Antimicrobial Resistance in Bangladesh

Training Manual

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Foreword

Antimicrobial resistance (AMR) is one of the emerging issues globally including Bangladesh as human and livestock health threats. Bangladesh are vulnerable to AMR issues for their poor surveillance health care facilities, unhygienic and unregulated conditions of the agriculture, livestock and aquaculture food production process, poor sanitation, widespread misuse and irrational antibiotics and prophylactics use in poultry, livestock and aquaculture industry. Therefore, people in the community acquiring resistance pathogens from food, environment, and wildlife sources.

The damaging effects caused by resistant pathogens are already responsible for an estimated 700,000 deaths per year globally, and future projections of the impact of unresolved AMR surpass the projected number of deaths caused by cancer by 2050. As AMR also could have a grave impact on the global economy, it potentially could pose a high direct and indirect cost to society.

Livestock is one of the most important sectors as the source of protein food for people through meat, milk, eggs, etc. Antimicrobials have been used in animal feed for about 70 years, not only to treat diseases, but also to boost growth, improve feed utilization and reduce mortality; in other words to obtain an improvement in productivity.

However, it is exacerbated by inappropriate and excessive use of antimicrobials in both human healthcare and the agriculture sector. Effectively addressing AMR requires the feed and livestock sectors to minimize the need for and use of antimicrobials.

I am happy to know that Livestock Division, BARC, Dhaka is going to organize the training pogram on Antimicrobial Resistance in Bangladesh from 26 -30 January 2025 for scientists of BLRI, BFRI, NIB; officers of DLS (LRI, CVH, CDIL, OC Lab.), DoF; and teachers from SAU, PSTU, BSMRAU and Gono Bishwabidyalay. A training manual will be published on the topic compiling the lecture notes of the senior and experienced persons in the field of Antimicrobial resistance. I think this training manual will be very helpful to the scientists, teachers, extension personnel and NGOs for combating AMR in Bangladesh.

I also acknowledge hard works and the sincere efforts of Dr. Mohammad Rafiqul Islam, Chief Scientific Officer, Livestock Division, BARC who was involved in this exercise. Finally, I would like to extend my sincere thanks to Livestock division, BARC for hard work in preparing this training manual and organizing training course.

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PREFACE

Antimicrobial resistance (AMR) has become an emerging issue in the developing countries as well as in Bangladesh. AMR is aggravated by irrational use of antimicrobials in a largely unregulated pluralistic health system.

The livestock sector of Bangladesh is endowed with 403 million terrestrial animals, which shares about 1.47% of the gross domestic product to the national economy. Both poultry and food-animal farming systems in Bangladesh are diversified from household small farms to medium and large-scale commercial farms. Due to the absence of adequate government animal healthcare system, farm owners mostly depend on informal and unqualified healthcare providers for the treatment of their animals. Therefore, irrationally prescribed and easy access to antibiotics leads to misuse, abuse, suboptimal, or overuse of these drugs in farms. Moreover, antibiotics are also used as prophylactic and sometimes as growth promoters, specifically in large-scale commercial farms of Bangladesh. The irrational, suboptimal, or overuse of antibiotics has resulted in the evolution of different species of pathogenic and zoonotic ABR bacteria in animal farming settings of Bangladesh. Unhygienic animal husbandry practices in Bangladesh are creating an important risk factor for disseminating these pathogenic and zoonotic ABR bacteria into humans and the environment.

Although, Disseminating the AMR knowledge and developing awareness of AMR consequences and its importance to the users in the field of livestock sector and research activities, a training program on Antimicrobial Resistance in Bangladesh, will be arranged by Livestock Division, Bangladesh Agricultural Research Council (BARC).

Scientists of BLRI, BFRI, NIB; teachers of SAU, PSTU, BSMRAU and Gono Bishawbidyalay; and officers of DLS and DoF will attend the training program. This training manual will be published with compilation of all lecture notes. I hope this manual would be very useful to participants as well as veterinary practitioners involved in livestock sector and research activities in the different fields.

I am grateful to all the training lecture contributors who work hard to put their excellent pieces of work into final shape. Finally, I would like to extend my sincere thanks to scientist of Livestock division, BARC for hard work in preparing this training manual and organizing training course.

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CONTENT

Antimicrobial resistance: A global threat

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Abstract:
Antibiotics in poultry production systems have resulted in the emergence of antibiotic resistant bacteria that can be transmitted to humans through food chain. In recent decades, antibiotic resistant strains of bacteria are considered as an emerging global threat to both animal and human health as infection with those bacteria can result in increasing incidence of treatment failure and severity of disease. In this article, mechanism of development of antibiotic resistance in bacteria in chickens, possible ways of entering those antibiotic resistant strains of bacteria in human food chain and its global threat will be shortly discussed.

Antibiotic Resistance and Multi-drug Resistance:
According to the Centers for Disease Control and Prevention (CDC), **antibiotic resistance** or antimicrobial resistance (AMR) is the ability of microbes to resist the effects of drugs- that is, the germs are not killed, and their growth is not stopped. According to the Clinical Laboratory Standards Institute (CLSI), a strain of a bacterium is defined as **multi-drug resistant** (MDR) strain when it is found non-susceptible to at least one agent in three or more antimicrobial different classes of antimicrobial agents.

Why globe is concerned on antimicrobial resistance?
AMR is one of the biggest challenges facing global public health. Antibiotic resistance is a growing concern that threatens the effective treatment of infectious diseases. Now-a-days antimicrobial resistance is a worldwide problem. The use and misuse of antimicrobial drugs in poultry accelerates the emergence of drug-resistant strains. Poor disease/infection control practices, inadequate sanitary conditions and inappropriate food-handling encourage the further spread of antimicrobial resistance. The selection and spread of resistant organisms in developing countries that can often be traced to complex socioeconomic and behavioral antecedents contribute to the escalating problem of antibiotic resistance in poultry.

Development of Antibiotic Resistance and Multi-drug Resistance:
Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: i) by a genetic mutation or ii) by acquiring resistance from another bacterium. Different genetic mutations yield different types of resistance. Some mutations enable the bacteria to produce potent chemicals or enzymes (eg. extended spectrum beta-lactamases-ESBL) that inactivate antibiotics, while other mutations eliminate the cell target that the antibiotic attacks. Bacteria can acquire antibiotic resistance genes from other bacteria in several ways-acquiring resistance genes through conjugation with resistant bacteria; acquiring resistance genes from bacteria through a virus; acquiring naked, "free" DNA directly from their environment.
Multidrug resistance in bacteria occurs by the accumulation of resistance (R) plasmids or transposons of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug type (Fig. 1). Any bacteria that acquire resistance genes, whether by spontaneous mutation or genetic exchange with other bacteria, have the ability to resist one or more antibiotics. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics.

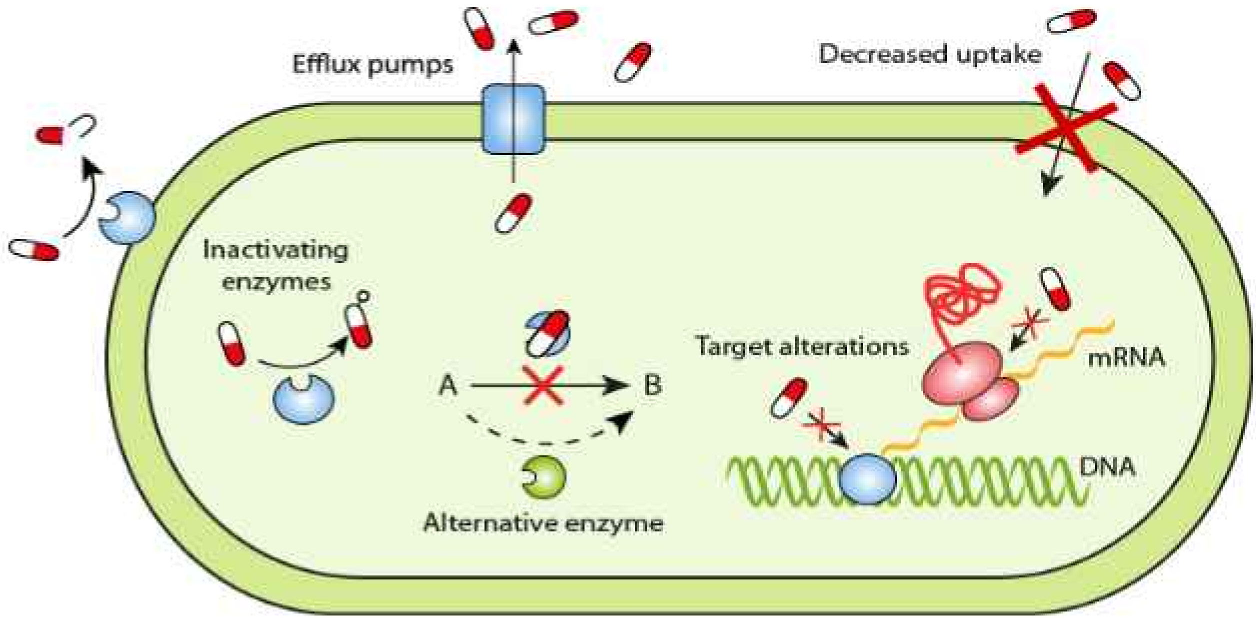

Fig. 1: Mechanism of development of antibiotic resistance in bacteria (Courtesy of Dr. E. Gullberg for providing the image).

Development of Resistant Bacteria in Chickens and Transfer in Humans:

In many developing countries including Bangladesh, indiscriminant use of antibiotics is practiced for the treatment of poultry diseases (and other animal diseases) which may develop antibiotic resistant bacteria in those animals. Chickens (like other animals) carry bacteria in their gut. Studies have shown that giving antibiotics to chickens kill many bacteria, but resistant bacteria can survive and multiply in the gut **(Fig. 2)**. Scientists around the world have provided strong evidence that resistant bacteria in chickens can enter into the human food chains through different ways and thus impose potential threat to human health around the world **(Fig. 3)**.

Fig. 2: Mechanism of development of resistant bacteria in chickens and other food animals (Adapted from CDC spotlight image).

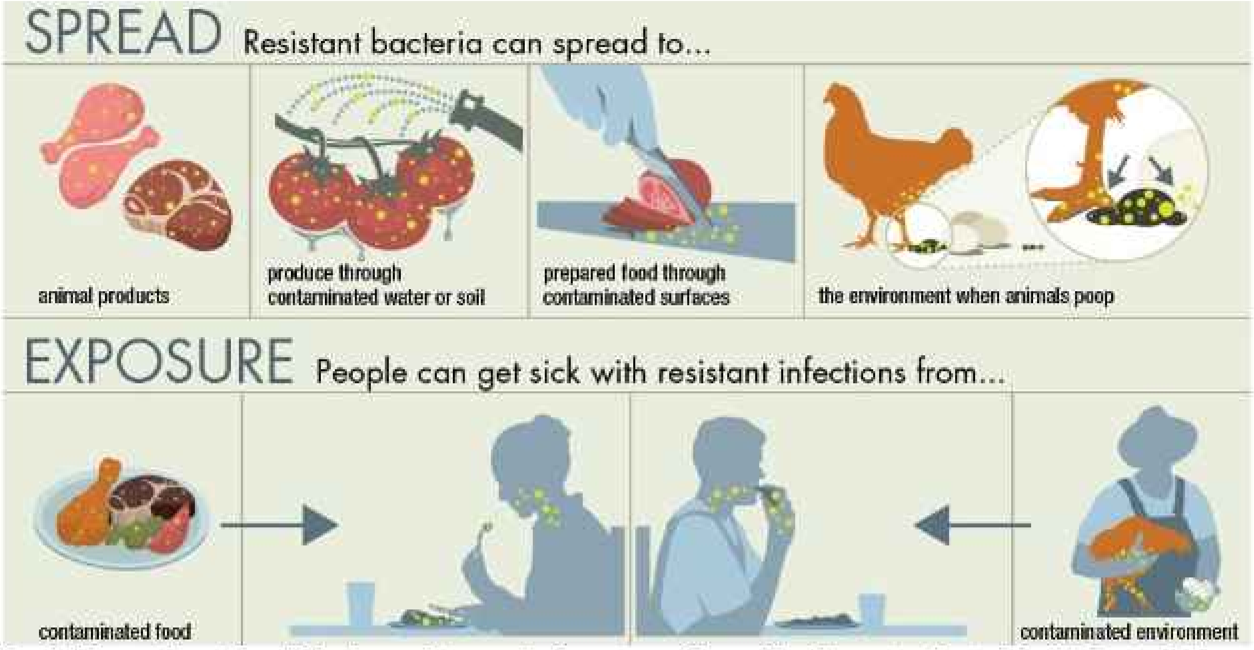

Fig. 3: Transfer of antibiotic resistance in humans: from the farm to the table (Adapted from CDC spotlight image).

Beginning of Antimicrobial Resistance:

Discovery	Emergence of Resistance
Penicillin-1943	1945
Vancomycin-1972	1988
Imipenem-1985	1998

Causes of emergence of antimicrobial resistant bacteria in poultry

In comparison to developed countries, the irrational use of antibiotics occurs more frequently in developing or least developed countries. Most of the developing countries anybody can buy any drug in any amount including antibiotics without prescription from the drug shops. In many cases, the sales man of the drug shops are prescribing and selling drug without any prescription from authorized physicians/veterinarians thus contributing finally to the development of resistance in microorganisms against commonly used antibiotics. Factors such as unregulated dispensing and manufacture of antimicrobials, truncated antimicrobial therapy, inadequate access to effective drugs and sometimes drugs of questionable quality and overall poverty are likely to be contributing to antimicrobial resistance. The widespread and inappropriate/irrational use of antibiotic and also continuous use of antibiotics in poultry feeds results in the development of a progressively antibiotic-resistant microbial ecosystem. Due to commercialization and introduction of exotic breeds of poultry, more intensive animal keeping and natural resistance against diseases reduces which results in more use of antibiotics. Especially in developing countries, small and medium poultry farms have minimum biosecurity practices, which increase disease incidences and subsequent continuous use of antibiotics. Apart from therapy and prophylaxis, antibiotics are consumed to increase growth and feed efficiencies in poultry. This is clearly indicated by the high prevalence of antibiotic resistance among the community.

Antimicrobial Resistance in poultry

Antimicrobial agents are widely administered in therapeutic treatment of poultry worldwide. In most cases poultry products & byproducts are marketed before the withdrawal period of antimicrobial drugs, even farmers sell their product during the treatment period with antibiotic. Antimicrobial drug residue in poultry food chain values is a growing issue worldwide. Resistance of microorganism to antimicrobial drug is increasing day-by-day due to indiscriminate use of antibiotic for the treatment of bacterial diseases of poultry. Many feed companies and farmers use antibiotics to poultry feed as a growth promoter by preventing infection, which may also contribute to antimicrobial resistance. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans. Several studies shown antimicrobial drug resistance viz. oxytetracycline (80-85%), doxycycline (85-90%), chlortetracycline (70-80%), ciprofloxacin (40-50%), amoxicillin (50-60%), sulphar drugs (40-70%) and erythromycin (70-80%) is very common in poultry. In recent studies, it was detected large number of multi-drug resistant *E. coli* in broiler and layer meat among which 62% were resistant to three classes of antibiotics, 46% were resistant to four classes of antibiotics and 30% were resistant to five classes of antibiotics. Among all MDR *E. coli* isolates, 6.8 % were ESBL producing. The highest frequency of antibiotic resistance and selection pressure occurs in poultry processing. This is dangerous not only for poultry but also for human. If the situation continues, there will be no effective antibiotic for treatment until new generation of antibiotic comes.

Containment of Antimicrobial Resistance

To overcome the present situation, it should take the energetic measures to slow down the emergence and spread of antimicrobial resistance, should include programmes on surveillance, education and research on AMR, and regulation of use of antimicrobials in the poultry sector. The efforts should include:

- Detection and awareness of problem of antimicrobial resistance by microbiology laboratory and public media.
- Research and surveillance (local, regional and international) on antimicrobial resistance in poultry should be established in collaboration with international organizations.
- Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria
- Antibiotic use guidelines and committee should be developed for each country.
- Antibiotic use in veterinary practices should be regulated and rationalized.
- Education on antibiotic resistance to manufacturers, prescribers, dispensers and consumers through continuing education and other means.
- Vaccines for preventing infectious diseases should be encouraged in all circumstances.
- Hygiene and sanitation should be improved and practiced.
- Value chain based standard practices to be formulated and practiced for different animal origin food products (milk, meat, egg and other value added products)
- Poultry slaughter and processing system to be strengthened.
- Nutritional status of the poultry population should be improved and maintained.
- One health approach considering human health, animal health and environmental health is required.

Conclusion:

Without any doubt, the introduction and use of antimicrobial agents in veterinary medicine had and still has an enormous impact on the health and welfare of animal species. But indiscriminant use of antibiotics in chickens in developing countries might generate antibiotic resistant bacteria and transfer those resistant bacteria in humans through entering into human food chains. Therefore, strict guidelines for the use of antimicrobials in chickens and other food animals and comprehensive antimicrobial drug administration monitoring systems should be urgently devised and implemented in developing countries to reduce the emergence of acquired multi-drug resistance in bacteria and to prevent the entrance of such bacteria into human food chain. Also, further research on alternative agents to antibiotics is needed.

National Antimicrobial Resistance (AMR) Surveillance Strategy in Animal Health Sector of Bangladesh

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Introduction

Antimicrobial resistance (AMR) has emerged as a major global health crisis, including in Bangladesh. AMR has resulted in an alarming increase in infections due to multidrug-resistant bacteria and limited the choice of antimicrobials for treatment, leading to adverse clinical and financial outcomes. For example, individuals who do not respond to traditional therapies may require expensive or alternative drugs. In addition, there are issues related to the high social cost of increased morbidity and mortality rates, longer hospital stays, increased health care costs, and, changes in empirical therapy. In most instances, resistance emerges because of the irrational use of antimicrobials. It is perpetuated by diverse risk factors; and is maintained within environments because of poor infection control practices.

Antimicrobial resistance surveillance is an essential component of combating and managing AMR, as outlined in the World Health Organization (WHO) Global Strategy for Containment of Antimicrobial Resistance (2001). More recently, in May 2015, the 68th World Health Assembly (WHA) adopted the "Global Action Plan on Antimicrobial Resistance" (GAP). The goal of the GAP is to ensure, for as long as possible, the continued, successful treatment and prevention of infectious diseases with effective, safe, quality-assured medicines that are used responsibly and accessible to all who need them.

Aim of the strategy

The National AMR Surveillance Strategy aims to establish a robust, cross-sectoral AMR surveillance system to generate data that inform policy decisions as well as the prudent production, distribution, and use of antimicrobials in the country.

Objectives of the surveillance system

The main objectives of the National AMR Surveillance Program are to:

- Establish a national surveillance system for monitoring the prevalence and evolving trends of AMR in organisms using a One Health approach.
- Facilitate evidence-based decision making for antimicrobial resistance containment.
- Establish a genomic-based AMR surveillance system.
- Make available surveillance data that can be combined with antimicrobial use (AMU) and antimicrobial consumption (AMC) data to facilitate evidence-based decision-making for antimicrobial resistance containment.

- Enable the surveillance of antibiotic resistance and antibiotic residues in the environment.
- Establish a national laboratory network with a monitoring and evaluation (M&E) framework,
- Contribute data to global and regional data-sharing mechanisms (e.g., the WHO Global Antimicrobial Resistance Surveillance System (GLASS) and the World Organisation for Animal Health (OIE) system).
- Promote research on antimicrobial resistance containment.

Target population, sampling point, and priority organisms for AMR Surveillance

The target population (species or sector to be sampled), sampling point in the production chain for surveillance, and a priority list of organisms are summarised in the Table 1.

Table 1: Summary of animal species, sampling point, preferred samples, and priority organisms

Species	Sampling point	Preferred samples	Organisms
Poultry broiler, layer, spent-hen, Sonali, Deshi, and other cross-bred chickens	Wet markets, live bird markets, poultry farms, poultry submitted to diagnostic centres (such as the Central Disease Investigation Laboratory or (CDIL), Field Disease Investigation Laboratories or FDIL)	Cloacal swab, oropharyngeal swab, caecal tonsil from dead birds, poultry meat, farm environmental samples (e.g. litter, water and dragging swab), live bird market environmental samples, poultry feed	• *Escherichia coli* • *Salmonella enterica* • *Campylobacter jejuni* • *Campylobacter coli* • *Enterococcus faecalis* • *Enterococcus faecium*
Cattle, sheep goats, and pet animals (dogs and cats)	Slaughterhouses, animal farms, district or *upazila* veterinary hospitals, teaching veterinary hospitals	Rectal swab, anorectal swab, nasal swab, swab from lesion, swab from oral cavity and perineal region, milk, faeces	• *Escherichia coli* • *Salmonella enterica* • *Staphylococcus aureus* • *OtherStaphylococcus (S. pseudintermedius)* • *Streptococcus canis* • *Pasteurella multocida*
Fish (cultured and wild-caught), shellfish (shrimps)	Markets, super shops, fish processing plants/ fish farms	Whole fish,skin surface, muscle, water sample (surface water, mid-water, bottom water), fisherman hand slime, draining water from fish display tray, fish processors' hand swab	• *Vibrio vulnificus* • *Vibrio parahaemolyticus* • *Vibrio cholerae* • *Listeria monocytogenes* • *Escherichia coli* • *Salmonella enterica* • *Staphylococcus aureus*

These recommendations are based on guidelines on AMR surveillance developed by the WHO, OIE, and FAO.

Antimicrobial panel to be used for Sensitivity Testing

Following antimicrobial disk has been chosen for the sensitivity testing of the AMR pathogens

Table 2: Antimicrobials to include in the susceptibility testing panel

Antimicrobial class	Antimicrobial	Recommended to include in the panel for	
		Gram-Positive Bacteria	Gram-Negative Bacteria
Aminoglycoside	Gentamicin	√	√
Carbapenem	Imipenem	√	√
Cephalosporin	Cefepime	√	√
Cephalosporin	Cefoxitin	√	√
Cephalosporin	Ceftazidime	√	√
Cephalosporin	Ceftriaxone	√	√
Cephalosporin	Cefuroxime	√	√
Cephalosporin	Cephalexin	√	√
Cephalosporin	Cephradine	√	√
Folate inhibitor	Trimethoprim	√	√
Folate inhibitor	Trimethoprim-sulfonamide	√	√
Glycopeptide	Vancomycin	√	N/A
Glycylcycline	Tigecycline	√	√
Lincosamide	Clindamycin	√	√
Macrolides	Azithromycin	√	√
Nitrofurantoin	Nitrofurantoin	√	√
Penicillin	Amoxicillin	√	√
Penicillin	Ampicillin	√	√
Penicillin	Flucloxacillin	√	N/A
Penicillin	Piperacillin	√	√
Phenicol	Chloramphenicol	√	√
Polymyxin	Colistin	N/A	√
Fluoroquinolone	Ciprofloxacin	√	√
Quinolone	Nalidixic Acid	√ (*Staphylococcus aureus* are naturally resistant to NA)	√
Tetracycline	Tetracycline	√	√

√ = Can be included; N/A = Not applicable

Laboratory methods

In light of available resources and existing capacity, AMR laboratory capacity to use the following methods should be strengthened:

1. **Disk diffusion** – Disk diffusion isthe core or routine AMR surveillance protocol used in the early stages of the national AMR surveillance programme due to its practicality and

sustainability (cost). Laboratories will test all isolates recovered from samples using this methodology.

2. **Broth dilution** – Broth dilution is complementary to disk diffusion. Representative isolates exhibiting unusual resistance patterns, such as multi-drug or multiclass resistant isolates or emerging resistance to certain antimicrobials (e.g., deemed critically important to human medicine), will be prioritised for testing using this method. The species-bacteria combination will be followed based on standard methods.
3. **Molecular testing** – Molecular testingis complementary to the above methodologies. Data derived from molecular testing will be used for source attribution and to provide an understanding of the epidemiology of antimicrobial-resistant organisms in Bangladesh. Laboratories will use molecular testing for multi-drug resistant isolates from representative samples as well as address specific research questions not addressed by routine surveillance. Specific resistance determinants will be tested based on phenotypic data.
4. **Automated antimicrobial susceptibility testing**– The National Reference Laboratories (NRLs) (CDIL and the Bangladesh Livestock Research Institute or BLRI) will carry out the automated AST using the VITEK 2 system. Automated AST will help validate the test results submitted by sentinel laboratories, as well as support NRLs in generating authentic AMR data in a short time.

Sampling Frame for AMR Surveillance

A) Poultry

As poultry is the most frequently consumed meat, chickens will be priority species for testing. Preliminary AMR data indicate no marked difference in the prevalence of resistance in all the antimicrobials included in the panel between the Sonali and the broiler chicken strains (i.e., developed by global genetic companies). For simplicity's sake, data will be aggregated and stratified by the strain of chicken. The prevalence estimates for *Campylobacter*, *Salmonella,* and *E. coli* in Bangladesh based on recent surveillance data. The preference of pathogens as well as expected sample size is presented in Table 3 and Table 4.

Table 3: Targeted quantity of birds to be sampled and sampling frequency (for animal health laboratories collectively)

Organisms	Sample frequency	Sample size/quarter or year
E. coli	Yearly	480 per year or 120 per quarter
Salmonella enterica	Yearly	480 per year or 120 per quarter
Campylobacter spp.	Yearly	480 per year or 120 per quarter
Enterococcus spp.	Yearly	480 per year or 120 per quarter

Table 4: Recommended bird sample size per quarter: 96 per laboratory

Location	No of samples per location	Designated laboratory
Dhaka	96	CDIL, Dhaka

Location	No of samples per location	Designated laboratory
	96	BLRI, Savar
Chattogram	96	FDIL, Feni
	96	PRTC, CVASU
Rajshahi	96	FDIL, Jaipurhut

Note: PRTC and FDILs will send one-third of their bacterial isolates to CDIL and BLRI for test validation.

B) Ruminants and pet animals

A combination of cattle, sheep, goats, dogs, and cats (the number of animals per ruminant species will be proportional to the production profile of the country). The recommended pathogens along with expected sample size is presented in Table 5 and Table 6.

