. Distribution and relationships of antimicrobial resistance determinants among extended-spectrum-cephalosporin-resistant or carbapenem-resistant Escherichia coli isolates from rivers and sewage treatment plants in India. Antimicrob Agents Chemother 2016 Mar 7;60(5):2972-2980. [doi: 10.1128/aac.01950-15]

19. Azam M, Jan A, Haq Q. bla CTX-M-152, a Novel Variant of CTX-M-group-25, Identified in a Study Performed on the Ambient Aquatic Ecosystem of the Indus River, Pakistan. Front Microbiol 2016;7:176 [FREE Full text] [doi: 10.3389/fmicb.2016.00176] [Medline: 26941715]

20. Poonia S, Singh TS, Tsering DC. Antibiotic susceptibility profile of isolates isolated from natural sources of water from rural areas of East Sikkim. Indian J Community Med 2014 Jul;39(3):156-160 [FREE Full text] [doi: 10.4103/0970-0218.137152] [Medline: 25136156]

21. Klein EY, Van Boeckel TP, Martínez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci U S A 2018 Apr 10;115(15):E3463-E3470. [doi: 10.1073/pnas.1717295115] [Medline: 29581252]

22. Chandy SJ, Mathai E, Thomas K, Faruqui AA, Holloway K, Lundborg CS. Antibiotic use and resistance: perceptions and ethical challenges among doctors, pharmacists and the public in Vellore, South India. Indian J Med Ethics 2013;10(1):20-27 [Medline: 23439193]

23. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 2015 May;112(18):5649-5654. [doi: 10.1073/pnas.1503141112] [Medline: 25792457]

24. Vinishuraj M, Kandeepan G, Rao K, Chand S, Kumbhar V. Occurrence, public health hazards and detection methods of antibiotic residues in foods of animal origin: A comprehensive review. Cogent Food Agric 2016 Sep 21;2(2):1-[FREE Full text] [doi: 10.1080/23319322016.1235458]

25. Sahu R, Saxena P. Antibiotics in Chicken Meat. Centre for Science and Environment. 2014. URL: https://www.cseindia.org/latest-study-by-cses-pollution-monitoring-lab-finds-antibiotic-residues-in-chicken-8498 [accessed 2020-10-09]

26. -. Chemical contaminants in milk and public health concerns: a review. Int J Dairy Sci 2007 Feb 1;2(2):104-115. [doi: 10.1073/pnas.1503141112] [Medline: 25792457]

27. Aarestrup F. Sustainable farming: get pigs off antibiotics. Nature 2012 Jun 27;486(7404):465-466. [doi: 10.1038/486465a] [Medline: 22739296]

28. Walia K, Madhumathi J, Veeraraghavan B, Chakrabarti A, Kapil A, Ray P, et al. Indian J Med Res 2019 Feb;149(2):164-179 [FREE Full text] [doi: 10.4103/ijnr.IJMR_226_18] [Medline: 31219080]

29. Harbarth S, Balkhy HH, Goossens H, Jarlier V, Kluytmans J, Laxminarayan R, et al. Antimicrobial resistance: one world, one fight!. Antimicrob Resist Infect Control 2015 Nov 18;4(1):49. [doi: 10.1186/s13756-015-0091-2] [Medline: 25792457]

30. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organisation. 2017. URL: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/ [accessed 2019-04-10]
Abbreviations

**ABR:** antibiotic resistance  
**AMR:** antimicrobial resistance  
**CRF:** Case Record Form  
**DDD:** defined daily dose  
**ESBL:** extended-spectrum beta-lactamase  
**IDI:** in-depth interview  
**MRSA:** methicillin-resistant Staphylococcus aureus  
**MSSA:** methicillin-sensitive Staphylococcus aureus  
**NDM:** New Delhi metallo-beta-lactamase  
**OPD:** outpatient department  
**VRSA:** vancomycin-resistant Staphylococcus aureus