Table 5: Targeted quantity of animals will be sampled and sampling frequency (for animal health laboratories collectively)

Organisms	Sample frequency	Sample size/quarter or year
E. coli	Yearly	96 per year or 24 per quarter
Salmonella enterica	Yearly	96 per year or 24 per quarter
Pasteurella multocida	Yearly	96 per year or 24 per quarter
*Staphylococcus aureus**	Yearly	96 per year or 24 per quarter
Streptococcus spp.	Yearly	96 per year or 24 per quarter

*will be screened for MRSA

Table 6: Recommended sample size per quarter: 24 per laboratory

Location	**No of samples per location**	**Designated laboratory**
Dhaka	24	CDIL, Dhaka
	24	BLRI, Savar
Chattogram	24	FDIL, Feni
	24	PRTC, CVASU
Rajshahi	24	FDIL, Jaipurhut

Note: PRTC and FDILs will send one-third of their bacterial isolates to CDIL and BLRI for test validation.

Preparation of different chemicals, reagents & bacteriological media for antimicrobial sensitivity testing

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Objectives:

Bacteria are grown on or in ***microbiological media*** of various types for antimicrobial sensitivity testing. Besides bacteriological media there are some chemical and reagents needed for AST procedure

Requirements:

- Sterile 0.85% NaCl
- 0.5 McFarland Standard
- Mueller-Hinton Agar plate
- Buffered peptone
- MacConkey agar plate
- Nutrient agar media
- Amoxicillin 20 µg
- Azithromycin 15 µg
- Chloramphenicol 30 µg
- Gentamicin 10 µg
- Colistin sulphate 10 µg
- Cefotaxime 30 µg
- Ceftazidime 30 µg
- Ciprofloxacin 5 µg
- Nitrofurantoin 300 µg
- Cefoxitin 30 µg
- Imipenem 10 µg
- Meropenem 10 µg
- Nalidixic 30 µg
- Tetracycline 30 µg
- Trimethoprim 5 µg

Procedure: Of the many media available, Müeller -Hinton (MH) agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing.

* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.

* It gives satisfactory growth of most non fastidious pathogens

* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.

2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.

3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.

4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).

5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic bags or sealed container, have been taken to minimize drying of the agar.

6. It may necessary to dry the plates prior to use, this is to avoid excess moisture, which may result in problems with fuzzy zone edges and/or haze within zones.

7. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35/37°C for 24 hours or longer.

Collection, transportation and preservation and culture of bacteriological samples for AMR surveillance

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Objectives:

To know the steps essential to collect and transport appropriate samples for AMR testing. During sample collection and transportation, some chemical, reagents and consumables are needed for AST procedure

Requirements:

- Buffered peptone
- MacConkey agar plate
- Sterile scissors
- Sterile forceps
- 15 ml Falcon tube
- Ziplock bags

Sample collection and transportation

Broiler/ Sonali layer chicken caecum samples should be collected from live bird market/ farm before antibiotic treatment. Caecums should be collected separately into sterile Ziplock bags using sterile scissors and tissue forceps and transported in a cool box for further processing. Samples should be labelled adequately including sample ID, date of collection, species and location or might put a sample ID and a separate sheet for other information.

General considerations

- Careful consideration must be given during the collection, containment, and storage of the specimens, including biosafety measures that must be in place to prevent contamination of the environment or exposure of other animals and humans to potentially infectious materials (see 2_SOP_Biosafety_BLRI).
- The reliability of the diagnostic testing is critically dependent on the specimen(s) being appropriate, of high quality, and representative of the disease process being investigated. Prior to sampling, consideration must be given to the type of specimen(s) needed including the purpose of the testing and the test technologies to be used.
- The volume or quantity of specimen must be sufficient to perform initial testing, to perform any subsequent confirmatory testing and to provide sufficient residual specimen for referral or archival purposes.
- Epidemiologically appropriate sampling plans should be developed prior to collection of specimens.
- Specimens must be collected according to a sound knowledge of the epidemiology and pathogenesis of the disease under investigation, or the disease syndrome to be diagnosed. This will lead to the sampling of tissues or fluids most likely to contain the infectious agent or evidence of the infection. Considerations will include the tissue predilection(s) or target organ, the duration and site of infection in each tissue type and the duration and route of

shedding, or the time frame in which evidence of past infection, such as an antibody response, can be detected reliably by the tests to be deployed. These considerations will also indicate the method(s) of collection to be used. In many herd or flock-based disease investigations it is beneficial to collect specimens from a healthy cohort for comparative epidemiological or baseline testing (e.g. case-control and cohort approaches for diagnostic testing) and for validation purposes.

- Tested as soon after collection as possible to minimize the negative impacts on test results caused by death of the targeted microorganism, bacterial overgrowth.

Procedure:

1. 1 gm of caecal content was added to 9 ml of buffered peptone water (BPW) and incubated at 37 °C±1 °C for 18-22 hours.
2. Following incubation, a loopful of culture was streaked on to MacConkey agar and incubated the agar plate at 37 ±1 °C for 24 hours.
3. The isolates were identified as *E. coli* on the basis of colony morphology on MacConkey agar.
4. Standard biochemical tests are conducted to confirm the identity of *E. coli* (lactose fermentation, citrate metabolism, methyl red Voges-Proskauer, urease production and indole production test.
5. Confirmed E. coli isolated colonies will be used for AMR assay.

Irrational use of antimicrobials and its hazard on human-animal and environment interface

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Infectious diseases remain among the leading causes of morbidity and mortality on our planet. The development of resistance in microbes- bacterial, viral, or parasites to therapeutics is neither surprising nor new. However, the scope and scale of this phenomenon is an ever-increasing multinational public health crisis as drug resistance accumulates and accelerates over space and time. Today some strains of bacteria and viruses are resistant to all but a single drug, and some may soon have no effective treatments left in the "medicine chest." The disease burden from multidrug-resistant strains of organisms causing AIDS, tuberculosis, gonorrhea, malaria, influenza, pneumonia, and diarrhea is being felt in both the developed and the developing world's alike.

The accelerating growth and global expansion of antimicrobial1 resistance (hereinafter referred to as AMR) is a demonstration of evolution in "real time" in response to the chemical warfare waged against microbes through the therapeutic and non-therapeutic uses of antimicrobial agents. After several decades in which it appeared that human ingenuity had outwitted the pathogens, multidrug-resistant "superbugs" have become a global challenge, aided and abetted by the use, misuse, and overuse of once highly effective anti-infective drugs. In the words of the "antimicrobial" is used inclusively to refer to any agent (including an antibiotic) used to kill or inhibit the growth of microorganisms (bacteria, viruses, fungi, or parasites). This term applies whether the agent is intended for human, veterinary, or agricultural applications.

It should be noted at the outset of this document that the meaning of the phrase "antimicrobial resistance" is wholly context-dependent. Most commonly, it refers to infectious microbes that have acquired the ability to survive exposures to clinically relevant concentrations of drugs that would kill otherwise sensitive organisms of the same strain. The phrase is also used to describe any pathogen that is less susceptible than its counterparts to a specific antimicrobial compound (or combination thereof). Resistance manifests as a gradient based on genotypic and phenotypic variation within natural microbial populations, and even microbes with low levels of resistance may play a role in propagating resistance within the microbial community as a whole (American Academy of Microbiology, 2009).

Pathogens resistant to multiple antibacterial agents, while initially associated with the clinical treatment of infectious diseases in humans and animals, are increasingly found outside the healthcare setting. Therapeutic options for these so-called community-acquired pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA) are extremely limited, as are prospects for the development of the next generation of antimicrobial drugs.

Antimicrobial Drug Resistance in Context

The History of Medicine:
2000 B.C.—Here, eat this root.
1000 A.D.—That root is heathen. Here, say this prayer.
1850 A.D.—That prayer is superstition. Here, drink this potion.
1920 A.D.—That potion is snake oil. Here, swallow this pill.
1945 A.D.—That pill is ineffective. Here, take this penicillin.
1955 A.D.—Oops … bugs mutated. Here, take this tetracycline.

1960–1999 A.D.—39 more "oops."... Here, take this more powerful antibiotic.
2000 A.D.—The bugs have won! Here, eat this root.
Anonymous, as cited by the World Health Organization (WHO, 2000a)

An Inevitable History

The use of antimicrobial drugs, no matter how well controlled, "inevitably leads to the selection of drug-resistant pathogens, "according to workshop speaker Julian Davies, of the University of British Columbia (Davies, 2009). As may be seen in the following illustration (Figure 1), there is no man-made defense that cannot be outmaneuvered by microbial evolution and adaptation. As speaker Gerard Wright of McMaster University observed, "there is no such thing as an irresistible antibiotic." (Dr. Wright's contribution to the workshop summary report can be found in Appendix A, pages 401-419.)

This characteristic of antimicrobial drugs has been well-known since the dawn of the antibiotic era over seven decades ago, and all too often has been either underestimated or ignored.

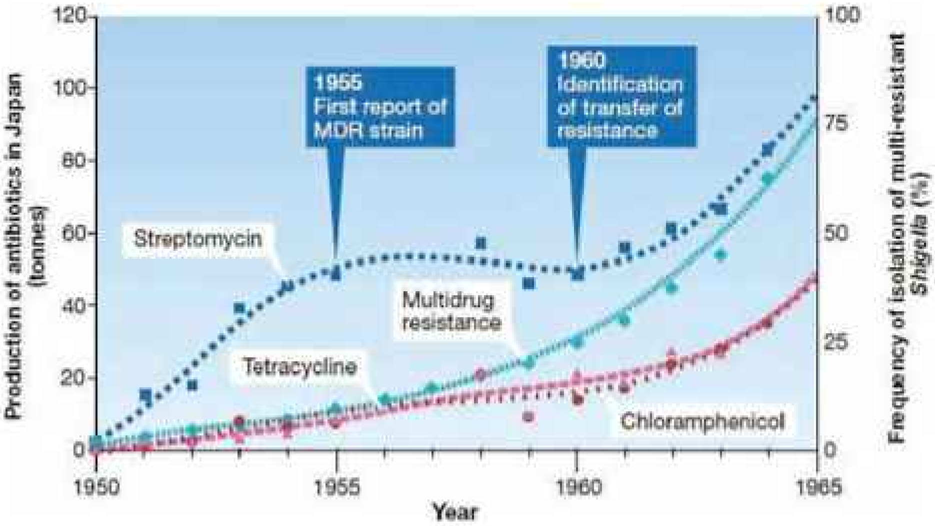

Figure 1: The relationship between antibiotic resistance development in *Shigella dysenteriae* isolates in Japan and the introduction of antimicrobial therapy between 1950 and 1965. In 1955, the first case of plasmid determined resistance was characterized. MDR = multidrug resistance. Transferable, multi-antibiotic, resistance was discovered five years later in 1960.

Hailed as a miracle drug when it was first introduced in 1943, penicillin was eagerly purchased by consumers who initially obtained it without a prescription following the conclusion of World War II (Stolberg, 1998). In a 1945 interview with the New York Times, penicillin's discoverer Alexander Fleming anticipated the development of drug-resistant bacterial strains. Indeed, penicillin-resistant strains were first isolated from patients in significant numbers a year later, in 1946.

Over the next several decades, researchers discovered and developed a range of antimicrobial agents and classes of compounds with antimicrobial properties, as illustrated in Figure 2. Like penicillin, some antimicrobial drugs were directly derived from soil microbes; others were synthesized or modified versions of naturally occurring antimicrobial products (Salmond and

Welch, 2008). Beginning in the early 1950s, antimicrobials were also widely adopted for non-human applications, most importantly as livestock feed additives (Davies, 2009).

Despite the warnings of Fleming and others to the contrary, in 1967, the Surgeon General of the United States, Dr. William H. Stewart, claimed that infectious diseases had been conquered through the development and use of antibiotics and vaccines and that therefore it was time to shift the U.S. government's attention and resources to the "War on Cancer" (Stewart, 1967; Stolberg, 1998).

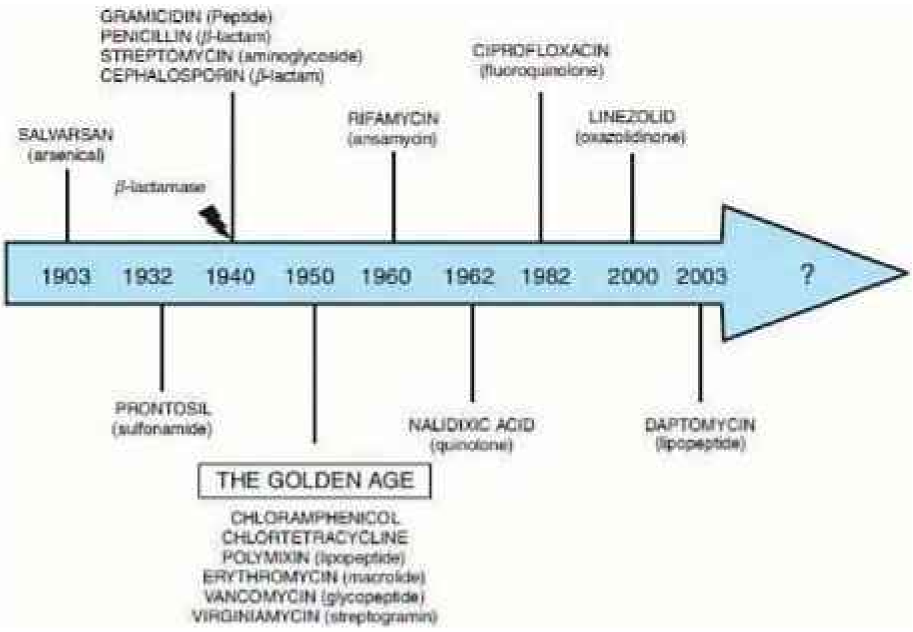

Figure 2. Major classes of antimicrobials and the year of their discovery
Source: Davies (2009), IOM (2009b).

The "Antibiotic Era" has been marked by a series of epidemics of resistant organisms including

- Penicillin-resistant Staphylococcus aureus,
- Methicillin-resistant Staphylococcus aureus (MRSA),
- Vancomycin-intermediate Staphylococcus aureus (VISA),
- Multi-drug-resistant (MDR) Vibrio cholerae,
- Multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis (hereinafter MDR- and XDR-TB),
- CTX-M2 resistant Escherichia coli and Klebsiella pneumoniae,
- Clostridium difficile, and many others.

Reports of new outbreaks of these so-called "superbugs" in the popular press are becoming increasingly commonplace events (Davies, 2009).

Numerous studies, reports, and review articles—several of which are cited

> Cefotaximases are β-lactamase enzymes named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (e.g., ceftazidime, ceftriaxone, cefepime). Rather than arising by mutation, cefotaximases represent examples of plasmid acquisition of β-lactamase genes normally found on the chromosome of Kluyvera species, a group of rarely pathogenic commensal organisms.

The Tragedy of the Commons

The phenomenon of AMR is ultimately both a global public health and environmental catastrophe, a "classic" example of the "tragedy of the commons" illustrated more than 40 years ago in a seminal article by the late ecologist Garrett Hardin (1968). Hardin's "tragedy of the commons" has proven to be a useful metaphor for understanding how we have come to be at the brink of numerous environmental catastrophes whether land use, global climate change, access to and availability of uncontaminated and abundant fresh water resources, or antimicrobial resistance. Simply stated, we face a serious dilemma an instance where individual rational behavior, acting without restraint to maximize personal short-term gain—can cause long-range harm to the environment, others and ultimately to oneself.

Many of the planet's natural resources are treated as a "commons," wherein individuals have the right to freely consume its resources and return their wastes to the collective environment. The "logic of the commons" ultimately results in its collapse with the concomitant demise of those who depend upon the commons for survival (Diamond, 2005). Like climate change (IOM, 2008a) and the global water crisis (IOM, 2009a), the emergence of drug-resistant microbes was catalyzed by rational behavior: humans acting without restraint to maximize personal short-term gain.

According to Baquero and Campos (2003), "antibiotics have been considered to be an inexhaustible common, both for prescribers and the general public," and the resulting over-consumption has produced a "net increase in antibiotic resistance and a likely reduction in the therapeutic efficacy of the drugs." If one person's misuse of a drug speeds up the evolution of resistant strains, while simultaneously decreasing his or her chance of being cured, then antimicrobial efficacy can be viewed as a scarce commodity in need of responsible management, on a par with energy, safe food, clean water, and climate stability. As Walker and coauthors (2009) observed, these and other resources in crisis comprise a nexus of "serious, intertwined global-scale challenges spawned by the accelerating scale of human activity." Addressing such challenges and their interactive effects, they contend, demands "cooperation in situations where individuals and nations will collectively gain if all cooperate, but each faces the temptation to take a free ride on the cooperation of others."

Antibiotics in Nature

Humans did not invent antibiotics; we merely observed often by accident that bacteria and other microorganisms produced biological compounds capable of killing or suppressing the growth and reproduction of other bacteria (Martinez, 2009). There are a variety of explanations for why microoganisms make antibiotics. A conventional ecological and evolutionary view holds that they enable organisms to kill or suppress the growth of competitors and to defend ecological niches (Salmond and Welch, 2008). It is also possible that these products serve other functions, such as signaling or nutrient sequestration (Martinez, 2009).

Some enzymes in the antibiotic biosynthetic pathways appear to have evolved millions to billions of years ago, which suggests that antibiotic-resistance genes and their cognate proteins are also ancient. For example, the bacterial metabolic pathways that produce both β-lactam antibiotics and the enzyme that foils them, β-lactamase, are thought to be more than 10 million years old (Spellberg et al., 2008a). Synthetic antibiotics (most of which are based on naturally-occurring bacterial products) target a variety of bacterial systems, as illustrated including those involved with cell wall synthesis, membrane integrity, transcription, and translation (Salmond and Welch, 2008; Walsh, 2003).

AMR Genes

Microbes have exchanged genes encoding resistance mechanisms for millennia. Genes conferring resistance to clinical antibiotics (but which also, presumably, provide other selective advantages to their hosts) exist in bacterial populations that have never encountered these compounds (Allen et al., 2010; IOM, 2009b; Salmond and Welch, 2008). The vast majority of antimicrobial resistance genes reside on mobile genetic elements such as insertion sequences, integrons, transposons, and plasmids, according to workshop speaker Henry "Chip" Chambers of the University of California, San Francisco. Bacteria readily acquire these genetic elements from the environment, exchange them through conjugation, and receive them via infection by bacterial viruses (bacteriophages, or phages) (Salmond and Welch, 2008). These processes used to acquire "novel" genetic elements are collectively referred to as "horizontal gene transfer." A mobile genetic element that confers selective advantages upon its host such as antibiotic resistance can spread widely, and may be expressed even when the antibiotic it deactivates is not present (O'Brien, 2002).

Source: https://www.nap.edu/read/12925/chapter/2#26

Application of vitek-2 for laboratory detection of AMR pathogen

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The VITEK 2 system has everything healthcare laboratories need for fast, accurate microbial identification, and antibiotic susceptibility testing.

The innovative VITEK 2 microbial identification system includes an expanded identification database, the most automated platform available, rapid results, improved confidence, with minimal training time.

The VITEK 2 system next-generation platform provides greater automation while increasing safety and eliminating repetitive manual operations. The rapid response time means results can be provided more quickly than with manual microbial identification techniques.

VITEK 2: Healthcare uses

- Microbial Identification - bacteria and yeast identification (ID)
- Antibiotic Susceptibility Testing (AST) and resistance mechanism detection
- Epidemiologic trending and reporting
- Most automated Microbial ID/Antibiotic Susceptibility Testing (AST) platform reduces set-up time and minimizes manual steps
- Ergonomic workflow decreases the risk of repetitive motion injuries
- Compact, sealed ID/AST cards enhance laboratory safety, minimizes waste and prevents biohazard spills
- Rapid microorganism identification (ID)
- Gram negative bacterial identification
- Gram positive bacterial identification
- Yeast identification
- Neisseria, *Haemophilus* and other fastidious Gram negative bacteria identification
- Anaerobic bacteria and coryneform bacteria identification
- Rapid, same-day antimicrobial susceptibility testing (AST) increases microbiology's relevance
- VITEK 2 and ETEST® meet a laboratory's antimicrobial susceptibility testing (AST) needs and deliver minimum inhibitory concentrations (MICs) for most organisms
- Gram negative antibiotic susceptibility testing (AST) cards
- Gram positive antimicrobial susceptibility testing (AST) cards
- Fluconazole antimicrobial susceptibility testing (AST) card for yeast susceptibility testing
- Resistance detection by phenotype identification
- Data management software and bidirectional interface with laboratory information system (LIS) allows for generation of epidemiology reports and antibiograms

Biosafety and Biosecurity of AMR laboratory

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Biosafety: Introduction

- Safety measures to minimize or prevent exposure to the person handling the agent, lab. and building occupants, the community and the environment.
  - Key Safety Measures:
    - good microbiological work practices
    - safety and containment equipment
    - facility design consideration

Biosecurity

- ➢ Set of preventive measures designed to reduce the risk of transmission of harmful biological agents.
- ➢ It ensure the security of areas containing hazardous pathogen/toxins or other assets.
- ➢ Components of a laboratory biosecurity program include:
  - Physical security
  - Personnel security
  - Material control & accountability
  - Transport security
  - Information security
  - Program management

Applications of Biosafety

- Research, including:
  - human & animal pathogens, select agents (bioterrorism)
  - toxins of biological origin

- rDNA research, Human Gene Transfer, plants, animals, large scale
- shipping, transport, import, export, permits
- training (BBP, TB, infection control, shipping, Biosafety, BSL-3, work practices, BSC's)
- field work, work abroad (feral animal, insects, arthropods)

• Clinical/Hospital Settings (helping sick people)

  - micro labs, in- and out-patient facilities, infirmaries
  - infection control, standard precautions, airborne precautions (TB, measles, varicella, flu)
  - social workers, divinity and law school volunteers, students

Principles of Laboratory Biosafety

Principles of Biosafety

The term "containment" is used in describing methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce exposure of laboratory employees, other persons and the outside environment to potentially hazardous or infectious agents. The three elements of containment include laboratory practices and techniques, safety equipment and facility design. Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good micro-biological technique and the use of appropriate safety equipment. Secondary containment, the protection of the external laboratory environment from exposure to infectious materials, is provided by a combination of facility design and operational practices.

A. Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards and be trained and proficient in the practices and techniques required for handling such material safely. The lab manager and lab supervisor is responsible for providing or arranging for appropriate training of personnel with assistance from EH&S. Additional measures may be necessary when standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure. The selection of additional safety practices is the responsibility of the lab manager and lab supervisor and must be commensurate with the inherent risk associated with the agent or procedures. An example of an additional practice would be a vaccination requirement. (See Appendix 5 for vaccination recommendations)

B. Safety Equipment

Such equipment includes biological safety cabinets and a variety of enclosed containers. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious aerosols generated by many microbiological procedures. Three types of BSC's (Class I, II, III) used in microbiological labs are described in Appendix 6. Openfronted Class I and Class II BSC's are partial containment cabinets which offer significant levels of protection to laboratory personnel and to the environment when used in conjunction with good microbiological techniques. The gas-tight Class III BSC provides the highest attainable level of protection to personnel and the environment. An example of an enclosed container is the capped centrifuge bottle which prevents the release of aerosols during centrifugation. Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses. These personal protective devices are often used in combination with biological safety cabinets and other devices which contain the agents, animals and materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective devices may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, production activities and activities relating to maintenance, service or support to the laboratory.

C. Facility Design

The design of the facility is critical for providing protection to persons outside the laboratory and in the community in the event that an infectious agent is accidentally released in the laboratory. It is the responsibility of the lab manager, EH&S, Physical Facilities and Facilities Planning to provide lab facilities commensurate with the function of the laboratory. The following information describes three facility designs, in ascending order of containment level.

1. **Basic Laboratory**
   This laboratory provides general space appropriate for work with defined viable agents which are not associated with disease processes in healthy adults or which do not colonize in humans. All activities are regularly conducted on the open bench using standard laboratory practices.
2. **Containment Laboratory**
   This laboratory provides general space appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practices. Work is commonly conducted on the open bench with certain operations confined to BSC's. Conventional laboratory designs are adequate. Areas known to be sources of general contamination such as animal rooms and waste staging areas should not be adjacent to media, processing areas, tissue culture laboratories or patient care activities. Public areas and general offices to which nonlaboratory staff require frequent access should be separated from spaces which primarily support laboratory functions.
3. **High Containment Laboratory**
   This laboratory has special engineering features which make it possible for laboratory workers to handle hazardous materials without endangering themselves, the community or the environment. The unique features which distinguish this laboratory from the basic and containment laboratories are the provisions for access control, a specialized ventilation system and vacuum line isolation (See Appendix 6). The high containment laboratory may be an entire building or a single module or complex of modules within a building. In all cases, the laboratory is separated by a controlled access zone from areas open to the public and laboratory personnel.

Containment
The term "containment" is used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The four elements of containment are administrative controls, work practices, personal protective equipment, and facility design.

The Principle of Containment

Containment is used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained.

Three elements of containment

1. Laboratory practices and techniques
2. Safety equipment
3. Facility design

A. Primary containment - Protection of personnel and the immediate laboratory environment using laboratory practices and techniques and safety equipment (i.e., the use of measures designed to reduce potential for escape, transmission and exposure).

B. Secondary containment - Protection of the environment external to the immediate laboratory through combination of primary containment and facility design safeguards.

C. Graphic illustration of the three elements of containment

LABORATORY-ASSOCIATED INFECTIONS

Laboratory-acquired infections (LAIs), also called occupational illness or laboratory-associated infections, are not new phenomena in microbiological laboratories. LAIs can arise in clinical laboratories as well as in animal facilities, R&D or production installations.

- 1940-Study Lab. Infection in human
- In 1950, bacteria were the causative agents for the majority of reported LAIs.
- From1979-2004, 1,448 infections were reported, where, 22 were death.
- Bacteria and viruses accounted for nearly 80% of the infections.
- 1984-1st Guideline on Biosafety by CDC

Most Frequently Reported LAIs 1979 - 2004

Infectious Agent	**Symptomatic Cases**	**Ranking Order 1930-1978**
Mycobacterium tuberculosis	199	6 (194 cases)
Arboviruses	192	8 (VEE - 146 cases)
Coxiella burnetii	177	2 (280 cases)
Hantavirus	155	
Brucella spp.	143	1 (426 cases)
Hepatitis B virus	82	3 (82 cases)
Shigella spp.	66	

Salmonella spp.	64	4 (258 cases)
Hepatitis C virus	32	*# 5 F. tularensis -225 cases;* *#7 B. dermatitidis -162 cases;* *#9 Ch. psittaci -116 cases;* *# 10 C. immitis -93cases*
Neisseria meningitidis	31	
Total	1,074	2,168

Reported LAIs 1979 - 2004

Category	Overt LAI	Subclinical LAI	Total LAI	No. of Deaths
Bacteria	598	60	658	17
Rickettsia	187	214	401	1
Viruses	608	430	1,038	18
Parasites	49	4	53	0
Fungi	6	0	6	0
Total	**1,448**	**663**	**2,156**	**36**

Laboratory Practices and Technique

A. Strict adherence to good microbiological practices and techniques (Aseptic Technique)
B. Sufficient training to all employees is provided by the supervisor
C. Hazard awareness and standard operating procedures (SOPs) established to manage them

Personal Protective Equipment

How to Put On (Don) PPE Gear

More than one donning method may be acceptable. Training and practice using your healthcare facility's procedure is critical. Below is one example of donning.

1. Identify and gather the proper PPE to don. Ensure choice of gown size is correct (based on training).
2. Perform hand hygiene using hand sanitizer.
3. Put on isolation gown. Tie all of the ties on the gown. Assistance may be needed by other healthcare personnel.
4. Put on NIOSH-approved N95 filtering facepiece respirator or higher (use a facemask if a respirator is not available). If the respirator has a nosepiece, it should be fitted to the nose with both hands, not bent or tented. Do not pinch the nosepiece with one hand. Respirator/facemask should be extended under chin. Both your mouth and nose should be protected. Do not wear respirator/facemask under your chin or store in scrubs pocket between patients.*

- Respirator: Respirator straps should be placed on crown of head (top strap) and base of neck (bottom strap). Perform a user seal check each time you put on the respirator.
- Facemask: Mask ties should be secured on crown of head (top tie) and base of neck (bottom tie). If mask has loops, hook them appropriately around your ears.

5. Put on face shield or goggles. When wearing an N95 respirator or half facepiece elastomeric respirator, select the proper eye protection to ensure that the respirator does not interfere with the correct positioning of the eye protection, and the eye protection does not affect the fit or seal of the respirator. Face shields provide full face coverage. Goggles also provide excellent protection for eyes, but fogging is common.
6. Put on gloves. Gloves should cover the cuff (wrist) of gown.
7. Healthcare personnel may now enter patient room.

How to Take Off (Doff) PPE Gear

More than one doffing method may be acceptable. Training and practice using your healthcare facility's procedure is critical. Below is one example of doffing.

1. Remove gloves. Ensure glove removal does not cause additional contamination of hands. Gloves can be removed using more than one technique (e.g., glove-in-glove or bird beak).
2. Remove gown. Untie all ties (or unsnap all buttons). Some gown ties can be broken rather than untied. Do so in gentle manner, avoiding a forceful movement. Reach up to the shoulders and carefully pull gown down and away from the body. Rolling the gown down is an acceptable approach. Dispose in trash receptacle. *
3. Healthcare personnel may now exit patient room.
4. Perform hand hygiene.
5. Remove face shield or goggles. Carefully remove face shield or goggles by grabbing the strap and pulling upwards and away from head. Do not touch the front of face shield or goggles.
6. Remove and discard respirator (or facemask if used instead of respirator). Do not touch the front of the respirator or facemask.*
    - Respirator: Remove the bottom strap by touching only the strap and bring it carefully over the head. Grasp the top strap and bring it carefully over the head, and then pull the respirator away from the face without touching the front of the respirator.
    - Facemask: Carefully untie (or unhook from the ears) and pull away from face without touching the front.
7. Perform hand hygiene after removing the respirator/facemask and before putting it on again if your workplace is practicing reuse.*

Biohazardous Waste: Segregation, Collection & Disposal Guide

This guide is intended to serve as a general informational guide. Please contact Robin Trundy, Biosafety Officer at 322-0927 for specific questions related to your waste and handling practices.

Biohazardous waste includes research-related wastes that are contaminated with recombinant or synthetic nucleic acids, agents infectious to humans, animals or plants, or fluids that may contain these contaminants. This waste needs to be collected, stored, treated and disposed of using practices that minimize spill and exposure risk for lab personnel, service workers and the general public. To support this principle, all biohazardous wastes need to be stored inside the lab while awaiting pickup.

This includes items which are sharp enough to puncture skin and contaminated with unsterilized biological materials. Example devices include:

- needles & lancets,
- scalpels & razor blades,
- glass slides,
- glass Pasteur pipettes,
- biologically-contaminated broken glass.

This category also includes all sharps-associated medical devices (i.e., syringes).

Collection & Storage

These devices need to be placed into a sharps container immediately after use. Sharps containers are biohazard-marked, solid-walled, puncture-proof containers that are leak-proof on the sides and bottom.

NOTE: Sharps containers must be containers designed for that purpose. Cardboard boxes or repurposed food/beverage containers are not acceptable!

Sharps container lids have a restricted access opening to prevent devices from being accessed once inside the container. Assure that the lid is properly and completely installed before using it for sharps collection. If the opening requires you to drop the device in vertically, it should be closed when the container is not in use. Keep the container free of visible contamination and store it in an upright position.

Treatment & Disposal

Permanently close the container when it is 3/4ths full or when items do not freely fall into the container, regardless of the fullness level. (Do not force objects into a container or shake the container to make more space!) Place full containers in the designated biohazardous waste pickup point.

If containers will be picked up by an outside vendor (i.e., Stericycle), place the closed container in the vendor-supplied waste container for pickup and disposal. Sharps containers must be autoclaved using a validated waste treatment cycle or incinerated for final treatment. At Vanderbilt, sharps containers are collected by Environmental Services or an outside vendor and ultimately treated and disposed of by an outside vendor.

Safety Equipment: Splash Protection

A large percentage of eye injuries are caused by direct contact with chemicals. These injuries often result from an inappropriate choice of personal protective equipment, that allows a chemical substance to enter from around or under protective eye equipment. Serious and irreversible damage can occur when chemical substances contact the eyes in the form of splash, mists, vapors, or fumes. When working with or around chemicals, it is important to know the location of emergency eyewash stations and how to access them with restricted vision.

When fitted and worn correctly, goggles protect your eyes from hazardous substances. A face shield may be required in areas where workers are exposed to severe chemical hazards.

Personal protective equipment devices for chemical hazards:

- Safety Goggles: Primary protectors intended to shield the eyes against liquid or chemical splash, irritating mists, vapors, and fumes.
- Face Shields: Secondary protectors intended to protect the entire face against exposure to chemical hazards.

Classification of Microrganisms by Risk Group

Microorganisms are classified into Risk Groups based on their potential impact on humans and the environment. These lists are not exhaustive, and microorganisms are generally classified as follows:

Risk Group 1 (RG1): Low individual and low community risk. These microorganisms are unlikely to cause disease.

Risk Group 2 (RG2): Moderate individual risk, limited community risk. These microorganisms are unlikely to be a significant risk to laboratory workers or the environment, but exposure may cause infection.

Risk Group 3 (RG3): High individual risk, limited/moderate community risk. These microorganisms usually cause serious disease and may present a significant risk to laboratory workers, but may only present a moderate risk of spreading amongst a community.

Risk Group 4 (RG4): High individual and high community risk. These microorganisms usually cause life-threatening disease and may be readily transmissable. Effective treatments are not usually available.

Three Common Ventilated Engineering Controls

Biological safety levels are ranked from one to four and are selected based on the agents or organisms on which the research or work is being conducted. Each level up builds on the previous level, adding constraints and barriers

Biosafety Level 1 (BSL-1)

Biosafety level one, the lowest level, applies to work with agents that usually pose a minimal potential threat to laboratory workers and the environment and do not consistently cause disease in healthy adults. Research with these agents is generally performed on standard open laboratory benches without the use of special containment equipment. BSL 1 labs are not usually isolated from the general building. Training on the specific procedures is given to the lab personnel, who are supervised by a trained microbiologist or scientist.

Standard microbiology practices are usually enough to protect laboratory workers and other employees in the building. These include mechanical pipetting only (no mouth pipetting allowed), safe sharps handling, avoidance of splashes or aerosols, and decontamination of all work surfaces when work is complete, e.g., daily. Decontamination of spills is done immediately, and all potentially infectious materials are decontaminated prior to disposal, generally by autoclaving. Standard microbiological practices also require attention to personal hygiene, i.e., hand washing and a prohibition on eating, drinking or smoking in the lab. Normal laboratory personal protective equipment is generally worn, consisting of eye protection, gloves and a lab coat or gown. Biohazard signs are posted and access to the lab is limited whenever infectious agents are present.

Biosafety Level 2 (BSL-2)

Biosafety level two would cover work with agents associated with human disease, in other words, pathogenic or infectious organisms posing a moderate hazard. Examples are the equine encephalitis viruses and HIV when performing routine diagnostic procedures or work with clinical specimens. Therefore, because of their potential to cause human disease, great care is used to prevent percutaneous injury (needlesticks, cuts and other breaches of the skin), ingestion and mucous membrane exposures in addition to the standard microbiological practices of BSL 1. Contaminated sharps are handled with extreme caution. Use of disposable syringe-needle units and appropriate puncture-resistant sharps containers is mandatory. Direct handling of broken glassware is prohibited, and decontamination of all sharps prior to disposal is standard practice. The laboratory's written biosafety manual details any needed immunizations (e.g., hepatitis B vaccine or TB skin testing) and whether serum banking is required for at-risk lab personnel. Access to the lab is more controlled than for BSL 1 facilities. Immunocompromised, immunosuppressed and other persons with increased risk for infection may be denied admittance at the discretion of the laboratory director.

BSL 2 labs must also provide the next level of barriers, i.e., specialty safety equipment and facilities. Preferably, this is a Class II biosafety cabinet or equivalent containment device for work with agents and an autoclave or other suitable method for decontamination within the lab. A readily available eyewash station is needed. Selfclosing lockable doors and biohazard warning signs are also required at all access points.

Biosafety Level 3 (BSL-3)

Yellow fever, St. Louis encephalitis and West Nile virus are examples of agents requiring biosafety level 3 practices and containment. Work with these agents is strictly controlled and must be registered with all appropriate government agencies.[2] These are indigenous or exotic agents that may cause serious or lethal disease via aerosol transmission, i.e., simple inhalation of particles or droplets. The pathogenicity and communicability of these agents dictates the next level of protective procedures and barriers. Add to all the BSL 2 practices and equipment even more stringent access control and decontamination of all wastes, including lab clothing before laundering, within the lab facility. Baseline serum samples are collected from all lab and other at-risk personnel as appropriate.

More protective primary barriers are used in BSL 3 laboratories, including solid-front wraparound gowns, scrub suits or coveralls made of materials such as Tyvek® and respirators as necessary. Facility design should incorporate self-closing double-door access separated from general building corridors. The ventilation must provide ducted, directional airflow by drawing air into the lab from clean areas and with no recirculation.

Biosafety Level 4 (BSL-4)

Agents requiring BSL 4 facilities and practices are extremely dangerous and pose a high risk of life-threatening disease. Examples are the Ebola virus, the Lassa virus, and any agent with unknown risks of pathogenicity and transmission. These facilities provide the maximum protection and containment. To the BSL 3 practices, we add requirements for complete clothing change before entry, a shower on exit and decontamination of all materials prior to leaving the facility.

The BSL 4 laboratory should contain a Class III biological safety cabinet but may use a Class I or II BSC in combination with a positive-pressure, air-supplied full-body suit. Usually, BSL 4 laboratories are in separate buildings or a totally isolated zone with dedicated supply and exhaust ventilation. Exhaust streams are filtered through high-efficiency particulate air (HEPA) filters, depending on the agents used.

We have touched on only the main issues and differences between BSL 1, 2, 3 and 4 laboratories. There are many other concerns and requirements addressed in the CDC manual, such as impervious, easy-to-clean surfaces; insect and rodent control; and total barrier sealing of all wall, floor and ceiling penetrations. Our goal was to introduce you to the different levels of biological safety practices and facility design considerations. Hopefully, you now have the

Occupational Health and Medical Surveillance

1. ***Pre-Employment Physical***

- Verify the individual is physically capable of handling the stresses of the job
- Establish range for normal vital signs
- Collect baseline serum, blood, and urine samples
- Provide necessary vaccinations (immunizations) based on risk

2. ***Annual Physicals***

- To determine continued fitness for the necessary job requirement
- Compare and reassess baseline vitals
- Collect new samples of serum, blood, and urine
- Provide necessary vaccinations (immunizations) and boosters

3. ***Emergency Response or Treatment***

- Laboratory exposure
- Accident or injury in the laboratory
- Have basis of comparison for post-exposure samples
- Provide treatment
- Re-examination of the patient before allowing them to return to work after a laboratory exposure
- Safety Responsibilities

Institution Management

- Appoint an individual to manage the biosafety program.
- Should encourage and promote the development, implementation and maintenance of a robust biosafety program – create manuals.
- Hold all line managers and supervisors accountable for the safety of their employees.
- All line managers and supervisors should have this responsibility incorporated in to their performance standard and are evaluated in this area as part of their annual performance evaluation.
- Provide the necessary resources to implement a biosafety program.

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Choice of antibiotics: Treatment for livestock and poultry

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Antibiotic resistance (AR) which is defined as the ability of an organism to resist the killing effects of an antibiotic to which it was normally susceptible and it has become an issue of global interest. This microbial resistance is not a new phenomenon since all microorganisms have an inherent capacity to resist some antibiotics. However, the rapid surge in the development and spread of AR is the main cause for concern. In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a contributing factor to the overall burden of AR has emerged. The extent of usage is expected to increase markedly over coming years due to intensification of farming practices in most of the developing countries. The main reasons for the use of antibiotics in food-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals.

Poultry is one of the most widespread food industries worldwide. Chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year. A large diversity of antimicrobials, are used to raise poultry in most countries. A large number of such antimicrobials are considered to be essential in human medicine. The indiscriminate use of such essential antimicrobials in animal production is likely to accelerate the development of AR in pathogens, as well as in commensal organisms. This would result in treatment failures, economic losses and could act as source of gene pool for transmission to humans. In addition, there are also human health concerns about the presence of antimicrobial residues in meat, eggs and other animal products.

Generally, when an antibiotic is used in any setting, it eliminates the susceptible bacterial strains leaving behind those with traits that can resist the drug. These resistant bacteria then multiply and become the dominating population and as such, are able to transfer (both horizontally and vertically) the genes responsible for their resistance to other bacteria. Resistant bacteria can be transferred from poultry products to humans via consuming or handling meat contaminated with pathogens. Once these pathogens are in the human system, they could colonize the intestines and the resistant genes could be shared or transferred to the endogenous intestinal flora, jeopardizing future treatments of infections caused by such organisms.

Use of antibiotic in animal production

Antimicrobials' use in animal production dates as far back as the 1910 when due to shortage of meat products, workers carried out protests and riots across America. Scientists at that time started looking for means of producing more meat at relatively cheaper costs; resulting in the use of antibiotics and other antimicrobial agents. With the global threat of antibiotic resistance and increasing treatment failures, the non-therapeutic use of antibiotics in animal production has been banned in some countries. Sweden is known to be the first country to ban the use of antimicrobials for non-therapeutic purposes between 1986 (for growth promotion) and 1988 (for prophylaxis). This move was followed by Denmark, The Netherlands, United Kingdom and other European Union countries. These countries also moved a step further and banned the use of all essential antibiotics as prophylactic agents in 2011.

Several other countries have withdrawn the use of some classes of antibiotics or set up structures that regulate the use of selected antibiotics in animal production. Despite these developments, it is currently estimated that over 60% of all antibiotics produced are used in livestock production, including poultry.

The use of antibiotics in poultry and livestock production is favorable to farmers and the economy as well because it has generally improved poultry performance effectively and economically but at the same time, the likely dissemination of antibiotic resistant strains of pathogenic and non-pathogenic organisms into the environment and their further transmission to humans via the food chain could also lead to serious consequences on public health.

Antimicrobial resistance

Bacteria counteract the actions of antibiotics by four well-known mechanisms, namely; enzyme modification, alteration in target binding sites, efflux activity and decreased permeability of bacterial membrane. This expression of resistance towards antibiotics by bacteria could either be intrinsic or acquired. Intrinsic resistance is due to inherent properties within the bacteria chromosome such as mutations in genes and chromosomally inducible enzyme production, whereas acquired resistance could be due to the transmission of resistance genes from the environment and/or horizontally transfer from other bacteria.

Antibiotic resistance of some selected organisms in poultry

Staphylococcus species

The bacterial genus Staphylococcus is a Gram-positive cocci and a facultative anaerobe which appears in clusters when viewed under the microscope. They are etiological agents of staphylococcosis, pododermatitis (bumblefoot) and septicaemia which affect mostly chicken and turkeys. Coagulase-negative species have also been implicated in human and animal infections.
β-lactams were considered the first line of drugs for treatment of staphylococcal infections but due to emergence of high level of resistance to these and other drugs, there are currently very few drugs available for treatment of these infections. Methicillin resistant Staphylococcus aureus (MRSA), now known as a superbug, is resistant to almost every available antibiotic used against Staphylococcus.

A study to detect the presence of MRSA in broilers, turkeys and the surrounding air in Germany reported the prevalence of MRSA in air as high as 77% in broilers compared to 54% in Turkeys. Ten different spa types were identified with spa type t011 and clonal complex (CC) 398 being the most prevalent. It was also found that for every farm, the same sequence types were present in both the birds and the environment. This pattern of resistance was also reported in India with 1.6% of staphylococcal isolates containing mecA resistant gene.
In Africa, studies carried out in Ghana and Nigeria have shown that livestock-associated Staphylococci are susceptible to amoxicillin/clavulanic acid, amikacin, ciprofloxacin, gentamycin and cephalexin, whereas in the US, most of the staphylococcal isolates were susceptible to rifampin, cotrimoxazole, gentamycin, vancomycin and chloramphenicol. It is worth noting that most of these organisms showed a high level of resistance to oxacillin and tetracycline, which would be disastrous if these oxacillin-resistant strains are transferred to humans.

Pseudomonas species

Pseudomonas is a genus of Gram-negative, aerobic bacteria that belongs to the family Pseudomonadaceae. The genus Pseudomonas is ubiquitous in soil, water and on plants. It consists of 191 subspecies belonging to species groups including P. fluorescens, P. pertucinogena, P. aeruginosa, P. chlororaphis, P. putida, P. stutzeri and P. syringae. Pseudomoniasis, which is an opportunistic P. aeruginosa infection, is common in poultry birds like chickens, turkeys, ducks, geese and ostriches where infections in eggs destroy embryos.

P. aeruginosa causes respiratory infection, sinusitis, keratitis/keratoconjuctivitis and septicemia and responsible for pyogenic infections, septicemia, endocarditis and lameness along with many diverse diseases. Infections may occur through skin wounds, contaminated vaccines and antibiotic solutions or needles used for injection. The disease may be systemic, affecting multiple organs and tissues or localized in tissues as infraorbital sinus or air sacs producing swelling of the head, wattles, sinuses and joints in poultry birds. P. aeruginosa has been isolated from many poultry farms and birds worldwide.

A study carried out in Ghana show that P. aeruginosa isolated from poultry litter were all susceptible to levofloxacin in the range of 20–100% and nearly 75% demonstrated intermediate susceptibility to aztreonam. The organisms showed resistance to cephalosporins, carbapenems, penicillins, quinolones, monobactam and aminoglycoside. Metallo β-Lactamase encoding genes (blaIMP, blaVIM) were not detected in any of the isolates but the class 1 integron which is known to carry multiple antibiotic resistant genes were detected in 89.4% of the multi-drug resistant strains. This is contrary to a report by Zhang and his Colleagues, who identified the blaVIM gene in P. aeruginosa and P. putida from chicken that resembled corresponding regions in clinical isolates of P. aeruginosa. These isolates were resistant to all β-lactam antibiotics tested, including meropenem, imipenem, aztreonam, and ceftazidime.

Another study in Nigeria reported that the P. aeruginosa isolates were highly resistant to β-lactams, tetracycline, tobramycin, nitrofurantoin and sulfamethoxazole-trimethoprim, while ofloxacin, imipenem and ertapenem were highly effective against the bacterial pathogens.

In Pakistan, a study which investigated the causative agents for necropsy in chicken, recorded a 28% prevalence for P. aeruginosa. These isolates were found to be 100% resistant towards ceftriaxone, meropenem, ciprofloxacin, erythromycin and colistin, while 60% sensitivity was observed against ampicillin sulbactam, ceftazidime, cefoperazone and rifampicin. Isolates exhibited variable multidrug resistance patterns to other antibiotics.

Escherichia species

Escherichia coli is a Gram-negative bacterium that has been known for ages to easily and frequently exchange genetic information through horizontal gene transfer with other related bacteria. Hence, it may exhibit characteristics based on the source of isolation. E. coli is a commensal organism living in the intestines of both humans and animals. However, some strains have been reported to cause gastrointestinal illnesses. Tetracycline which is commonly used in poultry has been reported to be one of the drugs bacteria are most resistant to. There is a reported tetracycline resistance in poultry even without the administration of this antibiotic. A study carried out on fecal isolates of E. coli in the Netherlands showed that there is a high level of multidrug resistance occurring in broilers, turkeys while majority of those from laying hens were susceptible. It was observed that the isolates from birds had high rates of resistance to amoxicillin alone and others had resistance to amoxicillin as well as oxytetracycline, streptomycin, sulfamethoxazole and trimethoprim.

E. coli had a prevalence of 46.98% among the other bacteria isolated in Ghana. All isolates showed some degree of resistance to ceftriaxone (1.34%), cefotaxime (0.67%), gentamycin (2.01%), cotrimoxazole (1.34%), tetracycline (2.01%) and ampicillin (3.36%). Resistant genes have been found in E. coli isolates from Nigeria and these include bla-TEM (85%), sul2 (67%), sul3 (17%), aadA (65%), strA (70%), strB (61%), catA1 (25%), cmlA1 (13%), tetA (21%) and tetB (17%) which conveyed resistance to the following antibiotics; tetracycline (81%), sulfamethoxazole (67%), streptomycin (56%), trimethoprim (47%), ciprofloxacin (42%), ampicillin (36%), spectinomycin (28%), nalidixic acid (25%), chloramphenicol (22%), neomycin (14%) gentamicin (8%). In this study the isolates were susceptible to amoxicillin-clavulanate, ceftiofur, cefotaxime, colistin, florfenicol and apramycin. Class 1 and 2 integrons were found in five (14%) and six (17%) isolates, respectively, while one isolate contained both classes of integrons. There is that suggestion that poultry production environments represent important reservoirs of antibiotic resistance genes such as qnrS that may spread from livestock production farms to human populations via manure and water.

Salmonella species

Salmonella spp. are Gram-negative, facultative anaerobic, non-spore forming, usually motile rods belonging to the Enterobacteriaceae family, which are found in the alimentary tract of animals. Fecal shedding allows Salmonella to be transmitted among birds in a flock. Salmonella spp. is widespread in poultry production. Prevalence varies considerably depending on country and type of production as well as the detection methods applied. It is known to be the etiological agent responsible for salmonellosis by Salmonella spp. in both humans and animals. Food-borne salmonellosis caused still occurs throughout the world. The risk factors associated with Salmonella infections and contamination in broiler chickens include contaminated chicks, size of the farm and contaminated feed and these risk increase when feed trucks are parked near the entrance of the workers' change room and when chicken are fed with meals. It also depends on age of the chicken, animal health, survival of organism in the gastric barrier, diet and genetic constitution of the chicken could also affect the colonization ability of Salmonella spp. in poultry.

Pullorum disease in poultry is caused by the S. pullorum. Transmission of the disease in birds can be vertical (transovarian) but also occurs through direct or indirect contact with infected birds via respiratory route or fecal matter or contaminated feed, water, or litter. Antimicrobials used to treat pullorum disease are furazolidone, gentamycin sulfate and antimetabolites (sulfadimethoxine, sulfamethazine and sulfamerazine).

Salmonella spp. have increasingly been isolated from poultry with prevalence of 2.7% in Brazil and the most common isolates were Salmonella enteritidis (48.8%), S. infantis (7.6%), S. typhimurium (7.2%), and S. heidelberg (6.4%). All the isolated strains were resistant to at least one class of antimicrobial and 53.2% showed multidrug resistance to three or more classes, with streptomycin (89.2%), sulfonamides (72.4%), florfenicol (59.2%), and ampicillin (44.8%).

Salmonella spp. are one of the commonest microbial contaminants in the poultry industry. In Ghana, there is high prevalence rate of 44.0% in poultry with main isolates being S.

kentucky (18.1%), S. nima (12.8%), S. muenster (10.6%), S. enteritidis (10.6%) and S. virchow (9.6%). Resistance of these isolates to the various antibiotics were nalidixic acid (89.5%), tetracycline (80.7%), ciprofloxacin (64.9%), sulfamethazole (42.1%), trimethoprim (29.8%) and ampicillin (26.3%).

Streptococcus species
Streptococcus is Gram-positive bacteria. Streptococcus gallolyticus is a common member of the gut microbiota in animals and humans; however, being a zoonotic agent, it has been reported to cause mastitis in cattle, septicemia in pigeons, and meningitis, septicemia, and endocarditis in humans. A study carried out in Japan isolated Streptococcus gallolyticus from pigeons with septicaemia. Most of the isolates were susceptible to vancomycin, penicillin G and ampicillin, while some were resistant to tetracycline, doxycycline and lincomycin. All the isolates were resistant to tetracycline had tet(M) and/or tet(L) and/or tet(O) genes.

Campylobacter species
Campylobacter jejuni and Campylobacter coli are the most prevalent disease causing species of the genus Campylobacter. They are mostly responsible for foodborne gastroenteritis in humans. Campylobacteriosis is often associated with handling of raw poultry or eating of undercooked poultry meat. Cross-contamination of raw poultry to other ready-to-eat foods via the cook's hands or kitchen utensils has been reported. Erythromycin is usually the drug of choice for the treatment of Campylobacter infections. However, fluoroquinolones, gentamicin, and tetracycline are also clinically effective in treating Campylobacter infections when antimicrobial therapy is required.

Resistance of *C. jejuni* and *C. coli* isolates to fluoroquinolones, tetracycline, and erythromycin has been reported. The increased resistance is partly due to the wide use of these antimicrobials in animal husbandary, especially in poultry. A study carried out by Elz˙bieta and his colleagues, in their quest to compare the prevalence and genetic background of antimicrobial resistance in Polish strains of C. jejuni and C. coli isolated from chicken carcasses and children reported a slight difference in resistance between human and chicken strains. The isolated Campylobacter strains were found to be resistant to gentamycin, tetracycline, ampicillin, ciprofloxacin and erythromycin and tet(O) gene and mutations in the gyrA genes were found to be associated with the observed antibiotic resistance in the study.

Another study carried out in Kenya isolated thermophilic Campylobacter species (C. jejuni and C. coli) from feces and clocal swabs of chicken. These isolates showed a high rate of resistance to nalidixic acid, tetracycline and ciprofloxacin of 77.4, 71.0 and 71.0%, respectively. Low resistance (25.8%) was detected for gentamicin and chloramphenicol and 61.3% of C. jejuni isolates exhibited multidrug resistance and 54.5% of the C. jejuni isolates possessed the tet(O) gene whereas all of C. coli had the tet(A) gene. C. jejuni and C. coli are the predominant species of Campylobacter usually isolated from poultry farms. In Ghana, other species such as Campylobacter lari, Campylobacter hyo-intestinalis and C. jejuni sub sp. doylei have been isolated from poultry. These organisms have been found to be resistant to β-lactams, quinolones, aminoglycosides, erythromycin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole and all isolated species were sensitive to imipenem.

Source:https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat/antibiotic-use-in-poultry-production-and-its-effects-on-bacterial-resistance; DOI: 10.5772/intechopen.79371

Use of prebiotics and probiotics as alternative to antibiotics in animal farming

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Enhancement of nutrient utilization coupled with productivity enhancement through alteration of the gastrointestinal microflora in animal (ruminant and non-ruminant) farming is an evolving strategy across the world, including Bangladesh. A new era in the biological science has been emerged following the greater understanding of the critical role of the "Newly Designated Organ" i.e., "Gut Microbiome" on health and disease in both animals and human. Even though, the gastrointestinal tract is sterile at the time of birth/ hatch in animals/ birds, nevertheless, it is being settled by numerous microorganisms with the advancement of age. According to the modern scientific dogma, the food producing animals are presumed to be the "Superorganism", the consortium of numerous *Eukarya, Bacteria, Archaea and Viruses*. In fact, both ruminants and non-ruminants evolved on the planet Earth predominated by microbes. In order to ensure survivability within this microbial world, animals developed mechanisms to support both eukaryotic and prokaryotic microbial communities on their numerous external and internal body surfaces leading to the development of an intimate relationship between the host animal and microbial communities during the course of evolution (Samanta et al., 2013). Upon examination on the function of gut microorganisms, it is amply clear that some of these teensy creatures have the ability to make animals productive and healthy; while the other members are responsible for declined nutrient utilization, lowering productivity and unsafe products, predisposition and progression of diseases and finally leads to loss of life. The former one offers the opportunity to the nutraceutical researchers for application of prebiotics and probiotic towards restoration of normal microflora or dominance of beneficial microorganism in the gastrointestinal tract followed by alteration of certain metabolic pathways to enable intended productivity and product quality (Prasad et al., 2014).

At the beginning of the early 20^{th} century, Ilyich Metchnikoff, a Russian scientist, observed good health coupled with longer life span among the Bulgarian farmers and he linked this fact with the consumption of fermented milk containing large number of Lactobacillus species and later on, he published the famous book entitled "The prolongation of life" based on those findings and he was bestowed with the "Nobel" prize in 1908. The particular book was the first scientific evidence to demonstrate improvement of human health through consumption of substance that favourably modulates the gastrointestinal microflora: the concept of probiotic (Fuller, 1992). Unlike probiotic, the prebiotic concept emerged only during the mid-nineties of the previous century (Samanta et al., 2015). The concept relies on the short chain oligosaccharides mediated alterations in the composition as well as metabolic signatures of the gastrointestinal microflora for better health and wellbeing (Gibson and Roberfroid, 1995).

History on antibiotic usages in livestock

The primary goal of the livestock farming is to produce safe and healthy food for human consumption after due contemplation to environment, animal welfare, consumer awareness, and public health (Prasad et al., 2014). To match the escalating demand for livestock and its products, the livestock sector of Bangladesh has adopted several scientific interventions in order to enhance nutrient utilization, productivity, product quality, as well as to control disease outbreak. Usages of antibiotic is one of such interventions adopted in the livestock production system (Samanta et al., 2018). Since the discovery of antibiotic during third decade of previous century, it has been playing a substantial role in the advancement and prosperity of livestock sector (Gadde et al., 2017). In fact, the growth promoting effects of antibiotic was demonstrated during the mid-forties of previous century. Birds receiving dried mycelia of *Streptomyces aureofaciens* (containing chlortetracycline) showed higher growth rate (Moore et al., 1946). Soon, non-therapeutic antibiotics usages become profitable in the animal industry because of their action mediated by reduction of overall numbers or diversity of the gut microflora, decreased competition for nutrients, and finally reduction of microbial metabolites (Castanon, 2007, Gadde et al. 2017). Additionally, antibiotics are also applied in livestock for treatment (therapeutic purpose) and prevention of common diseases (prophylactic use) (Landers et al., 2012). Currently, the usage of antibiotics in food producing animals is thought to be linked with major public health challenges i.e., development and transfer of AMR (WHO, 2012). This has led to the ban on the use of antibiotics in animal feeds by several countries since January 2006 and some countries are in the process of restricting the use of antibiotics in animal feed. The restriction on the usages of antibiotics as feed supplements has put up tremendous pressure over the livestock industry and driven intense research for alternatives. In light of those facts, prebiotics and probiotics have been emerged as viable alternatives to antibiotic growth promoters in livestock farming.

Evolution of prebiotic

Prebiotics are the class of bio-molecules grouped together by virtue of their indigestibility at the upper gut, but its fermentation at the hindgut to selectively promote the growth of beneficial gut microflora (Samanta et al., 2013). The term "Prebiotics" is coined by Gibson and Roberfroid (1995), who exchanged "pro" to "pre", which means "for" or "before" (Aida et al., 2009). Albeit, the term "prebiotic" emerges in the field of functional food science only during mid-nineties of previous century, nevertheless, historical evidences suggest that the people of ancient Indus valley civilization (3300 - 1700 BC) were cultivating and propagating the crops (wheat, barley, lentils, tubers) rich in bioactive molecules for better health and wellbeing (Samanta et al., 2011). The prebiotic is defined as "non-digestible food ingredients that beneficially affects the host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995).

With the growing global attention on research and development, it was proposed to revisit the definition and suggested to exclude the word 'non-digestible' from the domain of "prebiotic". The updated definition of prebiotic sounds as "a selectively fermented ingredient that allows specific change, both in the composition and or activity in the gastrointestinal tract microflora that confers benefits upon host wellbeing and health" (Gibson et al., 2004). In view of the growing attention on prebiotic application around the world, the Food and Agriculture Organization (FAO) of the United Nations called a technical meeting on "prebiotics" in 2007. It defines prebiotic as "a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (FAO, 2007). Thereafter, the definition of prebiotic undergoes several rounds of revision (Gibson, 2010, Bindels et al., 2015). In December, 2016, the International Scientific Association for Probiotics and Prebiotics (ISAPP) set up a panel to review the definition and scope of prebiotic. The panel suggested the definition of prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). The present theory of prebiotic expands the concept in order to incorporate non-carbohydrate substances, applications to body sites other than gastrointestinal tract, and diverse categories other than food.

The concept of prebiotic originated keeping in mind the gastrointestinal ecology of human being. Therefore, the criteria set to qualify a biomolecule as prebiotic may not be the same as it was being proposed by the inventors of the concept. Hence, the criteria (Samanta et al., 2007, 2013) for a compound to become a prebiotic in livestock sector could be as follows:

- ❖ Indigestibility by animal's own gastric or pancreatic enzymes;

- ❖ Selectively utilized by so called beneficial gut microflora (exception several rumen bacteria ferments prebiotics);
- ❖ Plant origin or produced by microbial enzymes;
- ❖ Non-absorption from the epithelial surface of gastrointestinal tract;
- ❖ Protects structural and functional integrity while passing through either acidic or alkaline pH of gastrointestinal tract;
- ❖ Exhibit its potentiality even at minute concentration;
- ❖ Remains intact while undergoing physical action of digestion process *i.e.,* mastication, chewing, mixing with several fluids etc.;
- ❖ Presence of chemicals bonds that is inaccessible to harmful gut microflora;
- ❖ No residue problems in livestock or their products;
- ❖ Non-carcinogenic;
- ❖ Its fermentation should not lead to generate metabolite of toxic nature;
- ❖ Easy to mix with other feed ingredients or micronutrient mixture.

Evolution of probiotic

The term 'probiotic' was introduced by the German scientist Werner Kollath in 1953 to define "active substances that are essential for a healthy development of life" (Gasbarrini, 2016). Indeed, it originates from two Latin words: 'Pro' means 'For' and 'Biotic' means 'Life', i.e., probiotics are for life. During the mid-sixties of previous century, probiotic was redefined as "substances secreted by one organism which stimulate the growth of another" (Lilly and Stillwell, 1965). Later on, the definition of probiotics emerged as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1992). According to FAO/WHO (2002), probiotics could be defined as "live microorganism which, when administered in adequate amounts, confer a health benefit on the host". The beneficial effects of probiotics are manifested by regulation of intestinal microbial homeostasis, stabilizing the gastrointestinal barrier function, secretion of bacteriocin, immunomodulatory effect, reduction of pro-carcinogenic enzymes, interference in pathogen colonization etc. Nevertheless, the modern history of probiotics commences at the beginning of twentieth century with the genius research of the Nobel laureate Elie Metchnikoff, the great Russian scientist.

In fact, the history of probiotic is as old as human civilization and is closely linked with the production and consumption of fermented food (Gasbarrini, 2016). The historical facts to consume fermented milk goes back to the ancient Egyptians cultures. The ancient people store the milk (cow, sheep, goat, horse, camel) in container prepared from the skin or the stomach of the same animal in order to permeate close contact between milk and gut bacteria; probably the ancestors of the acidophilus and bulgaricus. During those early days of human civilization, a storey is often described that a shepherd keeps milk in a goatskin bag and travels for a while in the desert of Turkey. After his return, he founds the milk in the form of "thick, creamy, and tasty custard" and toady we know it in the name of "yogurt". No matter, Whatsoever the origin, the positive health properties and therapeutic use of fermented milk (yogurt) has been recognized since time immemorial, long before the discovery of bacteria. Historical evidences during the period from 3000 and 2000 BC suggested that Hindu, Egyptians, Greeks, and Romans used fermented milk products. According to the Bible, Abraham offered to the Lord "veal, buns and sour milk." The Prophet Muhammad gave as a gift the first kefir grains to the ancestors of the mountaineers of Caucasus. Kefir is a drink rich in lactic acid bacteria and probiotics from the fermentation of milk. The concept of nutraceutical or functional food is also traced back to 460 to 375 BC as the then great philosopher opined that "Let the food be your medicine and medicine be your food". In light of those theories, consumption of different fermented food items becomes popular among people of several countries during ancient times and the same legacy is going on even during twenty first centuries in the name of either probiotic or prebiotic or synbiotic. According to Gaggia et al (2010), the salient features of probiotics are as follows:

- Non-toxic and non-pathogenic;
- Accurate taxonomic identification;
- Normal inhabitants of the targeted species;
- Resistance to gastric pH and bile;
- Persistence in the gastrointestinal tract;
- Adhesion to the gut epithelium or mucus;
- Compatibility with resident microflora;
- Production of antimicrobial substance;
- Antagonism against pathogenic microflora of gut;
- Immune modulating ability;
- Health promoting properties;
- Stability of characteristics during processing like storage and delivery;
- Acceptable organoleptic properties.

Common prebiotic

Albeit the prebiotic concept has been forwarded only during the mid-nineties of previous century, nevertheless, intensive research has resulted into development of several prebiotics within short timeframe. It could be produced by different process such as (i) extraction from plants (inulin from chicory root or global artichoke), (ii) extraction followed by enzymatic hydrolysis (xylan from lignocellulosic materials and thereafter its hydrolysis into xylooligosaccharides by endoxylanase), (iii) glycosylation reaction (fructo-oligosaccharides from sucrose by fructosyl transferase), (iv) hydrolysis and transgalactosylation (galacto-oligosaccharides from lactose by beta- galactosidase) etc. Table 1 outlines the different prebiotics and production process.

Table 1. Commonly available prebiotics

Prebiotic	Raw materials	Production method	Reference
Inulin	Chicory root	Water extraction	Chikkerur et al. (2019)
Short chain oligosaccharides	Inulin	Enzyme hydrolysis	Chikkerur et al. (2019)
Fructooligosaccharides	Sucrose	Enzyme catalysis	Sangeetha et al. (2005)
Galactooligosaccharides	Lactose	Enzyme hydrolysis and transgalactosylation	Splechtna et al. (2006)
Xylooligosaccharides	Cotton stalks	Alkaline extraction followed by enzyme/ acid hydrolysis	Samanta et al. (2020)
	Corn cobs		Samanta et al. (2012, 2014)
	Corn husks		Samanta et al. (2016)
	Tobacco stalks		Samanta et al. (2019)
	Sugarcane bagasse		Jayapal et al. (2013)

	Natural grass		Samanta et al. (2012a)
	Pigeon pea stalks		Samanta et al. (2013a)
Pectin oligosaccharides	Orange peels	Enzyme hydrolysis	Sabajanes et al. (2012)
Tagatose	D-galactose or arabinose	Microbial transformation	Roy et al. (2018)

Common probiotic

Probiotic organisms, owing to their beneficial roles, have occupied significant niche in the list of animal feed supplements. It could be either prokaryote (bacteria) or eukaryote (yeast) origin. Important genera for bacterial probiotics are: (1) Lactobacillus, (2) Enterococcus, (3) Bacillus, (4) Lactococcus, (5) Bifidobacteria, (6) Leuconostoc, (7) Pediococcus, (8) Propionibacterium, (9) Streptococcus. Among these genera of probiotics, Lactobacillus is one of the most tested and successful probiotic class that gained greater application in both human and livestock. Similar to Lactobacillus, the genus Enterococcus has been noticed as natural inhabitant in the gastrointestinal tract of animals (Fisher and Phillip, 2009). Due to its probiotic effect, different species of Enterococci are being used in cheese industry and silage making. In the eukaryotic category of probiotics, the important genera are (1) Saccharomyces, (2) Kluyveromyces, (3)Aspergillus. Amongst the several species of yeasts, *Saccharomyces boulardi is* commonly used as probiotic in both ruminants and non-ruminant species. Common probiotic species are listed in Table 2.

Table 2. Important species of probiotic used in livestock feeding

Origin	Genus	Species
I. Bacteria	1. Lactobacillus	*L. acidophilus, L. brevis, L. casei* *L. salivarius, L. rhamnosus, L. Plantarum, L. reutri, L. fermentum* *L. farmicinis, L. crispatus, L. amylovorus, L. murinus*
	2. Bifidobacterium	*B. lactis, B. longum, B. animalis, B. thermophilum, B. pseudolongum*
	3. Entercoccus	*E. faecalis, E. faecium*
	4. Lactococcus	*L. lactis*
	5. Leuconostoc	*L. citreum, L. lactis, L. mesenteroides*
	6. Pediococcus	*P. acidilactici, P. pentosaceus*
	7. Propinobacterium	*P. freudenreichii*
	8. Streptococci	*S. infentarius, S. salivarius, S. thermophilus*
	9. Bacillus	*B. cereus, B. subtilis, B. licheniformis*
II. Yeast	1. Saccharomyces	*S. cerevisiae (S. boulardii), S. pastorianus*
	2. Kluyveromyces	*K. fragilis, K. marxianus*
	3. Aspergillus	*A. Oryza, A. niger*

Scope of prebiotic to replace antibiotic

With the increase in the regulation on usages of antibiotic growth promoters coupled with growing consumer demands for "antibiotic free livestock products", the mission for alternative growth promoters has been intensified across the world. The success for developing alternatives to antibiotic growth promoters largely relies on better understanding the mechanism of action and intensive research with application advanced tools. Among the list of alternatives, prebiotic occupies significant niche as it addresses the issues of consumer, public health and environment. Prebiotics are class macromolecules that are derived from plants or synthesized by microbes or its enzymes. Hence, it qualifies to be considered as an alternative to antibiotic growth promoter from consumer perspective.

Mannan oligosaccharides (MOS) obtained from outer cell layer of *Saccharomyces cervisisae* is found to increase body weight gain, feed conversion efficiency, intestinal villi height, immune competence, jejunal gene expression in addition to alternation of gut microflora in poultry (Gadde et al., 2017). Supplementation of broiler diet with inulin resulted in higher population of *Lactobacillus* with substantial decrease in the population of *Campylobacter* and *Salmonella* (Yusrizal and Chen, 2003). It also improves body weight gain, feed conversion efficiency and carcass weight in broilers along with significant reduction in blood cholesterol levels. Supplementation of xylooligosaccharides in broiler birds increased beneficial gut microflora, decreased pathogenic bacteria, reduced blood cholesterol and glucose in broiler birds (Samanta et al., 2017). In swine, the supplementation of prebiotic @2% alters the gut microflora towards beneficial side and could act as alternative to antibiotic growth promoters (Samanta et al. 2015, 2018).

Scope of probiotic to replace antibiotic

The birds are exposed to several stressors (feed changes, transportation, processing at the hatchery, high stocking densities etc.) during its productive life. The stress conditions lead to imbalance in gut microbial composition that reduces the immune function and predisposes the birds for higher population of gastrointestinal pathogens such as Salmonella, Campylobacter, and Clostridium etc. Therefore, potential probiotic bacteria or yeast can be used to control those gut pathogens through establishment of healthy and beneficial microflora. The probiotic effect in poultry is mediated through competitive exclusion of *Clostridium jejuni, Listeria monocytogens, E. coli, Clostridum perfringens, Yersinia enterocolitica* (Schneitz, 2005).

Administration of Lactobacillus reduces the population of *Salmonella enteridis* in challenged broiler chicks as compared to control birds (Higgins et al., 2008). Similarly, application of Lactobacillus is found to reduce the mortality rates from 60 to 30% in birds, while challenged with necrotic enteritis (Hofacre et al., 2003). Broiler chicks supplemented with 10^8 CFU/g of diet with mixed probiotics *(Lactobacillus acidophilus, L. casei, Bifidobacterium thermophilus, Enterococcus faecium)* reflects reduction in the gut *Clostridium jejuni* (Willis and Reid, 2008).

There is an increasing interest to use probiotics for decreasing pathogen load and in ameliorating gastrointestinal disease symptoms in swine. During the productive life of pig, the animals are subjected to different stresses including weaning, transition of milk to plant polysaccharide diets, transport to production unit etc. This culminated in to break down of gut microbial homeostasis resulting in anorexia, diarrhoea, reduced growth rates and vulnerability to microbial disease. Daily dosing of piglets with *Enterococcus faecium* as probiotic supplements reduces incidence of diarrhoea and improves daily weight gains (Zeyner and Boldt, 2006). Similarly, administration of probiotic (*Lactobacillus plantarum* or Bacillus spp.) to piglets exhibits increase in the population of beneficial microflora and reduction the population of harmful microflora in the gastrointestinal tract

(Takahashi et al., 2007, Bhandari et al., 2008). Application of both Lactobacilli and Bifidobacterium after birth resulted in enhanced colonization by beneficial microflora, which limits feed induced mucosal atrophy, reduces pathogen load (*Clostridium perfringens*), incidence and severity of necrotizing enteritis (Siggers et al., 2008).

Neonatal diarrhoea is one of the major reasons for morbidity and mortality in young ruminants. Supplementation of calf diet with either *Lactobacillus acidophilus* or *Saccharomyces cerevisiae* reduces the incidence of diarrhoea (Agarwal et al., 2002). Inclusion of yeast in the diet of young calves also reduces the number of days with the symptoms of diarrhoea (Galvao et al., 2005). The probiotic supplementation, apart from reducing pathogens, resulted in enhanced performance of animals, improved live weight gains and rumen development in young calves (Adams et al., 2008). In addition to the positive influence during early growth phase, probiotics are shown to improve dry matter intake, immunity, and milk yield during lactation

Conclusion

Poor veterinary public health indicators, increasing income, over the counter sales of antibiotic without prescription, intensive animal production is converging to create the ideal platform for large scale selection and dissemination of AMR genes. Nevertheless, Bangladesh is not sole warrior in the battle ground of global threat on AMR, but it is also sharing and coordinating the defined and flagship activities along with the rest of the world. On the road of alternative growth promoters, prebiotic and probiotics have emerged as front runners among their counterparts. Now, it is the turn for the livestock sector that could accrue the benefits of recent scientific advancements coupled with initiation of multipronged activities including initiation of intense research on prebiotic, probiotic and symbiotic, real time data on antibiotics usages in livestock, awareness development, field demonstration in order to strengthen the fight against AMR.

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Molecular mechanism of antimicrobial resistance

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Antibiotics/antibacterial drugs are the most commonly used and abused antimicrobial agents in the management of bacterial infections globally. They have been used for more than 50 years to improve both human and animal health since and during the antibiotic golden age and post-antibiotic golden age. The discovery of the antibiotics and antibacterial agents revolutionized the treatment of infectious bacterial diseases that used to kill millions of people during the pre-antibiotic golden age worldwide. The major sources of antibiotics/ antibacterial agents include Streptomyces, Penicilliums, Actinomycetes and Bacilli.

During the post-antibiotic golden age, it has seen a massive antibiotic/antibacterial production and an increase in irrational use of these few existing drugs in the medical and veterinary practice, food industries, tissue cultures, agriculture and commercial ethanol production globally. The irrational drug use has been further exacerbated by the increased marketing and promotion of these drugs by the pharmaceutical companies thus increasing their accessibility in the public and hence their improper use. The lack of production and introduction of the newer and effective antibiotic/antibacterial drugs in clinical practice in the post-antibiotic golden age has seen an increase in the emergence of the resistant pathogenic bacterial infections creating a significant problem in the global health of humankind. The massive productions of the antibiotic/antibacterial drugs have contributed to the poor disposal of these drugs and hence many of them are discharged in various water bodies contributing to the environmental antibiotic/antibacterial drug pollution. In the environment, these drugs exert pressure on the environmental bacteria by destroying useful bacteria that are responsible for the recycling of the organic matter and as well as promoting the selection of the resistant pathogenic bacteria that can spread in human and animal population thus causing an increase in the observed bacterial disease burden and hence a significant global public health problem. The resistant bacterial diseases lead to the high cost, increased occurrence of adverse drug reactions, prolonged hospitalization, the exposure to the second and third-line drugs like in MDR-TB and XDR-TB that leads to toxicity and deaths as well as the increased poor production in agriculture and animal industry and commercial ethanol production.

Bacteria must disrupt a step in the action of the antimicrobial agent to survive in the presence of an antibiotic. This may involve preventing antibiotic access into the bacterial cell or perhaps removal or even degradation of the active component of the antimicrobial agent. No single mechanism of resistance can explain why all bacteria are resistant to a particular antibiotic. In fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent, or multiple mechanisms in different bacteria may achieve the same results.

CLASSES OF ANTIBIOTICS AND THEIR SITES OF ACTION ON BACTERIA

The different antibiotics/antibacterial drugs have various targets on the bacteria including 1) cell wall and cell membranes, 2) ribosomes, 3) nucleic acids, 4) bacterial cellular metabolism and 5) bacterial cellular enzymes. There many different mechanisms by which these agents inhibits the multiplication and growth, and the destruction of bacteria. Among these include 1) Inhibition of cell wall synthesis such as beta lactams, 2) Disruption of cell-membrane function, 3) Inhibition of protein synthesis (both 50S and 30S) 4) Inhibition of nucleic acid synthesis both the DNA synthesis and RNA synthesis and 5) action as antimetabolites.

Several different mechanisms may work together to confer resistance to a single antimicrobial agent.

Strategy 1: Preventing Access

Antimicrobial compounds almost always require access into the bacterial cell to reach their target site, where they can interfere with the normal function of the bacterial organism. Porin channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane of Gram-negative bacteria. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls. For example, one variety of Gram-negative bacteria reduces the uptake of certain antibiotics—such as aminoglycosides and ß-lactams—by modifying the cell membrane porin channel frequency, size, and selectivity. Prohibiting entry in this manner will prevent these antimicrobials from reaching their intended targets that, for aminoglycosides and ß-lactams, are the ribosomes and the penicillin-binding proteins (PBPs), respectively.

This mechanism has been observed in:

- *Pseudomonas aeruginosa* against carbapenems (ß-lactam antibiotics)
- *Enterobacter aerogenes* and *Klebsiella* spp. against carbapenems
- Vancomycin intermediate-resistant *S. aureus* or VISA strains with thickened cell wall trapping vancomycin
- Many Gram-negative bacteria against aminoglycosides
- Many Gram-negative bacteria against quinolones

Strategy 2: Eliminating Antimicrobial Agents from the Cell by Expulsion Using Efflux Pumps

To be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria possess membrane proteins that act as an export or efflux pump for certain antimicrobials, extruding the antibiotic out of the cell as fast as it can enter. This results in low intracellular concentrations that are insufficient to elicit an effect. Some efflux pumps selectively extrude specific antibiotics such as macrolides, lincosamides, streptogramins, and tetracyclines, whereas others (referred to as multiple drug resistance pumps) expel a variety of structurally diverse anti-infectives with different modes of action.

This strategy has been observed in:

- *E. coli* and other Enterobacteriaceae against tetracyclines
- Enterobacteriaceae against chloramphenicol
- Staphylococci against macrolides and streptogramins
- *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones

Efflux pumps are variants of membrane pumps possessed by all bacteria, both pathogenic and nonpathogenic, to move lipophilic or amphipathic molecules in and out of their cells. Some efflux pumps are used by antibiotic-producing bacteria to pump antibiotics out of their cells as fast as the antibiotic is made. This constitutes an immunity protective mechanism for the bacteria to prevent being killed by its own chemical weapon.

Strategy 3: Inactivation of Antimicrobial Agents via Modification or Degradation

Another means by which bacteria preserve themselves is by destroying the active component of the antimicrobial agent. A classic example is the hydrolytic deactivation of the ß-lactam ring in penicillins and cephalosporins by the bacterial enzymes called ß-lactamases. The process inactivates penicilloic acid, causing it to be ineffective in binding to PBPs, thereby protecting the process of cell wall synthesis.

This strategy has been observed in:

- Enterobacteriaceae against chloramphenicol (acetylation)
- Gram-negative and Gram-positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation).

The first antibiotic resistance mechanism described was penicillinase, an enzyme from bacteria able to destroy penicillin. It was first reported by Abraham and Chain in 1940. Less than 10 years after the clinical introduction of penicillins, penicillin-resistant *Staphylococcus aureus* was observed in a majority of Gram-positive infections in people. The initial response by the pharmaceutical industry was to develop ß-lactam antibiotics that were unaffected by the specific ß-lactamases secreted by *S. aureus*. However, as a result, bacterial strains producing ß-lactamases with different properties began to emerge, as well as those with other resistance mechanisms. This cycle of resistance counteracting resistance continues even today.

Strategy 4: Modification of the Antimicrobial Target

Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, despite the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place.

This strategy has been observed in:

- Staphylococci against methicillin and other ß-lactams (changes or acquisition of different PBPs that do not sufficiently bind ß-lactams to inhibit cell wall synthesis)
- Enterococci against vancomycin (alteration in cell wall precursor components to decrease binding of vancomycin)
- *Mycobacterium* spp. against streptomycin (modification of ribosomal proteins or 16S rRNA)
- Mutations in RNA polymerase resulting in resistance to the rifamycins
- Mutations in DNA gyrase resulting in resistance to quinolones

Some Examples of Bacterial Resistance Due to Target Site Modification

- Alteration in PBPs reducing affinity of ß-lactam antibiotics (Methicillin-Resistant *Staphylococcus aureus*, *S. pneumoniae*, *Neisseria gonorrhoeae*, Group A streptococci, *Listeria monocytogenes*)
- Changes in peptidoglycan layer and cell wall thickness reducing activity of vancomycin: Vancomycin-resistant *S. aureus*
- Changes in vancomycin precursors reducing activity of vancomycin: *Enterococcus faecium* and *E. faecalis*
- Alterations in DNA gyrase subunits reducing activity of fluoroquinolones: Many Gram-negative bacteria
- Alteration in topoisomerase IV subunits reducing activity of fluoroquinolones: Many Gram-positive bacteria, particularly *S. aureus* and *Streptococcus pneumoniae*
- Changes in RNA polymerase reducing activity of rifampicin: *Mycobacterium tuberculosis*

Examples of intrinsic resistance and their respective mechanisms

Organisms	Natural Resistance Against	Mechanism
Anaerobic bacteria	Aminoglycosides	Lack of oxidative metabolism to drive uptake of aminoglycosides

Aerobic bacteria	Metronidazole	Inability to anaerobically reduce metronidazole to its active form
Gram-positive bacteria	Aztreonam	Lack of penicillin binding proteins (PBPs) for aztreonam to bind and inhibit
Gram-negative bacteria	Vancomycin	Lack of uptake resulting from inability of vancomycin to penetrate outer membrane
Klebsiella spp.	Ampicillin	Production of ß-lactamase enzymes that destroy ampicillin before the drug can reach the PBPs
Stenotrophomonas maltophilia	Imipenem	Production of ß-lactamase enzymes that destroy imipenem before the drug can reach the PBPs
Lactobacilli and *Leuconostoc*	Vancomycin	Lack of appropriate cell wall precursor target to allow vancomycin to bind and inhibit cell wall synthesis
Pseudomonas aeruginosa	Sulfonamides, trimethoprim, tetracycline, or chloramphencicol	Lack of uptake resulting from inability of antibiotics to achieve effective intracellular concentrations
Enterococci	Aminoglycosides	Lack of sufficient oxidative metabolism to drive uptake of aminoglycosides
All cephalosporins	Lack of PBPs for cephalosporins to bind and inhibit	

Various antibiotics with their mode of action and bacterial mechanism of resistance.

	Antibiotic Mode of Action	Bacterial Mechanism of Resistance
ß-Lactams	Target and bind to penicillin-binding proteins (PBPs), inhibiting bacterial cell wall synthesis	– Enzymatic destructions of ß-lactam rings – Target (PBP) modification – Reduced intracellular accumulation
Glycopeptides	Inhibit the last stages of cell wall assembly by preventing cross-linking reactions	– Target modification – Production of false targets
Quinolones	Target DNA gyrase and topoisomerase IV of the bacteria and inhibit the necessary step of supercoiling	– Target modification – Reduced intracellular accumulation
Aminoglycosides	Target and bind to the 30s ribosomal subunit to cause misreading of the genetic code which results in inhibition of protein synthesis	– Antibiotic (structural) modification – Target modification – Reduced uptake
Macrolides	Target and bind to 50s ribsomal subunit to inhibit translocation and transpeptidation process, resulting in inhibition of protein synthesis	– Reduced intracellular uptake – Target modification
Tetracyclines	Target and bind to 30s ribosomal subunit to prevent aminoacyl-	– Reduced intracellular

	Antibiotic Mode of Action	Bacterial Mechanism of Resistance
	tRNA to attach to RNA-ribosome complex, inhibiting protein synthesis	accumulation – Target modification
Rifampicins	Interacts with the ß-subunit of the bacterial RNA polymerase to block RNA synthesis	– Target modification
Sulfonamides	Targets dyhydropteroate synthase (DHPS) and prevents addition of para-aminobenzoic acid (PABA), inhibiting folic acid synthesis	– Target modification

Mechanisms of Resistance Against Different Antimicrobial Classes.

Antimicrobial Class	Mechanism of Resistance	Specific Means to Achieve Resistance	Examples
ß-lactams Examples: penicillin, ampicillin, mezlocillin, peperacillin, cefazolin, cefotaxime, ceftazidime, aztreonam, imipenem	Ensymatic destruction	**Destruction of ß-lactam rings by ß-lactamase enzymes.** With the ß-lactam ring destroyed, the antibiotic will no longer have the ability to bind to PBP (penicillin-binding protein), and interfere with cell wall syntheses.	Resistance of staphylococi to penicillin; resistance of Enterobacteriaceae to penicillins, cephalosporins, and aztreonam
Altered target	**Changes in penicillin binding proteins.** Mutational changes in original PBPs or acquisition of different PBPs will lead to inability of the antibiotic to bind to the PBP and inhibit cell wall synthesis	Resistance of staphylococci to methicillin and oxacillin	
Decreased uptake	**Porcin channel formation is decreased.** Since this is where ß-lactams cross the outer membrane to reach the PBP of Gram-negative bacteria, a change in the number or character of these channels can reduce ß-lactam uptake		
Glycopeptides Example: vancomycin	Altered target	**Alteration in the molecular structure of cell wall precursor components** decreases binding of vancomycin so that cell wall synthesis is able to continue	Resistance of enterococci to vancomycin
Aminoglyosides	Enzymatic modification	**Modifying enzymes** alter various sites on the	Resistance of many Gram-positive and Gram

Examples: gentamicin, tobramycin, amikacin, netilmicin, streptomycin, kanamycin		aminoglycoside molecule so that the ability of this drug to bind the ribosome and halt protein synthesis is greatly diminished or lost entirely.	negative bacteria to aminoglycosides
Decreased uptake	**Change in number or character of porin channels** (through which aminoglycosides cross the outer membrane to reach the ribosomes of gram-negative bacteria) so that aminoglycoside uptake is diminished.	Resistance of variety of Gram-negative bacteria to aminoglycosides	
Altered target	**Modification of ribosomal proteins or of 16s rRNA.** This reduces the ability of aminoglycoside to successfully bind and inhibit protein synthesis	Resistance of *Mycobacterium* spp to streptomycin	
Quinolones Examples: ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin	Decreased uptake	Alterations in the outer membrane diminishes uptake of drug and/or activation of an "efflux" pump that removes quinolones before intracellular concentration is sufficient for inhibiting DNA metabolism.	Resistance of Gram negative and staphylococci (efflux mechanism only) to various quinolones
Altered target	Changes in DNA gyrase subunits decrease the ability of quinolones to bind this enzyme and interfere with DNA processes	Gram-negative and Gram-positive resistance to various quinolones	

Acquired Resistance

Acquired resistance is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. This can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes, or from a combination of these two mechanisms. Successful gene change and/or exchange may involve mutation or horizontal gene transfer by transformation, transduction, or conjugation.

Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of a bacterial species and require laboratory methods for detection. These same methods are used for monitoring rates of acquired resistance as a means of combating the emergence and spread of acquired resistance traits in pathogenic and nonpathogenic bacterial species.

Mechanism of acquired resistance via gene change or exchange

Antibiotics exert selective pressure on bacterial populations by killing susceptible bacteria, allowing strains with resistance to an antibiotic to survive and multiply. These traits are vertically passed on to subsequently reproduced cells and become sources of resistance. Because resistance traits are not necessarily eliminated or reversed, resistance to a variety of antibiotics may be accumulated over time. This can lead to strains with multiple drug resistance, which are more difficult to eliminate due to limited effective treatment options.

In this section, we'll be discussing acquired resistance as it pertains to:

- Mutations
- Horizontal Gene Transfer
- Detecting Antimicrobial Resistance
  Lab Approaches and Strategies
- Test Methods in Detecting Antimicrobial Resistance
- Examples of Antibiotic Sensitivity Testing Methods

Mutations

A mutation is a spontaneous change in the DNA sequence that may lead to a change in the trait for which it's coded. Any change in a single base pair may lead to a corresponding change in one or more of the corresponding amino acids, which can then change the enzyme or cell structure and consequently affect the affinity or effectiveness activity of related antimicrobials.

In prokaryotic genomes, mutations frequently occur due to base changes caused by exogenous agents, DNA polymerase errors, deletions, insertions, and duplications (Gillespie, 2002).

Horizontal Gene Transfer

Horizontal gene transfer, or the process of swapping genetic material between neighboring bacteria, is another means by which resistance can be acquired. Many of the antibiotic resistance genes are carried on plasmids, transposons, or integrons that act as vectors to transfer genes to other similar bacterial species. Horizontal gene transfer may occur via three main mechanisms: transformation, transduction, or conjugation.

Mechanisms of Gene Exchange: Conjugation

Transformation involves the process in which bacteria uptake short fragments of DNA. Transduction involves transfer of DNA from one bacterium into another via bacteriophages. Conjugation involves transfer of DNA via sex pilus and requires cell-to-cell contact.

Conjugation was first described in 1946 by Lederberg and Tatum, based on studies showing that the intestinal bacteria *E. coli* use a process resembling sex to exchange circular, extrachromosomal elements, now known as plasmids.

Examples of acquired resistance through mutations and horizontal gene transfer, including resistance observed and mechanism involved.

Acquired Resistance Through	Resistance Observed	Mechanism Involved
Mutations	*Mycobacterium tuberculosis* resistance to rifamycins	Point mutations in the rifampin-binding region of *rpoB*
Resistance of many clinical isolates to fluoroquinolones	Predominantly mutation of the quinolone-resistance-determining-region (QRDR) of GyrA and ParC/GrlA	
E. coli, Hemophilius	Mutations in the chromosomal	

influenzae resistance to trimethoprim	gene specifying dihydrofolate reductase	
Horizontal gene transfer	Staphylococcus aureus resistance to methicillin (MRSA)	Via acquisition of mecA genes which is on a mobile genetic element called "staphylococcal cassette chromosome" (SCCmec) which codes for penicillin binding proteins (PBPs) that are not sensitive to ß-lactam inhibition
Resistance of many pathogenic bacteria against sulfonamides	Mediated by the horizontal transfer of foreign folP genes or parts of it	
Enterococcus faecium and *E. faecalis* resistance to vancomycin	Via acquisition of one of two related gene clusters VanA and VanB, which code for enzymes that modify peptidoglycan precursor, reducing affinity to vancomycin	

Biological Versus Clinical Resistance

Biological resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is said to have achieved clinical resistance. It is important to note that biological resistance and clinical resistance do not necessarily coincide. From a clinical laboratory and public health perspective, biologic development of antimicrobial resistance is an ongoing process, while clinical resistance is dependent on current laboratory methods and established cutoffs.

Molecular Detection of AMR and Virulence Genes in Bacteria

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Lab Approaches and Strategies

Some points to consider when deciding whether or not to conduct antimicrobial susceptibility testing should include:

- Clinical relevance of the isolate
- Purity of the isolate
- Logical panel of antimicrobial agents to be tested (e.g., do not include antibiotics to which the isolate is known to have intrinsic resistance)
- Availability of test methodology, resources, and trained personnel
- Standardization of testing
- Valid interpretation of results
- Cost efficiency
- Effective means to communicate results and interpretation to end-users
- Public health impact

Most often, interpretation is reduced to whether the isolate is classified as susceptible, intermediately susceptible, or resistant to a particular antibiotic. It should, however, be remembered that these *in vitro* procedures are only approximations of *in vivo* conditions, which can be very different depending on the nature of the drug, the nature of the host, and the conditions surrounding the interaction between the antibiotic and the target pathogen. One critical aspect is following standardized, quality-controlled procedures that can generate reproducible results.

Aspects of quality control include:

- Standardized bacterial inoculum size and physiological state
- Culture medium (nutrient composition, pH, cation concentration, blood and serum supplements and thymidine content)
- Incubation conditions (atmosphere, temperature, duration)
- Concentration of antimicrobials for testing
- Routine testing of prescribed quality control strains

Because of the required culture time, antimicrobial susceptibility testing by the above methods may take several days, which is not ideal, particularly in critical clinical cases demanding urgency. Often practitioners may use locally established antibiograms as a guideline for therapy. An antibiogram is a compiled susceptibility report or table of commonly isolated organisms in a particular hospital, farm, or geographic area, which can serve as a useful guideline in therapy before actual culture and susceptibility data becomes available for reference. In some cases, specific resistance gene detection by PCR or direct enzyme testing can provide earlier susceptibility information (Example: mecA detection in methicillin-resistant staphylococci).

Testing Methods for Detection of Antimicrobial Resistance

There are several antimicrobial susceptibility testing methods available today and each one has its respective advantages and disadvantages. They all have the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotic treatment. These data may be used as guidelines for treatment, or as indicators of emergence and spread of resistance on a population level based on passive or active surveillance. Some examples of antibiotic susceptibility testing methods are:

- Dilution (broth and agar)
- Disk-diffusion
- Gradient diffusion (E-test)
- Automated systems (Vitek)
- Mechanism-specific tests (such as ß-lactamase detection test and chromogenic cephalosporin test)
- Resistance gene detection (PCR and DNA hybridization)

Selection of the appropriate method will depend on the intended degree of accuracy, convenience, urgency, availability of resources, availability of technical expertise, and cost. Interpretation should be based on veterinary standards whenever possible rather than on human medical standards due to applicability. Among these available tests, the two most commonly used methods in veterinary laboratories are the agar disk-diffusion method and the broth microdilution method.

Examples of Antibiotic Sensitivity Testing Methods

1. Dilution (broth and agar)

The broth dilution method involves placing the isolate into several separate broth solutions containing an antimicrobial agent in a series of varying concentrations. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited, as evidenced by the absence of visible bacterial growth, is recorded as the minimal inhibitory concentration (MIC). The test is only valid if the positive control shows growth and the negative control shows no growth. A procedure similar to broth dilution is agar dilution. The agar dilution method follows the same principle of establishing the lowest concentration of a serially diluted antibiotic for which bacterial growth is still inhibited.

2. Disk-diffusion

Because of convenience, efficiency, and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinics.

A growth medium—usually Mueller-Hinton agar—is first evenly seeded throughout the plate with the isolate of interest that has been diluted to a standard concentration (approximately $1–2 \times 10^8$ colony forming units per ml). Commercially prepared disks, each of which is preimpregnated with a standard concentration of a particular antibiotic, are evenly dispensed and lightly pressed onto the agar surface. The antibiotic being tested diffuses outward from the diffusion disk and creates an antibiotic concentration gradient in the agar. The highest concentration of antibiotic is found closest to the diffusion disk with decreasing amount of antibiotic present, further and further from the disk.

The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition. This approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. The zone is measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible, or resistant. The

MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate, or resistant.
On this agar plate, a bacterial isolate is tested for resistance to each of twelve different antibiotics. The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic.

3. **Gradient diffusion** (E-test)
The e-test is a commercially available test that uses a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration. This method is a convenient quantitative test of antibiotic resistance. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.

4. **Automated systems**
Several commercial systems provide conveniently prepared and formatted microdilution panels, instrumentation and automated plate readings. These methods are intended to reduce technical errors and lengthy preparation times. Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading, and interpretation. Although these systems are rapid and convenient, one major limitation for most laboratories is the cost associated with the purchase, operation, and maintenance of the machinery.
5. **Mechanism-specific tests**
Resistance may also be established through tests that directly detect the presence of a particular resistance mechanism. For example, ß-lactamase detection can be accomplished using an assay such as the chromogenic cephalosporinase test.
6. **Resistance gene detection** (PCR and DNA hybridization)
Since resistance traits are genetically encoded, we can sometimes test for the specific genes that confer antibiotic resistance. Even though nucleic acid-based detection systems are generally rapid and sensitive, it is important to remember that the presence of a resistance gene does not necessarily equate to treatment failure, as resistance is also dependent on the mode and level of expression of these genes.
Some of the most common molecular techniques used for antimicrobial resistance detection are as follows:

- **Polymerase chain reaction (PCR):** One of the most commonly used molecular techniques for detecting certain DNA sequences. This involves several cycles of sample DNA denaturation, annealing of specific primers to the target sequence, if present, and extension of the DNA sequence as facilitated by a thermostable polymerase. This leads to replication of a duplicate DNA sequence, which is visibly detectable by gel electrophoresis via a DNA-intercalating chemical that fluoresces under UV light.
- **DNA hybridization:** DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine, or uracil for RNA). To take advantage of this, a labeled probe with a known specific sequence can pair up with opened or denatured DNA from the test sample, as long as their sequences complement each other. If hybridization occurs, the probe labels the DNA hybrid with a detectable radioactive isotope, antigenic substrate, enzyme, or chemiluminescent compound. If no target sequence is present or the isolate does not have the specific gene of interest, no probe attachment will occur, and therefore no signals will be detected.

Culturing and antimicrobial sensitivity testing (AST) using disk diffusion method

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Objectives:

To establish a standard and uniform procedure for antibiotic Sensitivity test by Disc Diffusion Method

Requirements:

- Specimen: isolated Bacteria
- Muller Hilton Agar (MH Agar)
- Sterile inoculating loop
- Powder free hand gloves
- Mask, apron, mask, biohazard bag
- 0.5 McFarland standard
- Sterile normal saline
- Sterile cotton swab sticks
- Antibiotic discs

Procedure

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.
2. If the surface of the agar has visible liquid present, set the plate inverted, agar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30minutes).
3. Appropriately label each MH agar plate for each organism to be tested.
4. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
5. Suspend the organism in 2ml of sterile saline.
6. Vortex the saline tube to create a smooth suspension.
7. Adjust the turbidity of this suspension to a 0.5McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
8. Use this suspension within 15minutes of preparation.
9. Dip a sterile swab into the inoculums tube
10. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.
11. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculums.
12. Rim the plate with the swab to pick up any excess liquid

13. Discard the swab into an appropriate container.
14. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.
15. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multi disk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps.)
16. To use a multi disk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid.
17. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate.
18. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petridish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid.
19. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.
20. To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.
21. Using the forceps carefully remove one disk from the cartridge
22. Partially remove the lid of the petridish. Place the disk on the plate over one of the darkspots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air
23. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. and g. above
24. Once all disks are in place, replace the lid, invert the plates, and place them in a 37°C air incubator for 16 to 18 hours.

Culturing and antimicrobial sensitivity testing (AST) using disk diffusion method

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Objectives:

To establish a standard and uniform procedure for antibiotic Sensitivity test by Disc Diffusion Method

Requirements:

- Specimen: isolated Bacteria
- Muller Hilton Agar (MH Agar)
- Sterile inoculating loop
- Powder free hand gloves
- Mask, apron, mask, biohazard bag
- 0.5 McFarland standard
- Sterile normal saline
- Sterile cotton swab sticks
- Antibiotic discs

Procedure

25. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.
26. If the surface of the agar has visible liquid present, set the plate inverted, agar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30minutes).
27. Appropriately label each MH agar plate for each organism to be tested.
28. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
29. Suspend the organism in 2ml of sterile saline.
30. Vortex the saline tube to create a smooth suspension.
31. Adjust the turbidity of this suspension to a 0.5McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
32. Use this suspension within 15minutes of preparation.
33. Dip a sterile swab into the inoculums tube
34. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.
35. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculums.
36. Rim the plate with the swab to pick up any excess liquid

37. Discard the swab into an appropriate container.
38. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.
39. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multi disk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps.)
40. To use a multi disk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid.
41. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate.
42. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petridish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid.
43. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.
44. To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.
45. Using the forceps carefully remove one disk from the cartridge
46. Partially remove the lid of the petridish. Place the disk on the plate over one of the darkspots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air
47. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. and g. above
48. Once all disks are in place, replace the lid, invert the plates, and place them in a 37°C air incubator for 16 to 18 hours.

AWaRe categorization and therapeutic application in farming practices

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WHO AWaRe Classification Database was developed on the recommendation of the WHO Expert Committee on Selection and Use of Essential Medicines. It includes details of 180 antibiotics classified as Access, Watch or Reserve, their pharmacological classes, Anatomical Therapeutic Chemical (ATC) codes and WHO Essential Medicines List status. It is intended to be used as an interactive tool for countries to better support antibiotic monitoring and optimal use.

The database also lists those antibiotics whose use is not recommended by WHO namely fixed-dose combinations of multiple broad-spectrum antibiotics that lack evidence-based indications for use or recommendations in high-quality international guidelines. Use of these antibiotics should be actively discouraged through several measures.

AWaRe classifies antibiotics into three stewardship groups: Access, Watch and Reserve, to emphasize the importance of their optimal uses and potential for antimicrobial resistance.

Access Group Antibiotics

This group includes antibiotics that have activity against a wide range of commonly encountered susceptible pathogens while also showing lower resistance potential than antibiotics in the other groups. The Access group includes 48 antibiotics,19 of which are included individually on the WHO Model List of Essential Medicines as first- or second -choice empiric treatment options for specified infectious syndromes.

Watch Group Antibiotics

This group includes antibiotics that have higher resistance potential and includes most of the highest priority agents among the Critically Important Antimicrobials for Human Medicine and/or antibiotics that are at relatively high risk of selection of bacterial resistance. Antibiotics in Watch group should be prioritized as key targets of stewardship programs and monitoring. The Watch group includes 110 antibiotics, 11 of which are included individually on the WHO Model List of Essential Medicines as first- or second -choice empiric treatment options for specified infectious syndromes.

Reserve Group Antibiotics

This group includes antibiotics and antibiotic classes that should be reserved for treatment of confirmed or suspected infections due to multi-drug-resistant organisms. Antibiotics in **Reserve** group should be treated as "last resort" options, which should be accessible, but their use should be tailored to highly specific patients and settings, when all alternatives have failed or are not suitable. These medicines could be protected and prioritized as key targets of national and international stewardship programs involving monitoring and utilization reporting, to preserve their effectiveness. 22 antibiotics have been classified as Reserve group. Seven Reserve group antibiotics are listed individually on the WHO Model List of Essential Medicines.

Why WHO developed AWaRe

Improving use of antibiotics through antibiotic stewardship is one of the key interventions necessary to curb the further emergence and spread of antimicrobial resistance (AMR). It is also important for ensuring appropriate treatment. For that reason, WHO in 2017 introduced the Access, Watch, Reserve ("AWaRe") classification of antibiotics in its Essential Medicines List. The classification is a tool for antibiotic stewardship at local, national and global levels with the aim of reducing antimicrobial resistance.

Improving use of antibiotics for universal health coverage

Access to quality, safe and affordable medicines and health products is a key contribution to Universal Health Coverage (UHC) and the triple billion target set by WHO's 13th General Program of Work (GPW). Within the 13th GPW is an indicator, based on AWaRe, which specifies a country-level target of at least 60% of antibiotic consumption being from medicines in the Access Group. This indicator was included to monitor access to essential medicines and progress towards UHC.

Measuring antibiotic consumption, e.g. by quantifying the use of antibiotics in each of the AWaRe categories (relative or absolute) allows some inference about the overall quality of antibiotic use in a given country. Countries should first compare national / regional antibiotic use using absolute consumption data, and then relative use according to AWaRe categories. The combination of both absolute and relative consumption by category allows simple benchmarking (e.g. an overuse of Watch antibiotics can become immediately apparent and a reduction in Watch antibiotics can be identified as a target for antibiotic stewardship interventions) and assessment of trends over time (to evaluate the impact of interventions).

The AWaRe Classification Database can assist policy makers in adopting AWaRe as a tool to support setting performance targets and guide optimal use of antibiotics in countries. This tool can also be adopted by clinicians to monitor antibiotic use and implement surveillance activities at local level, and inform the development of antibiotic treatment guidelines.

Access group antibiotics

This group includes antibiotics that have activity against a wide range of commonly encountered susceptible pathogens while also showing lower resistance potential than antibiotics in the other groups. Selected Access group antibiotics are recommended as essential first or second choice empiric treatment options for infectious syndromes reviewed by the EML Expert Committee and are listed as individual medicines on the Model Lists of Essential Medicines to improve access and promote appropriate use.

Antibiotic	Class	ATC Code	Category	Listed on EML 2019
Amikacin	Aminoglycosides	J01GB06	Access	Yes
Amoxicillin	Penicillins	J01CA04	Access	Yes
Amoxicillin/clavulanic Acid	Beta lactam - beta lactamase inhibitor	J01CR02	Access	Yes
Ampicillin	Penicillins	J01CA01	Access	Yes
Ampicillin/sulbactam	Beta lactam - beta lactamase inhibitor	J01CR01	Access	No
Bacampicillin	Penicillins	J01CA06	Access	No
Benzathine benzylpenicillin	Penicillins	J01CE08	Access	Yes
Benzylpenicillin	Penicillins	J01CE01	Access	Yes

Cefacetrile	First-generation cephalosporins	J01DB10	Access	No
Cefadroxil	First-generation cephalosporins	J01DB05	Access	No
Cefalexin	First-generation cephalosporins	J01DB01	Access	Yes
Cefalotin	First-generation cephalosporins	J01DB03	Access	No
Cefapirin	First-generation cephalosporins	J01DB08	Access	No
Cefatrizine	First-generation cephalosporins	J01DB07	Access	No
Cefazedone	First-generation cephalosporins	J01DB06	Access	No
Cefazolin	First-generation cephalosporins	J01DB04	Access	Yes
Cefradine	First-generation cephalosporins	J01DB09	Access	No
Cefroxadine	First-generation cephalosporins	J01DB11	Access	No
Ceftezole	First-generation cephalosporins	J01DB12	Access	No
Chloramphenicol	Amphenicols	J01BA01	Access	Yes
Clindamycin	Lincosamides	J01FF01	Access	Yes
Clometocillin	Penicillins	J01CE07	Access	No
Cloxacillin	Penicillins	J01CF02	Access	Yes
Dicloxacillin	Penicillins	J01CF01	Access	No
Doxycycline	Tetracyclines	J01AA02	Access	Yes
Flucloxacillin	Penicillins	J01CF05	Access	No
Gentamicin	Aminoglycosides	J01GB03	Access	Yes
Mecillinam	Penicillins	J01CA11	Access	No
Metronidazole (IV)	Imidazoles	J01XD01	Access	Yes
Metronidazole (oral)	Imidazoles	P01AB01	Access	Yes
Nafcillin	Penicillins	J01CF06	Access	No
Nitrofurantoin	Nitrofurantoin	J01XE01	Access	Yes
Oxacillin	Penicillins	J01CF04	Access	No
Penamecillin	Penicillins	J01CE06	Access	No
Phenoxymethylpenicillin	Penicillins	J01CE02	Access	Yes
Pivampicillin	Penicillins	J01CA02	Access	No
Pivmecillinam	Penicillins	J01CA08	Access	No
Procaine benzylpenicillin	Penicillins	J01CE09	Access	Yes
Spectinomycin	Aminocyclitols	J01XX04	Access	Yes
Sulfadiazine/trimethoprim	Trimethoprim - sulfonamide combinations	J01EE02	Access	No
Sulfamethizole/trimethoprim	Trimethoprim - sulfonamide combinations	J01EB02	Access	No
Sulfamethoxazole/trimethoprim	Trimethoprim - sulfonamide combinations	J01EE01	Access	Yes
Sulfametrole/trimethoprim	Trimethoprim - sulfonamide combinations	J01EE03	Access	No
Sulfamoxole/trimethoprim	Trimethoprim - sulfonamide combinations	J01EE04	Access	No
Sultamicillin	Beta lactam - beta lactamase inhibitor	J01CR04	Access	No
Tetracycline	Tetracyclines	J01AA07	Access	No
Thiamphenicol	Amphenicols	J01BA02	Access	No

Watch group antibiotics

This group includes antibiotic classes that have higher resistance potential and includes most of the highest priority agents among the Critically Important Antimicrobials for Human Medicine and/or antibiotics that are at relatively high risk of selection of bacterial resistance. These medicines should be prioritized as key targets of stewardship programs and monitoring. Selected Watch group antibiotics are recommended as essential first or second choice empiric treatment options for a limited number of specific infectious syndromes and are listed as individual medicines on the WHO Model Lists of

Antibiotic	**Class**	**ATC code**	**Category**	**Listed on EML 2019**
Arbekacin	Aminoglycosides	J01GB12	Watch	No
Azithromycin	Macrolides	J01FA10	Watch	Yes
Azlocillin	Penicillins	J01CA09	Watch	No
Biapenem	Carbapenems	J01DH05	Watch	No
Carbenicillin	Carboxypenicillins	J01CA03	Watch	No
Cefaclor	Second-generation cephalosporins	J01DC04	Watch	No
Cefamandole	Second-generation cephalosporins	J01DC03	Watch	No
Cefbuperazone	Second-generation cephalosporins	J01DC13	Watch	No
Cefcapene pivoxil	Third-generation cephalosporins	J01DD17	Watch	No
Cefdinir	Third-generation cephalosporins	J01DD15	Watch	No
Cefditoren pivoxil	Third-generation cephalosporins	J01DD16	Watch	No
Cefepime	Fourth-generation cephalosporins	J01DE01	Watch	No
Cefetamet pivoxil	Third-generation cephalosporins	J01DD10	Watch	No
Cefixime	Third-generation cephalosporins	J01DD08	Watch	Yes
Cefmenoxime	Third-generation cephalosporins	J01DD05	Watch	No
Cefmetazole	Second-generation cephalosporins	J01DC09	Watch	No
Cefminox	Second-generation cephalosporins	J01DC12	Watch	No
Cefodizime	Third-generation cephalosporins	J01DD09	Watch	No
Cefonicid	Second-generation cephalosporins	J01DC06	Watch	No
Cefoperazone	Third-generation cephalosporins	J01DD12	Watch	No
Ceforanide	Second-generation cephalosporins	J01DC11	Watch	No
Cefoselis	Fourth-generation cephalosporins	to be assigned	Watch	No
Cefotaxime	Third-generation cephalosporins	J01DD01	Watch	Yes
Cefotetan	Second-generation cephalosporins	J01DC05	Watch	No
Cefotiam	Second-generation cephalosporins	J01DC07	Watch	No
Cefotiam hexetil	Second-generation cephalosporins	J01DC07	Watch	No
Cefoxitin	Second-generation cephalosporins	J01DC01	Watch	No
Cefozopran	Fourth-generation cephalosporins	J01DE03	Watch	No
Cefpiramide	Third-generation cephalosporins	J01DD11	Watch	No
Cefpirome	Fourth-generation cephalosporins	J01DE02	Watch	No
Cefpodoxime proxetil	Third-generation cephalosporins	J01DD13	Watch	No
Cefprozil	Second-generation cephalosporins	J01DC10	Watch	No
Ceftazidime	Third-generation cephalosporins	J01DD02	Watch	Yes
Cefteram pivoxil	Third-generation cephalosporins	J01DD18	Watch	No
Ceftibuten	Third-generation cephalosporins	J01DD14	Watch	No
Ceftizoxime	Third-generation cephalosporins	J01DD07	Watch	No
Ceftriaxone	Third-generation cephalosporins	J01DD04	Watch	Yes
Cefuroxime	Second-generation cephalosporins	J01DC02	Watch	Yes
Chlortetracycline	Tetracyclines	J01AA03	Watch	No
Ciprofloxacin	Fluoroquinolones	J01MA02	Watch	Yes
Clarithromycin	Macrolides	J01FA09	Watch	Yes
Clofoctol	Phenol derivatives	J01XX03	Watch	No
Delafloxacin	Fluoroquinolones	J01MA23	Watch	No

Dibekacin	Aminoglycosides	J01GB09	Watch	No
Dirithromycin	Macrolides	J01FA13	Watch	No
Doripenem	Carbapenems	J01DH04	Watch	No
Enoxacin	Fluoroquinolones	J01MA04	Watch	No
Ertapenem	Carbapenems	J01DH03	Watch	No
Erythromycin	Macrolides	J01FA01	Watch	No
Fleroxacin	Fluoroquinolones	J01MA08	Watch	No
Flomoxef	Second-generation cephalosporins	J01DC14	Watch	No
Flumequine	Fluoroquinolones	J01MB07	Watch	No
Fosfomycin (oral)	Phosphonics	J01XX01	Watch	No
Fusidic Acid	Steroid antibacterials	J01XC01	Watch	No
Garenoxacin	Fluoroquinolones	J01MA19	Watch	No
Gatifloxacin	Fluoroquinolones	J01MA16	Watch	No
Gemifloxacin	Fluoroquinolones	J01MA15	Watch	No
Imipenem/cilastatin	Carbapenems	J01DH51	Watch	No
Isepamicin	Aminoglycosides	J01GB11	Watch	No
Josamycin	Macrolides	J01FA07	Watch	No
Kanamycin	Aminoglycosides	J01GB04	Watch	No
Latamoxef	Third-generation cephalosporins	J01DD06	Watch	No
Levofloxacin	Fluoroquinolones	J01MA12	Watch	No
Lincomycin	Macrolides	J01FF02	Watch	No
Lomefloxacin	Fluoroquinolones	J01MA07	Watch	No
Lymecycline	Tetracyclines	J01AA04	Watch	No
Meropenem	Carbapenems	J01DH02	Watch	Yes
Metacycline	Tetracyclines	J01AA05	Watch	No
Mezlocillin	Penicillins	J01CA10	Watch	No
Micronomicin	Aminoglycosides	to be assigned	Watch	No
Midecamycin	Macrolides	J01FA03	Watch	No
Minocycline (oral)	Tetracyclines	J01AA08	Watch	No
Moxifloxacin	Fluoroquinolones	J01MA14	Watch	No
Neomycin	Aminoglycosides	J01GB05	Watch	No
Netilmicin	Aminoglycosides	J01GB07	Watch	No
Norfloxacin	Fluoroquinolones	J01MA06	Watch	No
Ofloxacin	Fluoroquinolones	J01MA01	Watch	No
Oleandomycin	Macrolides	J01FA05	Watch	No
Oxytetracycline	Tetracyclines	J01AA06	Watch	No
Panipenem	Carbapenems	to be assigned	Watch	No
Pazufloxacin	Fluoroquinolones	J01MA18	Watch	No
Pefloxacin	Fluoroquinolones	J01MA03	Watch	No
Pheneticillin	Penicillins	J01CE05	Watch	No
Piperacillin	Penicillins	J01CA12	Watch	No
Piperacillin/tazobactam	Beta lactam - beta lactamase inhibitor (anti-pseudomonal)	J01CR05	Watch	Yes
Pristinamycin	Streptogramins	J01FG01	Watch	No
Prulifloxacin	Fluoroquinolones	J01MA17	Watch	No
Ribostamycin	Aminoglycosides	J01GB10	Watch	No
Rifabutin	Rifamycins	J04AB04	Watch	No
Rifampicin	Rifamycins	J04AB02	Watch	No
Rifamycin	Rifamycins	J04AB03	Watch	No
Rifaximin	Rifamycins	A07AA11	Watch	No
Roxithromycin	Macrolides	J01FA06	Watch	No

Rufloxacin	Fluoroquinolones	J01MA10	Watch	No
Sisomicin	Aminoglycosides	J01GB08	Watch	No
Sitafloxacin	Fluoroquinolones	J01MA21	Watch	No
Sparfloxacin	Fluoroquinolones	J01MA09	Watch	No
Spiramycin	Macrolides	J01FA02	Watch	No
Spiramycin/metronidazole	Combination of antibiotics	J01RA04	Watch	No
Streptomycin	Aminoglycosides	J01GA01	Watch	No
Sulbenicillin	Penicillins	J01CA16	Watch	No
Tebipenem	Carbapenems	J01DH06	Watch	No
Teicoplanin	Glycopeptides	J01XA02	Watch	No
Telithromycin	Macrolides	J01FA15	Watch	No
Temocillin	Carboxypenicillins	J01CA17	Watch	No
Ticarcillin	Carboxypenicillins	J01CA13	Watch	No
Tobramycin	Aminoglycosides	J01GB01	Watch	No
Tosufloxacin	Fluoroquinolones	J01MA22	Watch	No
Vancomycin (IV)	Glycopeptides	J01XA01	Watch	Yes
Vancomycin (oral)	Glycopeptides	A07AA09	Watch	Yes

Reserve group Antibiotics

This group includes antibiotics and antibiotic classes that should be reserved for treatment of confirmed or suspected infections due to multi-drug-resistant organisms. Reserve group antibiotics should be treated as "last resort" options. Selected Reserve group antibiotics are listed as individual medicines on the WHO Model Lists of Essential Medicines when they have a favourable risk-benefit profile and proven activity against "Critical Priority" or "High Priority" pathogens identified by the WHO Priority Pathogens List1, notably carbapenem resistant Enterobacteriaceae. These antibiotics should be accessible, but their use should be tailored to highly specific patients and settings, when all alternatives have failed or are not suitable.

These medicines could be protected and prioritized as key targets of national and international stewardship programs involving monitoring and utilization reporting, to preserve their effectiveness.

Antibiotic	Class	ATC code	Category	Listed on EML 2019
Aztreonam	Monobactams	J01DF01	Reserve	No
Ceftaroline fosamil	Fifth-generation cephalosporins	J01DI02	Reserve	No
Ceftazidime-avibactam	Third-generation cephalosporins	J01DD52	Reserve	Yes
Ceftobiprole medocaril	Fifth-generation cephalosporins	J01DI01	Reserve	No
Ceftolozane-tazobactam	Fifth-generation cephalosporins	J01DI54	Reserve	No
Colistin	Polymyxins	J01XB01	Reserve	Yes
Dalbavancin	Glycopeptides	J01XA04	Reserve	No
Dalfopristin-quinupristin	Streptogramins	J01FG02	Reserve	No
Daptomycin	Lipopeptides	J01XX09	Reserve	No
Eravacycline	Tetracyclines	J01AA13	Reserve	No
Faropenem	Penems	J01DI03	Reserve	No
Fosfomycin (IV)	Phosphonics	J01XX01	Reserve	Yes
Linezolid	Oxazolidinones	J01XX08	Reserve	Yes
Meropenem-vaborbactam	Carbapenems	J01DH52	Reserve	Yes
Minocycline (IV)	Tetracyclines	J01AA08	Reserve	No
Omadacycline	Tetracyclines	to be assigned	Reserve	No

Oritavancin	Glycopeptides	J01XA05	Reserve	No
Plazomicin	Aminoglycosides	to be assigned	Reserve	Yes
Polymyxin B	Polymyxins	J01XB02	Reserve	Yes
Tedizolid	Oxazolidinones	J01XX11	Reserve	No
Telavancin	Glycopeptides	J01XA03	Reserve	No
Tigecycline	Glycylcyclines	J01AA12	Reserve	No

Source:https://www.who.int/medicines/news/2019/WHO_releases2019AWaRe_classification_antibiotics/en/

Rational basis of antibiotic therapy: What do we need to know?

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Introduction

Antimicrobial agents are widely used in food-animal production for disease prevention and treatment in animals, to control disease spread, to prevent contamination of the food chain via horizontal and vertical transfer of antimicrobial resistance, and to increase productivity. However, their overuse in humans and animals leads to the emergence of antimicrobial resistance, a general term that encompasses decreased and poor efficacy of antimicrobials to treat disease. Recent projections revealed that by 2050 global livestock production would fall by 3–8% each year, as result of which annual global gross domestic product will decline by 1.1–3.8%. Due to rise in disease incidence, low income countries will be affected more severely, with a predicted rise of extremely poor people from 6.2 to 18.7 million by 2030. Rise in frequency of treatment failures have been reported in treatments with infections caused by multi-, extensive-, and pan-drug resistant bacteria. Once antimicrobials (antibiotics) normally used against bacteria lose their efficacy to treat disease, it becomes necessary to use others, so-called "reserve" or "last resort" options that are often more expensive and/or toxic preparations. In several developing countries, antimicrobial consumption is expected to rise considerably due to increase in meat consumption, from Indonesia (202%) and Nigeria (163%) to Vietnam (157%) and Peru (160%), by 2030. Organization for Economic Cooperation and Development (OECD) estimated that antimicrobials used in food-animal production will increase by 67% globally, i.e., from 63,000 in 2010 to 106,000 tonnes by 2030 an increase of 67%. Thus, overuse of antimicrobials in the food-animal production sector gives rise to antimicrobial resistance in animal pathogens, leading to increase in therapy failure with a negative effect on animal health and welfare. The immediate cost of withdrawal of non-therapeutic antimicrobials at animal level, without adjustments in production processes, may decrease the feed efficiency, growth, survival, and number of animals born.

Genesis of antibiotic resistance and its spread across geographical boundaries

The World Health Organization (WHO) has emphasized the need for an integrated and coordinated global effort to control antibiotic resistance. In 2001, the World Health Organization Global Strategy for Containment of Antimicrobial Resistance has provided a framework of interventions to slow the emergence and reduce the spread of antimicrobial-resistant microorganisms across geographical boundaries and species. For understanding the genesis and spread of antimicrobial resistance across species and increase in resistance burden, the following sub-heads points the focus.

Development and spread of antimicrobial resistance

The development of resistance in microbes arises in two ways: (i) intrinsic resistance, which occurs when the microbial species is able to innately resist the activity of an antimicrobial agent (by preventing either the entry or binding of the antimicrobial agent); and (ii) acquired resistance, in which once-susceptible microbial species mutate or obtain genes from other microbe, to acquire resistance. Antimicrobial resistance cannot be prevented because every time antimicrobials are used, the effective lifespan of that antimicrobial drug is shortened. In general, few categories of pathogen are responsible for a large portion of resistant infections in humans. One of them is New Delhi metallo-β-lactamase-1 (NDM-1) gene which confers broad resistance to most antibiotics, including carbapenems, and can be transferred to a wide variety of bacterial species. Another is resistant Gram-negative bacteria which carry extended-spectrum beta-lactamase enzymes (ESBLs), responsible for high levels of resistance to some of the most commonly prescribed antibiotics.

Antibiotic use in livestock and resistance

Livestock contributes for over a fourth of India's total agricultural output, and 4% of the gross domestic product (GDP). India is one of the top consumers of antibiotics in agriculture worldwide, which accounts for 3% of global consumption, which is estimated to double in 2030. Resistant microbes and residues have been detected in living bovines, chickens, honey, pigs, horses, donkeys and mules, and fish and shellfish. In cattle, resistant strains of coagulase-negative staphylococci, Escherichia coli, and Staphylococcus aureus, extended-spectrum beta-lactamase (ESBL) and New Delhi metallo-beta-lactamase (NDM-1) genes, have been reported. E. coli, S. aureus, enterococci, Pasteurella multocida, Campylobacter jejuni, and Salmonella, including ESBL-producing strains have been found in poultry. The chances for antimicrobial resistant microbes in the race for survival are in direct proportion to the volume of antibiotics used, this makes it more critical to examine current habits and encourage rational and conservative use of antimicrobials. Due to antimicrobial resistance, easy-to-treat infections are becoming difficult or impossible to cure, with an unambiguous global increase in both livestock mortality and treatment costs.

Use of antimicrobials for different purposes

Therapeutic use of antimicrobials is meant for treatment of diseases. However, if a few animals are found to be sick, often the whole flock or herd will be treated (known as meta-phylaxis or sub-therapeutic) to prevent the disease spreading. Thus, there is not always a clear distinction between treatment and prevention. In this condition, treatment usually occurs at high doses for a relatively short period of time. Prophylactic treatment is done for prevention of disease. The treatment of animals is done with low, sub-therapeutic doses of antibiotics via feed or drinking water, even in the absence of any signs of disease but when there is risk of infection. Treatment can be given over a period of several weeks, and sometimes longer. Antibiotics are also used for growth promotion. Here, very low sub-therapeutic doses of antibiotics are given to animals (particularly intensively kept pigs and poultry) in their feed, in order to increase their growth-rate and productivity. Treatment is continuous and it lasts for a long time.

Use of antimicrobials in food animals

A study revealed that annually, 45, 148, and 172 mg/kg antimicrobials are consumed by cattle, chicken, and pigs, respectively, to produce each kilogram of their meat. The global consumption of antimicrobials estimated to increase by 67% from 2010 to 2030, i.e., from $63,151 \pm 1560$ to $105,596 \pm 3605$ tons. At present time, more antibiotics are used worldwide in poultry, swine, and cattle production than in the entire human population. In aquaculture, antibiotics are used for therapeutic and prophylactic purposes often in high concentrations because bacteria travel in water easily, here antibiotics are not used for growth promotion. In the BRICS countries (Brazil, Russia, India, China and South Africa), antibiotic use in animals is expected to double by 2030. Use of antibiotics, particularly in chickens, is expected to triple in India by 2030.

Use of antibiotics in dairying

The antibiotic residues are at alarming rate in dairying in India. A study by Ramakrishna and Singh in 1985 revealed that streptomycin was found in 6% milk samples in Haryana. One decade later, in Hyderabad, Secunderabad, and surrounding villages dairy farmers were surveyed on antibiotic use practices. Among 38 dairy farmers, about 50% of them used oxytetracycline to treat diseases such as mastitis and fever; the survey revealed that oxytetracycline residues were found in 9% samples from markets and 73% individual animals, while no residues were found in government dairy samples. A survey conducted by the National Dairy Research Institute near Bangalore in 2000 revealed that tetracyclines, gentamycin, ampicillin, amoxicillin, cloxacillin, and penicillin were commonly used to treat dairy animals and mastitis was treated with beta-lactam class of antibiotics. The prevalence of antibiotic residues in milk samples has been found to be higher in silo and tanker samples as compared to market and commercial pasteurized milk samples. These findings prove that that antibiotic are used in dairy animals in these regions, though details of the frequency, duration, and reasons for use and overuse are not well recognized.

Use of antibiotics in poultry

The level of resistance in Indian poultry is reported to be high for many antibiotics. A recent study conducted by members of the Global Antibiotic Resistance Partnership reported significant differences in the resistance pattern of broiler farms of Punjab with level of antibiotics used in normal poultry production. Results revealed that antibiotic use in broiler farms were likely to be more than 20 times to harbor-resistant E. coli, and prevalence of multi-drug resistance was much higher which was found 94% in broiler farms. In meat shops of Bikaner (Rajasthan), 96% of chicken samples contained S. aureus ($n = 48$), which were sensitive to ciprofloxacin, doxycycline, and gentamycin, and all were resistant to ampicillin, cloxacillin, and tetracycline.

Transfer of antimicrobial resistance from livestock to humans

Farm workers and slaughterers are at high risk of exposure to resistant antimicrobials due to direct contact with infected animals. Handling pigs and poultry while working in a farm environment puts farm workers at risk of picking up resistant bacteria from the animals' bodies or their feces. A study in the Netherlands in 2001–2002 revealed the same genetic patterns of resistance in E. coli samples from turkeys and broiler chickens, their farmers and slaughterers. Consumption of food contaminated with resistant bacteria such as Salmonella, Campylobacter, and E. coli can increase the resistant bacteria in the human beings. Contamination of meat from fecal material getting onto the carcass during the slaughter and evisceration process, during the removal of animal gut, can contaminate other foods in domestic or restaurant/catering kitchens. The European Food Safety Authority (EFSA) revealed in 2010 that live chickens colonized with Campylobacter are 30 times more likely to contaminate meat as compared to uninfected birds. Resistant bacteria can be transferred in water, soil, and air because animals excrete a significant amount of antibiotics they are administered, which make manure a potential source of both antibiotics and antibiotic-resistant bacteria that can enter soil and groundwater.

Rationale and approaches to limit the spread of antimicrobial resistance

Synchronization of international, national, and local approaches is advised for control and prevention of antimicrobial resistance. Promoting the rational use of antimicrobials, control on over-the-counter availability of antimicrobials, improvement of hygiene, prevention of infection, and control are the major recommended approaches. Thus, proper understanding of mechanism of resistance and accordingly innovation in development of new drugs is the need of the hour. A multidisciplinary, collaborative, regulatory approach is demanded for combating antimicrobial resistance.

Reasons to focus on antibiotics' use in livestock vis-à-vis antibiotic overuse and resistance

For decades, meat industry has fed antibiotics to chickens, pigs, and cattle for their weight gain and disease prevention in the stressful and unhygienic conditions that is prevalent in industrialized animal agriculture production facilities. A strong scientific consensus asserts that this practice fosters antibiotic resistance in bacteria, which is detrimental to human health (HSUS Report). Food animals are quite susceptible to benign or commensal opportunistic microbes, so they are often exposed to antimicrobials, such as the antibiotic, for disease treatment and prevention, sub-therapeutic purpose and prophylactic purpose to promote growth and improve feed efficiency. Many of these antimicrobials used to treat diseases common to both livestock species and humans closely resemble drugs used in this species. On the one hand, these miraculous antimicrobial drugs are pillars of modern medicine to prevent and diagnose dangerous bacterial infections and save lives. On the other hand, the overuse, injudicious use, and misuse of these antimicrobial drugs have spawned the evolution of life-threatening bacteria that is making the current antibiotics reserve useless. Thus, antimicrobial resistance can be defined as the ability of microbes, such as bacteria and fungi, to grow and continue to multiply even in the presence of administered antimicrobial with purpose to kill or limit their growth (NIAID).

Philosophy of judicious use of antimicrobials in line with animal welfare

Animal agriculture by human needs to be predicated on ethical judgments where sub- or non-therapeutic use of antibiotics on food producing animals on ethical judgment scale seems to be objectionable. The problem is that food-animal producers do not realize the ethics in their business because they claim that the conditions and processes in the factory farm are not a matter of ethics but of a societal necessity to fulfil the feed demand of the population. These producers seem to fail to realize the ethical dimensions of their practices, not only for food safety issues for consumers, but also welfare issues for their animals. Any policy judgment including the danger of tolerable resistance or the level of animal abuse tolerable for the sake of the benefits from antibiotics overuse in animal feed is the subject of ethical judgment. Lack of treatment protocols and solidarity of animal from herd and stopping the course of treatment after apparently realizing the disappearance also comes under the purview of animal welfare.

Delineating the use of antibiotics by farmers from farm-to-fork

Low income countries should follow the approaches of World Health Organization, World Organization for Animal Health, and the Food and Agriculture Organization of the United Nations, which recommends to implement national action plans encircling human, food animal, dairy animals, and environmental sectors to formulate appropriate policies, interventions, and activities that could address the prevention and containment of antimicrobial resistance from farm-to-fork. Suitable interventions should be designed, which include the following fields and coverage.

Use of antimicrobials by farmers

In strong sense, there is dependence among piggery farmers on antimicrobials to sustain production, improve farm performance, and maintain health status. Lack of concern about the harmful effects of antimicrobial use on their own and public health was identified among pig producers as a result of a reduction in the curative ability of antimicrobials and the selection of antimicrobial resistance bacteria. A study conducted in Danish system revealed that 82% of antimicrobials sold by pharmacies were direct to individuals on prescription with specifications for use, 78% of antimicrobials sold by pharmacies used for pigs, and 20% for cattle. The overuse of antibiotic has exploited this miracle drug to such an extent that a study in the Netherlands revealed that 79% of farmers used antibiotics routinely and 18% occasionally extended antibiotic treatment. The choice of progressive farmers for adopting prudent use of antibiotics by avoiding routine use of antibiotics was perceived as good practice by fellow farmers. This was followed by repeating the initial label treatments. There are certain specific antibiotics which are used by farmers to treat animals without veterinary consultation (e.g., gentamicin in Ohio). Thus, improving information flow from Veterinarians to farmers may be the most effective means of promoting prudent use of antibiotics on dairy farms. Subjective norms and moral obligations together, in which perceived moral obligations to peers, clients, and the regulatory norm setting sector associated with the feedlot industry increase social pressures to use antibiotics in acutely sick, chronically sick, and high-risk feedlot cattle.

Understanding the antimicrobials overuse in small dairy farms

The incidence of death of farm workers due to treatment failure attributed by antimicrobial resistance is likely much higher in developing countries where more people live in close contact with livestock, where food hygiene is not well practiced. A report by WHO revealed that in developing countries throughout the world, even less than 50% of human are treated according to standard treatment protocol, and prescribing patterns were found substandard regardless of the type of prescriber. Antibiotic-resistant food-borne infections, emergence of new multi-resistant strains of bacteria, and spread of resistant genes are some main areas of risk due to indiscriminate and overuse of antibiotics.

Antimicrobial resistance and intensive animal farming

The basic reason for increase in antimicrobial resistance in food animals is factory farming. In intensive pig and poultry production, animals are reared in confined and overcrowded conditions, usually with no outdoor access, and they are bred and managed for maximum production yield, i.e., to grow faster in size and number or to produce more meat, milk, and eggs. This forces them to compromise their health and their immune responses and encourage infectious disease to develop and spread easily among these livestock. Without the aid of drugs for disease prevention, it would not be possible to keep the animals productive in the intensive conditions, in which they are often kept and managed without proper care by the livestock keeper. Earlier, the policy-makers of 50 years ago permitted antibiotics to be used for non-therapeutic reasons in animal production, often in spite of scientific misgivings, which can be perceived as a serious mistake now. Fifty years later, while the evidence continues to be disputed by some sections of the industry, the actual and potential damage to public health is acknowledged by scientists and policy-makers due to the spread of antibiotic resistance among livestock species and human being (vertical and horizontal transfer of resistance).

Synchronized efforts by stakeholders to reduce the pace of spread of antimicrobial resistance

All the stakeholders including veterinarians, paravets, farmers, and pharmaceutical companies should be made aware about their interacting roles from antimicrobial prescription to use, in which decision made by one stakeholders affects the worth and value of choices and decision for other stakeholder. These aspects can be highlighted under following sub-heads.

One Health approach to combat antimicrobial resistance

One Health approach recognizes that human, animals, and ecosystem health are inextricably linked to each other. It came in to light because many factors have changed the interaction between humans, animals, and environment. Thus, for achieving the mutual optimal health outcomes, it needs the cooperation of human health, livestock, and environment health. Resistance to infectious diseases increase the cost of treatment as well as serious biosecurity concerns due to spread of antibiotic resistance. Thus, the animal production is hampered due to rise in incidence of infections.

Antimicrobial stewardship

The primary focus of an antimicrobial stewardship program is to optimize the use of antimicrobials to achieve the best treatment outcomes, reduce the risk of infections, reduce or stabilize levels of antibiotic resistance, and promote livestock safety. Creating an antimicrobial stewardship program needs baseline information, including institutional use of antimicrobial. This would help to identify recurrent problems with antimicrobial use at the institution and frames the problems that need to be addressed. The antimicrobial stewardship efforts should focus on improving adherence to documentation standards, optimizing the use of antimicrobials, appropriateness of drug dosing, halting treatment of asymptomatic bacteria and microbes, and minimizing the length of surgical prophylaxis.

Recommendations to control antibiotic overuse

Recognizing that antibiotic resistance is a reality crossing the geographical boundaries of the world, in developing countries, the prevalence of resistant microbes will rise over time, which demands urgent action. Vaccinations to prevent various disease falls into this category of recommendation, but their "antibiotic-sparing" effects are often overlooked because these are of secondary importance. Restricting the use of antimicrobials in livestock and poultry for non-therapeutic use, particularly growth promotion, could be beneficial. There is a need to eliminate irrational or inappropriate use, enforce prescription only laws, and eliminate over-the-counter antibiotic purchases, surveillance, distribution of Standard Treatment Guidelines (STGs), antibiotic sensitivity testing, checklists for surgical procedures, educating farmers and other stakeholders about appropriate use of antibiotics, and improving antibiotic supply chain and quality (Global Antibiotic Resistance Partnership (GARP)-India Working Group 2011). For gaining better understanding and subsequent action toward antimicrobial resistance, detailed social science research is needed to gather information on the processes of diagnosis, prescription, use of antimicrobials, the application of treatments besides antimicrobials, and the processes of data generation. Thus, sub-optimal use, potential users, and food chain pinch points could be identified. There is a general scantiness of data on on-farm application and use of antimicrobials. The tools for recording on-farm medicine use, such as paper spread sheets and computerized entries, may be of practical use to farmers in the health management of their animals/birds or to veterinarians in providing an accurate picture of how prescribed medicines are actually used.

Prescription of antimicrobials

Prescription of antibiotics are strongly influenced by the demand of farmers for antibiotics, fear of veterinarians blamed if antimicrobials later prove unnecessary, the expectation of farmers to be prescribed antimicrobials, confidence of veterinarians in diagnosis. Thus, prescription decisions are strongly influenced by multifactorial non-clinical influences such as farmer pressure and cost of drug, etc., to some extent. Also, variations are present in beliefs of veterinarians regarding efficacy of systemic antibiotics for dry-cow therapy results in very different decisions being taken on farm and considerable discrepancies in treatment. Thus, it raises concern of the consistency and appropriateness of antibiotic prescription by them. Antibiotic sensitivity testing should be preferred before prescribing the antibiotics.

Conclusion

The overuse of antimicrobials in livestock is leading to decline in antimicrobial effectiveness against infections in animals and eventually in humans. Use of antimicrobials purely as growth promoters and prophylactic purposes should be avoided and initiatives should be taken to phase out the sub-therapeutic use of antimicrobials. Injudicious use, overuse, and indiscriminate use of antimicrobials should be avoided. The obtaining of antibiotics from over—the counter sales should be checked and antimicrobial conservation practices should be encouraged to control the indiscriminate prescription and use of antimicrobials. Suitable strategies and policies should be formulated in line with the World Organisation for Animal Health and World Health Organization initiatives which call for harmonious efforts among stakeholders of different countries. Suitable extension outreach and continuing programmes should be devised to promote awareness among stakeholders about judicious use of antimicrobials and educate farmers, veterinarians, and consumers on the potential risk of antimicrobial resistance. There is need for surveillance and monitoring to track rates of antimicrobial use in veterinary sector, increase in resistance, and spread of antimicrobial residues in food chain.

Source: https://www.intechopen.com/books/livestock-health-and-farming/antimicrobial-resistance-and-rational-use-of-antimicrobials-in-livestock-developing-countries-perspe

Hands on training on antimicrobial sensitivity interpretation

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Objectives:

To assess the sensitivity of a specific organism to specific antibiotics.

Requirements:

- Powder free hand gloves
- Mask, apron, biohazard bag
- Calibrated scale (mm) or slide calipers
- Marker
- Data recording sheet
- Black background

Procedure:

1. Following incubation, measure the zone sizes to the nearest milli meter using a ruler or caliper; include the diameter of the disk in the measurement
2. When measuring zone diameter, always round upto the next millimeter.
3. All measurements are made with the un aided eye while viewing the back of the petridish. Hold the plate a few inches above a black, nonreflecting surface illuminated with reflected light
4. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading
5. Record the zone size on the recording sheet.
6. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter.
7. Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If it is determined by repeat testing that the phenomenon repeats itself, the organism must be considered resistant to that drug.
8. Using the published CLSI guidelines, determine the susceptibility or resistance of the organism to each drug tested. Note that there are different charts for different organisms. Abbreviated charts specific for the author's suggested organisms and antimicrobial disks to use are provided below.

9. For each drug, indicate on the recording sheet whether the zone size is susceptible(S), intermediate (I), or resistant(R) based on the interpretation chart.

Table: Zone Diameter Interpretive Standards for *E. coli*

Sl. No.	Antimicrobial Agents	Disk content	Zone Diameter Interpretative Criteria (mm)			ESBL positive interpretative criteria	References
			S	I	R		
1	AML (Amoxycillin)	10 µg	≥17	14-16	≤13		CLSI*M100-S25, January 2015: Table 2A
2	AZM (Azithromycin)	15 µg	≥13	-	≤12		CLSI* M100-S25, January 2015: Table 2A
3	C (chloramphenicol)	30 µg	≥18	13-17	≤12		CLSI* M100-S25, January 2015: Table 2A
4	CN (Gentamicin)	10 µg	≥15	13-14	≤12		CLSI* M100-S25, January 2015: Table 2A
5	CT (colistinsulphate)	10 µg	≥14	-	≤11		A. C. Gales, A. O. Reis, and R. N. Jones, "Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines," *Journal of Clinical Microbiology*, vol. 39, no. 1, pp. 183–190, 2001.
6	CTX (Cefotaxime)	30 µg	≥26	23-25	≤22	≤27	CLSI* M100-S25, January 2015: Table 2ACLSI*; Table 3A for ESBLinterpretation
7	CAZ (Ceftazidime)	30 µg	≥21	18-20	≤17	≤22	CLSI* M100-S25, January 2015: Table 2A CLSI*; Table 3A for ESBL interpretation
8	CIP (Ciprofloxacin)	5 µg	≥21	16-20	≤15		CLSI* M100-S25, January 2015: Table 2A
9	F (Nitrofurantoin)	300 µg	≥17	15-16	≤14		CLSI* M100-S25, January 2015: Table 2A
10	FOX (Cefoxitin)	30 µg	≥18	15-17	≤14		CLSI* M100-S25, January 2015: Table 2A
11	IMP (imipenem)	10 µg	≥23	20-22	≤19		CLSI* M100-S25, January 2015: Table 2A
12	MEM (Meropenem)	10 µg	≥23	20-22	≤19		CLSI* M100-S25, January 2015: Table 2A
13	NA (Nalidixic acid)	30 µg	≥19	14-18	≤13		CLSI* M100-S25, January 2015: Table 2A
14	TE (Tetracycline)	30 µg	≥15	12-14	≤11		CLSI* M100-S25, January 2015: Table 2A
15	WS (Trimethoprim)	5 µg	≥16	11-15	≤10		CLSI* M100-S25, January 2015: Table 2A

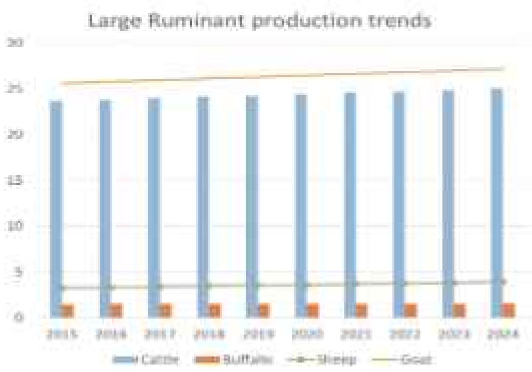
Initiative of Department of livestock Services Against AMR

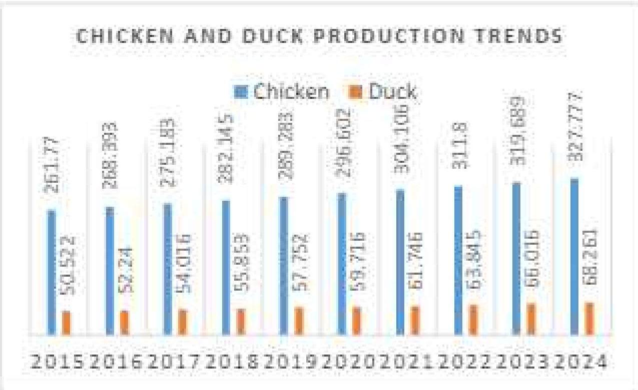
Dr.Md.Shahinur Alam
Chief Scientific Officer (Ectoparasite Section), 48-kazi alauddin road, Dhaka

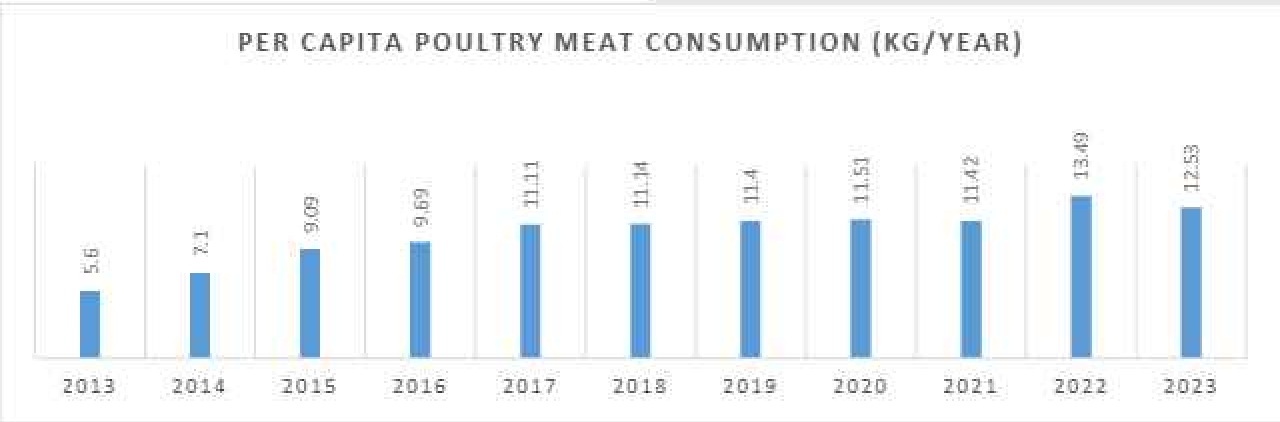
The Vision of **Department of Livestock Services is 'ensure safe, adequate and quality protein for all'. DLS** in Bangladesh plays an important role in addressing the growing threat of **Antimicrobial Resistance (AMR).** AMR is a significant challenge both for animal, human and environmental health. Besides this Animal originated protein are the vital sources for food security, employment generation, and livelihood improvement. It plays a great role in national economy. In the fiscal year 2023-24 the contribution of livestock in gross Domestic product (GDP) was (constant Prices) 1.80%, GDP growth rate in constant Prices 3.15%, Share of Livestock in Agricultural GDP (Constant prices) 16.33%, GDP volume (Current prices) 820.14 Billion BDT. Apart from 20% people directly and 50% peoples are partly involve in This sector. The demand for animal-originated protein in Bangladesh is increasing daily due to several socio-economic and demographic factors. These changes reflect the country's evolving dietary patterns, population growth, and economic progress. Economic growth and rising incomes enable more people to afford diverse and protein-rich diets at the same time rapid urbanization led to changing food consumption patterns. On the other hand Public health campaigns and educational initiatives have improved awareness of the importance of a balanced diet, including adequate protein intake.

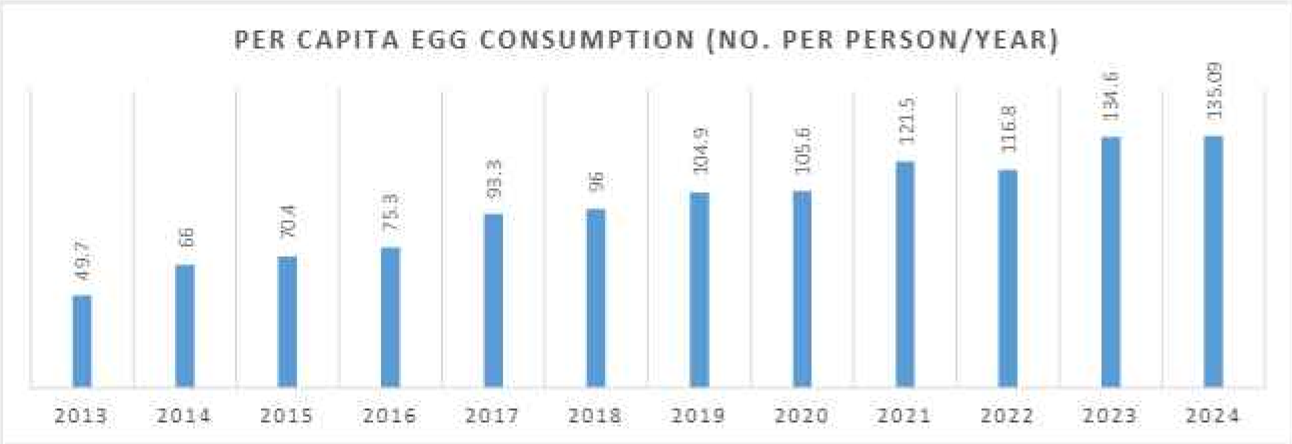
Traditional plant-based diets are increasingly supplemented or replaced by animal proteins as consumer preferences shift. This dietary transition is driven by changing tastes, exposure to global food trends, and aspirations for a more diverse diet. Younger populations, who make up a significant portion of Bangladesh's demographic, have different dietary preferences, often favouring protein-rich fast foods and meat-based dishes. Religious practices and cultural preferences for meat-based dishes like beef, chicken, and eggs are also boosting the demand. To fulfil the growing demand of animal protein department of of livestock services working closely. As a result production and productivity has increased surprisingly.

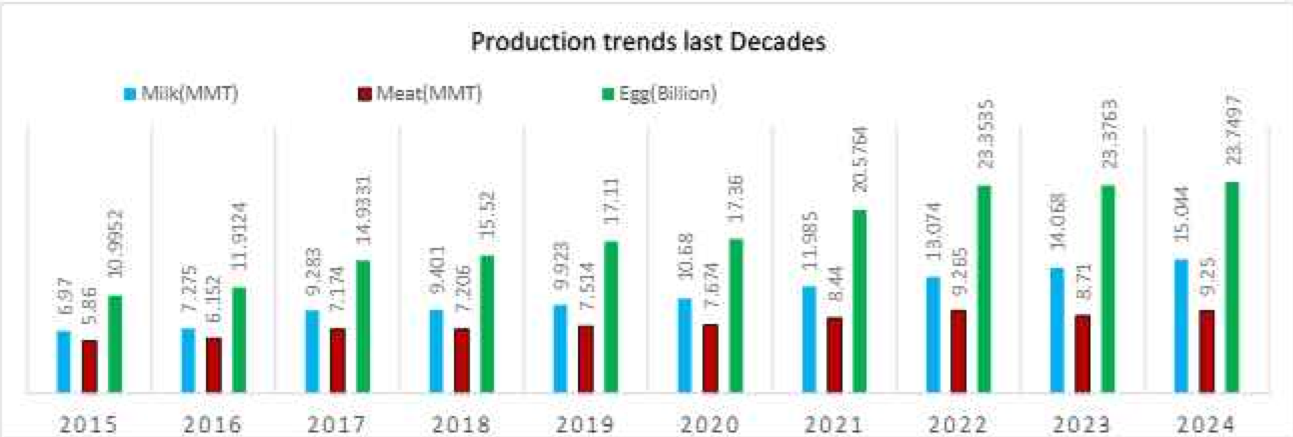
According to Livestock Economy, MOFL-2023-2024 population of livestock are Cattle 25.013 M, Buffalo 1.524 M, Goat 27.117 M, Sheep 3.903 M, Duck 68.261M, and Poultry 327.777M.





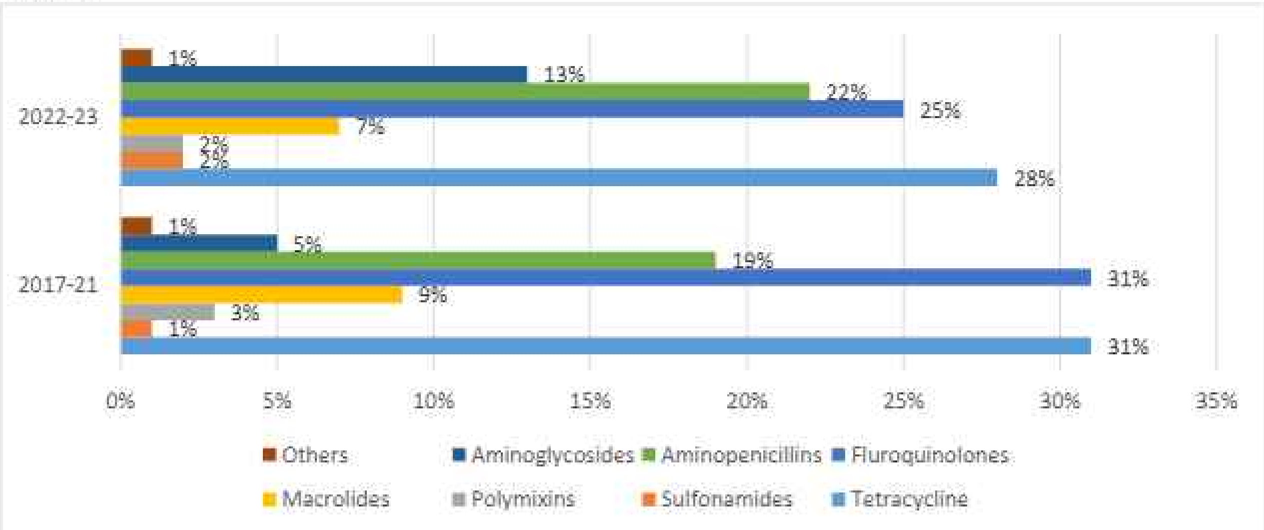


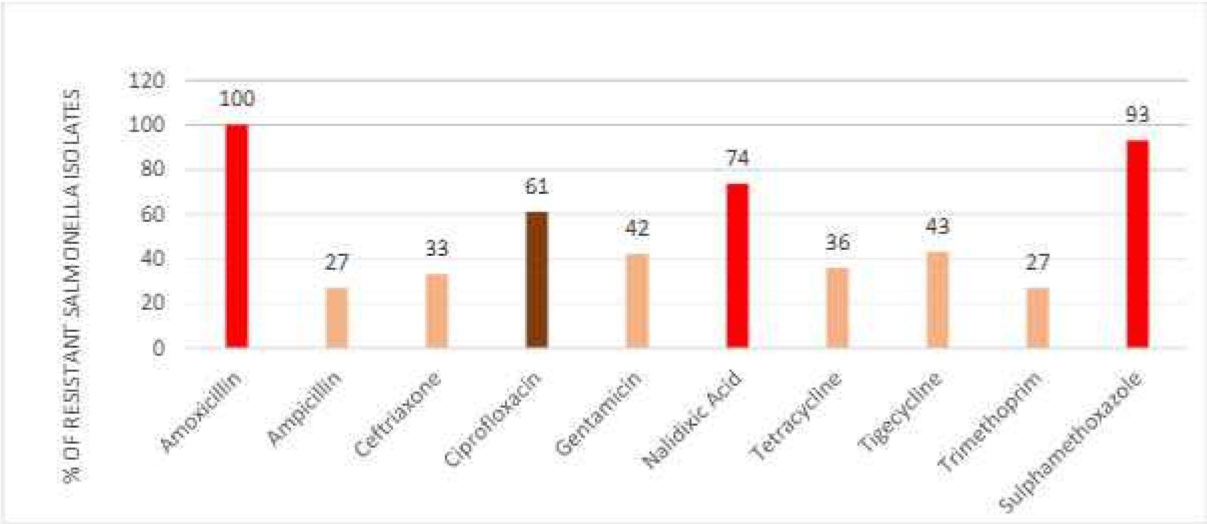




Since the fiscal year 1971-1972 to 2023-2024 Milk production has increased 15 folds, Meat production 18 folds and egg 15 folds.

AMR Situation in DLS: **Proportion of Antibiotic Class documented via poultry farm assessment (FAO Report)**





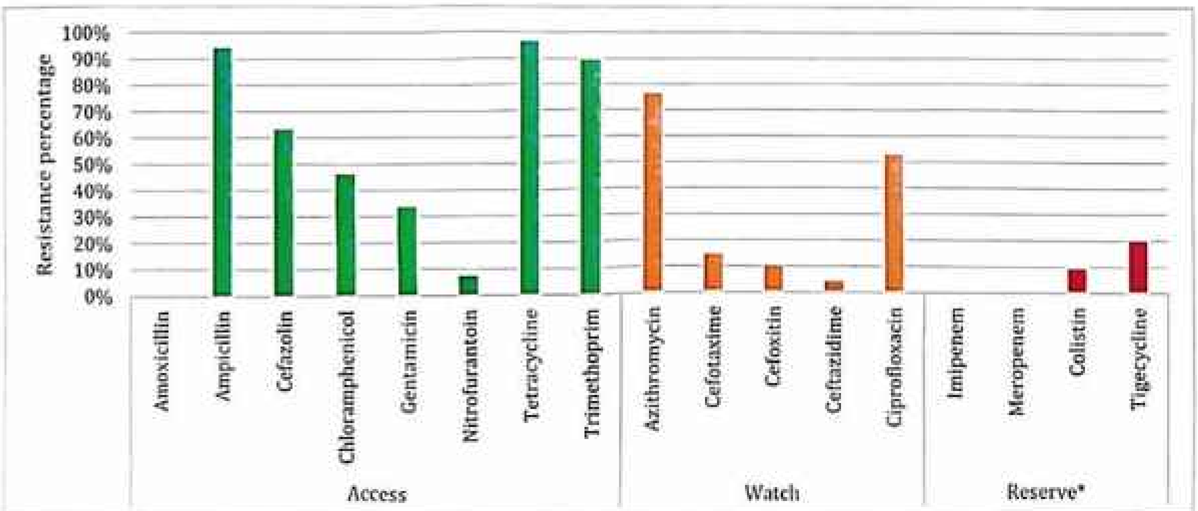


Figure 8: Percentage of resistance to antibiotics in *E. coli* isolates from slaughtered chickens in LBMs according to WHO AWaRe classification between 2021-2023

Major Activities of DLS:

- Increase Production and productivity of safe animal protein.
- Treatment, Control and prevention of Animal and poultry diseases.
- Improvement of Animal nutritional status.
- Creation of skilled manpower and human resource development.
- Quality control of Animal feed and fodder.
- Creation of market linkage, value addition and entrepreneurship development.
- Poverty reduction by self employment generation.
- Good animal husbandry practices for safe and sustainable production.
- Identifying Research needs and technology adoption.
- Formulation, updating and implementation of necessary laws, rules, policies and guidelines.
- Strengthening Public private collaboration.
- Build a strong collaboration with national and international organization.

The uncontrolled spread of AMR can severely impact on animal health, productivity, and the sustainability of the livestock sector.

To combate AMR DLS has taken several initiatives focusing on prevention, control, and awareness in the livestock sector. The major initiatives are:

1. **Integration with the National Action Plan (NAP) on AMR:** The DLS actively participates in implementing Bangladesh's **National Action Plan on AMR (2021-2026)**, which aims to reduce antimicrobial misuse in humans, animals, and the environment. This includes promoting responsible use of antimicrobials and strengthening multi-sectoral collaboration under the **One Health** approach.
2. **Awareness and Capacity Building Programs:** Conducting awareness campaigns for farmers about the risks of antimicrobial misuse and the benefits of alternative measures such as vaccination, probiotics, and improved biosecurity. Educating veterinarians on antimicrobial stewardship and best practices for prescribing antimicrobials.
3. **Antimicrobial Stewardship Programs:** The DLS has developed standered treatment guidelines for poultry and large animal to encourage the judicious use of antimicrobials in veterinary practices. This includes advocating for prescription-only use and discouraging over-the-counter sales of antibiotics for livestock and poultry.
4. **AMR Surveillance in Livestock and Poultry:** DLS Has Developed Bangladesh Animal Health Intelligence system(BAHIS) for AMU Surveillance.
5. **AMR Surveillance in Livestock and Poultry: Use of** Antibiotic in feed is completely prohibited by Animal& fish feed act 2010.
6. 33 different Veterinary drugs has Banned by DGDA and All doses form of Colistin has been banned for Livestock use.
7. DLS has Strengthened CDIL as AMR reference Lab and capacitated five sentinel lab for AST-Jopurhat and Feni, Shirajgonj, Barishal, and Chattogram.
8. Disease control and eradication program has taken and implemented with the help of development project.
9. Registration of all feed mill and commercial farm are going on.
10. Implementing regulatory activities with the help of local administration.

By addressing AMR through these initiatives, the DLS aims to safeguard the health of animals and humans, ensure sustainable livestock production, and preserve the efficacy of antimicrobials for future generations.

AMR/AMU Situation in Bangladesh

Md. Abu Sufian, PhD
Director General
Department of Livestock Services

Mission & Vision of DLS

Mission

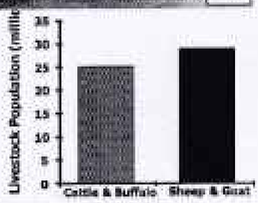
❖ Providing safe, adequate and quality animal protein for all

Vision

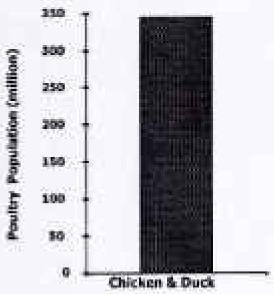
❖ Increasing the production and productivity of livestock and the need for animal protein through value addition.

Livestock Populations

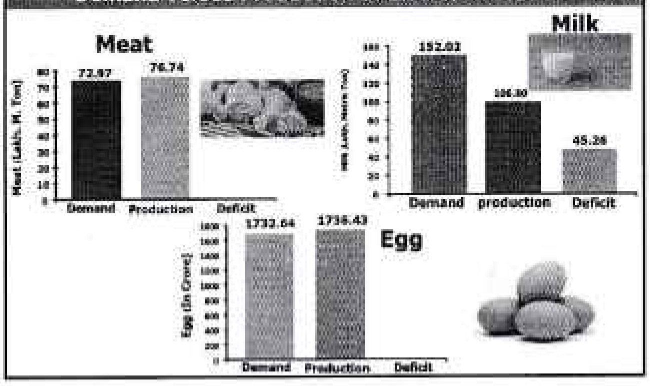




Livestock Populations




Demand versus Production of Livestock Product



FAO-OIE-WHO Tripartite Alliance at Global level

- Three organizations have worked together to prevent, detect, control and eliminate health threats
- A formal alliance facilitates putting the "One Health" vision into practice

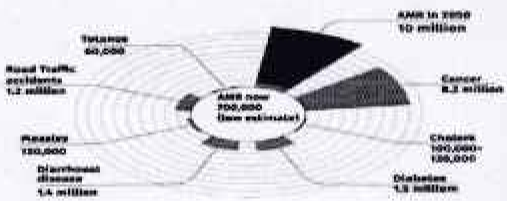
Antimicrobial Drugs

Fading Miracle?

Antimicrobial Resistance (AMR)

- One of the most serious and growing threats to public health.
- The misuse of antibiotics can adversely impact the health of patients **who are not even exposed to them.**

Comparative analysis of death due to common conditions



Recent modeling by the Independent Review on AMR, has predicted that AMR will cause an additional 10 million deaths per year by 2050.

Antibiotics
Antivirals
Antifungals
Antiparasitics

"Spread Awareness, Stop Resistance"

Achievement and Way Forward of AMR Surveillance in Animal Health Sector

The National AMR Surveillance Strategy of Bangladesh (Draft)- 2020-2025

Awareness in Antibiotic Use

CHALLENGES TO OVERCOME...

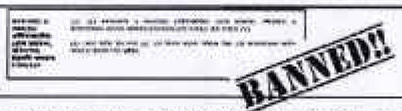
Awareness in Alternative to Antibiotic Use

DLS gives the permission on Importation of Alternative to antibiotic which is used in Feed Industry

International collaboration, alternatives to antibiotics and research on Antimicrobial Resistance (AMR)

Activities carried out by DLS

Ban on Use of Antibiotics in animal & fish feed

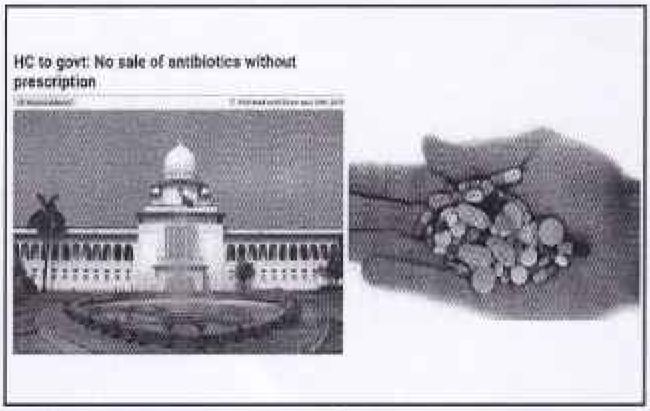


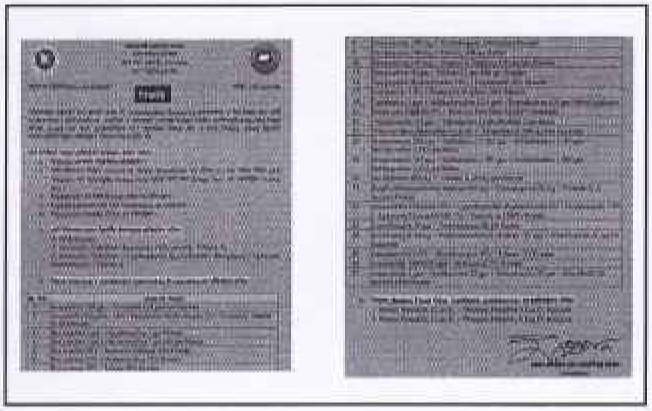
The Fish & Animal Feed Act, 2010 'Prohibits the use of antibiotics, growth hormones, steroids or other harmful chemicals in animal feed. For violating this law, a person might face up to one year's imprisonment or up to Tk- 50,000 (~650 USD) in fine or both

Monitoring and supervisions in the field levels

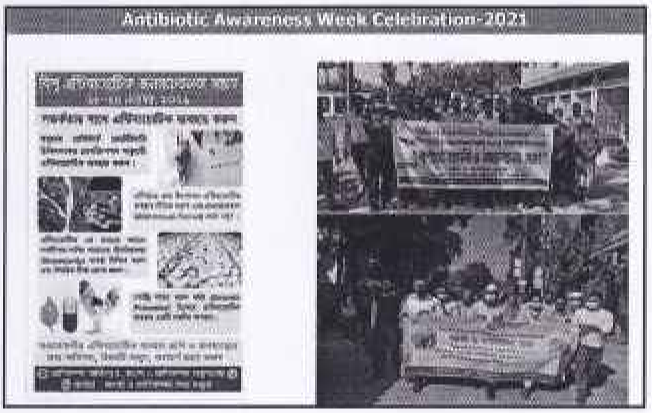
Legal Action carried out by DLS

- **Mobile court is ongoing countrywide specially in the feed mills to monitor the quality of feed and AMs as growth promoter**
- **In 2019, the total mobile court was 325**


HC to govt: No sale of antibiotics without prescription




Antibiotic Awareness Week Celebration

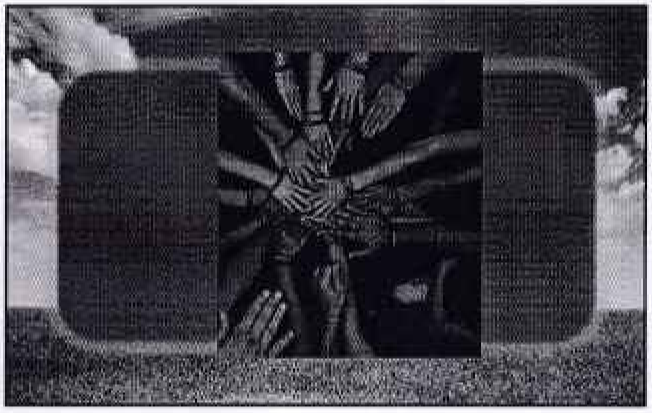

Antibiotic Awareness Week Celebration-2021

Challenges

- ❖ Injudicious use of antibiotics by the quacks in treatment or preventive procedure
- ❖ Dealer/Feed seller based contract farming
- ❖ Failure to monitor withdrawal period of antibiotics used
- ❖ Biosafety & biosecurity in the farm level
- ❖ Inadequate veterinary coverage

Way Forward

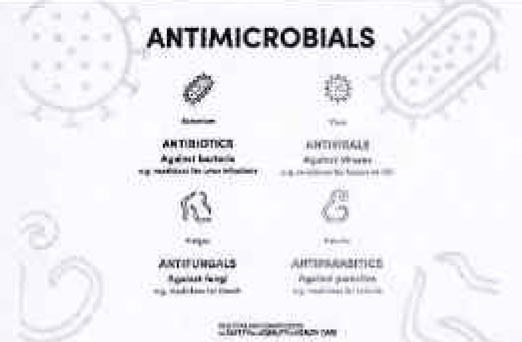
- ❖ Improve biosafety & biosecurity in the farm level
- ❖ Identify essential AMs for animal health sectors
- ❖ Conduct advocacy and orientation of all stakeholders
- ❖ Development of STG for Poultry and Large Animal
- ❖ Strengthening laboratory capacity
- ❖ Data sharing among the sectors



Thank You!

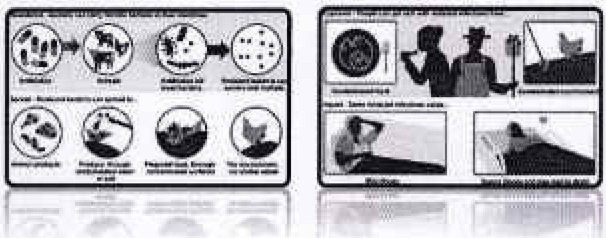
Antimicrobial resistance (AMR) affecting both human health and the agriculture

Md. Latiful Bari, Ph.D.
latiful@du.ac.bd
CARS, DU



The routes of transmission of AMR between farm animals, the wider environment and humans.

How Antibiotic-Resistant Bacteria Can Develop and Spread

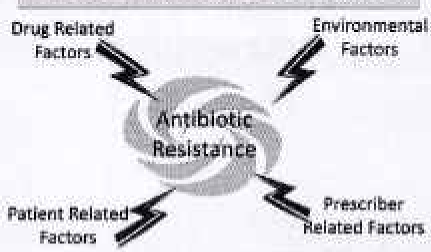


Antimicrobial agents

- Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity.

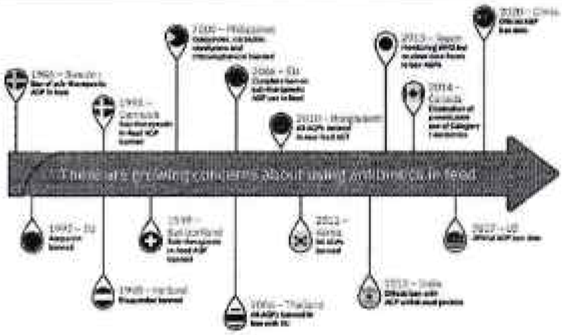
1. Agents that inhibit cell wall synthesis.
2. Depolarize the cell membrane.
3. Inhibit protein synthesis.
4. Inhibit nuclei acid synthesis.
5. Inhibit metabolic pathways in bacteria.

Factors of Antibiotic Resistance

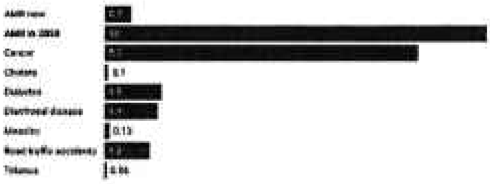


1. Environmental Factors

1. Huge populations and overcrowding
2. Rapid spread – increased travelling
3. Poor sanitation
4. Increases community acquired resistance
5. Ineffective infection control program
6. Increasing national and international travel.
7. Widespread use of antibiotics in animal husbandry and agriculture and as medicated cleansing products.



Yearly deaths attributable to antimicrobial resistance (AMR) comparing to other causes (million)



Antimicrobial agent and antimicrobial resistance in the agricultural environment A case study of Japan

- In 1954, USDA focused on the potential use of streptomycin, tetracycline, and actidione as a pesticide.
- In agriculture, it is a vital compound to control bacterial plant pathogens, such as *Pseudomonas* spp., *Streptomyces* spp., and *Erwinia* spp. in vegetable or fruit production.
- In addition, streptomycin treatment promotes seedlessness and the enlargement of grape berries, like gibberellic acid treatment.

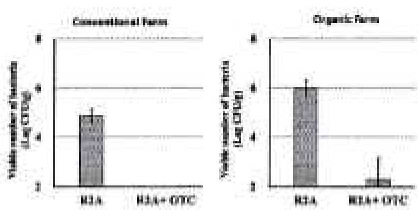
The population size of streptomycin-resistant bacteria in agricultural soil

Sampling Place (Prefecture)	Soil Property	Cultivation History	SM Resistant Bacteria (Log CFU/g)
Hokkaido	Brown lowland soil	Onion, Conventional	4.07
Fukushima	Brown lowland soil	Peach, Conventional	4.00
Wakayama	Black soil	Japanese apricot, Conventional	3.00
[illegible]	Brown lowland soil	Lettuce, Organic	3.47

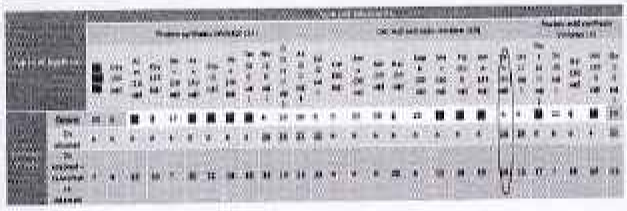
- Even in organic farms managed for more than 20 years, there was no significant difference in the population size of streptomycin resistant bacteria between conventional and organic farm soil.
- This result suggests that resistant bacteria to streptomycin exist in agricultural soil environments as natural microbial flora even without using streptomycin as an agricultural chemical.

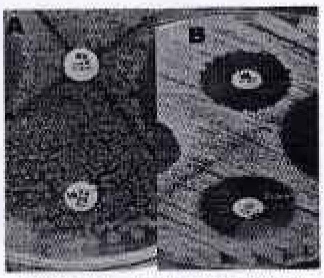
Oxytetracycline, applied as a pesticide since 1956 in Japan

Organic inorganic lettuce leaves



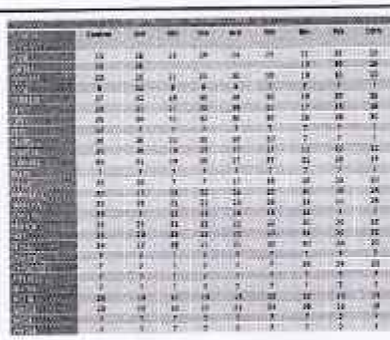
- A large population of resistant bacteria to concerned antibiotics were found in observed specimens, irrigation water, potting soil, fresh produce, and agricultural field soil, even without the application of antibiotics as agricultural chemicals.
- The resistant bacteria to concerned antibiotics in agricultural specimens may have existed initially in each sample and were not directly affected by pesticide application in the agricultural field.

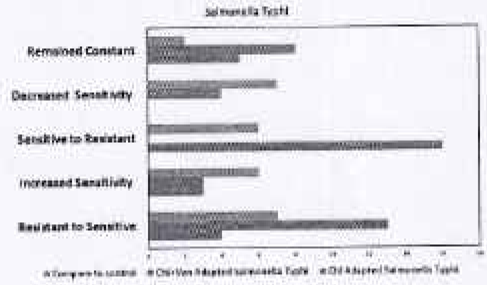




A: No zone of inhibition found around the discs on control bacterium lawn;
B: Clear zone of inhibition around the VA and NV discs on Chloramphenicol adapted bacterium

Cell cycle to explain AMR





Adaptation to Chloramphenicol (30 µg/ml) to S. typhi to become sensitive to the following antibiotics (7)

Serial no.	Names	Mechanism of action	Control	Chloramphenicol adapted S. typhi
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	Kanamycin [illegible]	[illegible]	[illegible]	[illegible]

Antibiotic Resistance

Some microorganisms may 'born' resistant, some 'achieve' resistance by mutation or some have resistance 'thrust upon them' by plasmids

Some are born great, some achieve greatness or some have greatness thrust upon them

Why resistance is a concern

- Resistant organisms lead to treatment failure
- Increased mortality
- Resistant bacteria may spread in Community
- Low level resistance can go undetected
- Added burden on healthcare costs
- Threatens to return to pre-antibiotic era
- Selection pressure

Thanks

Training Program
on
Antimicrobial Resistance in Bangladesh

Date	:	26-30 January 2025
Venue	:	Central Disease Investigation Laboratory, Kazi Alauddin Road, Dhaka Bangladesh Livestock Research Institute, Savar, Dhaka
Training Organizer	:	Livestock Division, Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka
Participants	:	Twenty five teachers/researchers/veterinarians from DLS (LRI, CVH, CDIL, QC Lab), BLRI, BFRI, NIB, DoF, SAU, PSTU, BSMRAU and Gono Bishwabidyalay
Course Director	:	Dr. Mohammad Rafiqul Islam, Member Director, Planning and Evaluation Division; and Chief Scientific Officer, Livestock Division, BARC, Farmgate, Dhaka
Course Coordinator	:	Dr. Masud Rana, Principal Scientific Officer, Livestock Division, BARC, Farmgate, Dhaka

Inaugural Session

Time	Events
09:00-09:30 A.M.	Registration
09:30-09:35 A.M.	Telawat-E-Quran
09:35-09:45 A.M.	Welcome address **Dr. Md. Golam Azam Chowdhury** Principal Scientific Officer, Central Disease Investigation Laboratory, Dhaka
09:45-09:55 A.M.	Address by the Special Guest **Dr. Md. Abu Sufiun** Director General, Department of Livestock Services, Farmgate, Dhaka
09:55-10:10 A.M.	Address by the Chief Guest **Dr. Nazmun Nahar Karim**, Executive Chairman, Bangladesh Agricultural Research Council, Farmgate, Dhaka
10:10-10:25 A.M.	Address by the Chairperson **Dr. Mohammad Rafiqul Islam**, Member Director, Planning and Evaluation Division; and Chief Scientific Officer, Livestock Division, Bangladesh Agricultural Research Council, Farmgate, Dhaka.
10:25-10:45 A.M.	Refreshment

Day 1: 26 January 2025

Time	Topics	Speaker
10:45-11:45 A.M.	AMR/AMU Situation in Bangladesh	Dr. Md. Abu Sufiun, Director General, Department of Livestock Services

Time	Topics	Speaker
11:45-01:00 P.M.	Antimicrobial resistance: A global threat	Dr. Mohammad Rafiqul Islam, Member Director (P&E) and Chief Scientific Officer (Livestock), BARC
01:00-02:00 P.M.	Lunch and prayer	
02:00-03:00 P.M.	National Antimicrobial Resistance (AMR) Surveillance initiative in Animal Health	Dr. Md. Nure Alam Siddiky, Deputy Team Lead, The Fleming Fund, Bangladesh Country Grant, DAI Global, Dhaka
03:00-05:00 P.M. **Practical:** Group A	Preparation of different chemicals, reagents & bacteriological media for antimicrobial sensitivity testing.	Dr. Md. Golam Azam Chowdhury, Principal Scientific Officer, Central Disease Investigation Laboratory
03:00-05:00 P.M. **Practical:** Group B	Collection, Transportation, preservation and processing of bacteriological samples for AMR surveillance	Dr. Mst. Shamima Akter, ULO, Central Disease Investigation Laboratory

Day 2: 27 January 2025 (Field Visit at AMR Laboratory, BLRI)

09:15-10:00 A.M.	BLRI laboratory resources for combating AMR challenges in Bangladesh	Dr. Shakila Faruque, Director General Bangladesh Livestock Research Institute
10:00-11:00 A.M.	Irrational use of antimicrobials and its hazard on human-animal and environment interface	Dr. Mohammed Abdus Samad, Principal Scientific Officer, Transboundary Animal Diseases Research Center, BLRI
11:00-11:15 A.M.	Tea break	
11:15-12:15 P.M.	Application of vitek-2 for laboratory detection of AMR pathogen	Dr. Mohammed Abdus Samad, Principal Scientific Officer, Transboundary Animal Diseases Research Center, BLRI
12:15-01:00 P.M.	Good laboratory practices in AMR detection	Dr. Mohammed Abdus Samad, Principal Scientific Officer, Transboundary Animal Diseases Research Center, BLRI
01:00-02:00 P.M.	Lunch and prayer	
02:00-04:00 P.M. (**Practical**)	Quality Assurance (QA) and Quality Control (QC) in AMR detection in AMR Reference laboratory	Dr. Md. Rezaul Karim Senior Scientific Officer, Animal Health Research Division, BLRI

Day 3: 28 January 2025

09:15-10:00 A.M.	Biosafety and biosecurity for AMR laboratory	Dr. Mohammad Showkat Mahmud, Associate Professor and Head, Dept. of Microbiology, Gono Bishwabidyalay, Dhaka

Time	Topics	Speaker
10:00-11:00 A.M.	Choice of antibiotics: Treatment for livestock and poultry	Professor Dr. Kazi Rafiqul Islam, Vice-Chancellor, PSTU, Patuakhali
11:00-11:15 A.M.	Tea break	
11:15-12:15 P.M.	Use of probiotics and prebiotics alternative to antibiotics in animal farming	Professor Dr. Kazi Rafiqul Islam, Vice-Chancellor, PSTU, Patuakhali
12:15-01:00 P.M.	AMR: Effects on Human, Animal and Environmental Health	Dr. Latiful Bari, Director, CARS, DU
01:00-02:00 P.M.	Lunch and prayer	
02:00-04:00 P.M. **Practical:** Group A	Collection, Transportation, preservation and processing of bacteriological samples for AMR surveillance	Dr. Mst. Shamima Akter, ULO, Central Disease Investigation Laboratory
02:00-04:00 P.M. **Practical:** Group B	Preparation of different chemicals, reagents & bacteriological media for antimicrobial sensitivity testing.	Md. Golam Azam Chowdhury, Principal Scientific Officer, Central Disease Investigation Laboratory

Day 4: 29 January 2025

09:30-10:30 A.M.	Molecular mechanism of antimicrobial resistance	Dr. Jahangir Alam, Chief Scientific Officer, Animal Biotechnology Division, NIB
10:30-11:30 A.M.	Molecular detection of AMR and virulence genes in Bacteria	Dr. Jahangir Alam, Chief Scientific Officer, Animal Biotechnology Division, NIB
11:30-11:45 A.M.	Tea break	
11:45-12:45 P.M.	Initiatives of Department of Livestock Services against AMR	Dr. Md. Shahinur Alam, Chief Scientific Officer, Ectoparasite section, Central Disease Investigation Laboratory Department of Livestock Services
12:45-02:15 P.M.	Lunch and prayer	
02:15-04:15 P.M. **Practical:** Group A	Culturing and antimicrobial sensitivity testing (AST) using disk diffusion method	Md. Golam Azam Chowdhury, Principal Scientific Officer, Central Disease Investigation Laboratory
02:15-04:15 P.M. **Practical:** Group B	Culturing and antimicrobial sensitivity testing (AST) using disk diffusion method	Dr. Sukesh Chandra Badhy, ULO, Central Disease Investigation Laboratory

Day 5: 30 January 2025

09:15-10:00 A.M.	AWaRe categorization and therapeutic application in farming practices	Professor Dr. Md. Mahmudul Hasan Sikder, Dept. Pharmacology, Faculty of Veterinary Science, BAU

Time	Topics	Speaker
10:00-11:00 A.M.	Rational basis of antibiotic therapy: What do we need to know? AMU guidelines	Professor Dr. Md. Mahmudul Hasan Sikder, Dept. Pharmacology, Faculty of Veterinary Science, BAU
11:00-11:30 A.M.	Tea break	
11:30-01:00 P.M.	AMR situation in E. Coli and other bacteria in Chickens	Dr Malay Kumar Sur,Chief Scientific Officer, Virology Section, Central Disease Investigation Laboratory
01:00-02:00 P.M.	Lunch and prayer	
02:00-04:00 P.M. **Practical:** Group A	Hands on training on antimicrobial sensitivity interpretation Hands on training on Bacterial specie identification using MALDI-TOF	Md. Golam Azam Chowdhury, Principal Scientific Officer, Central Disease Investigation Laboratory
02:00-04:00 P.M. **Practical:** Group B	Hands on training on antimicrobial sensitivity interpretation Hands on training on Bacterial specie identification using MALDI-TOF	Dr. Sukesh Chandra Badhy, ULO, Central Disease Investigation Laboratory
04:00-05:00 P.M.	**CLOSING CEREMONY AND CERTIFICATE DISTRIBUTION**	

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