Antibiotic use and resistance in the food and agricultural sectors in Bangladesh

– present risk factors and possible improvements

Användning av antibiotika och antibiotikaresistens inom livsmedels- och jordbrukssektorerna i Bangladesh – nuvarande riskfaktorer och möjliga förbättringar

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Swedish University of Agricultural Sciences, SLU
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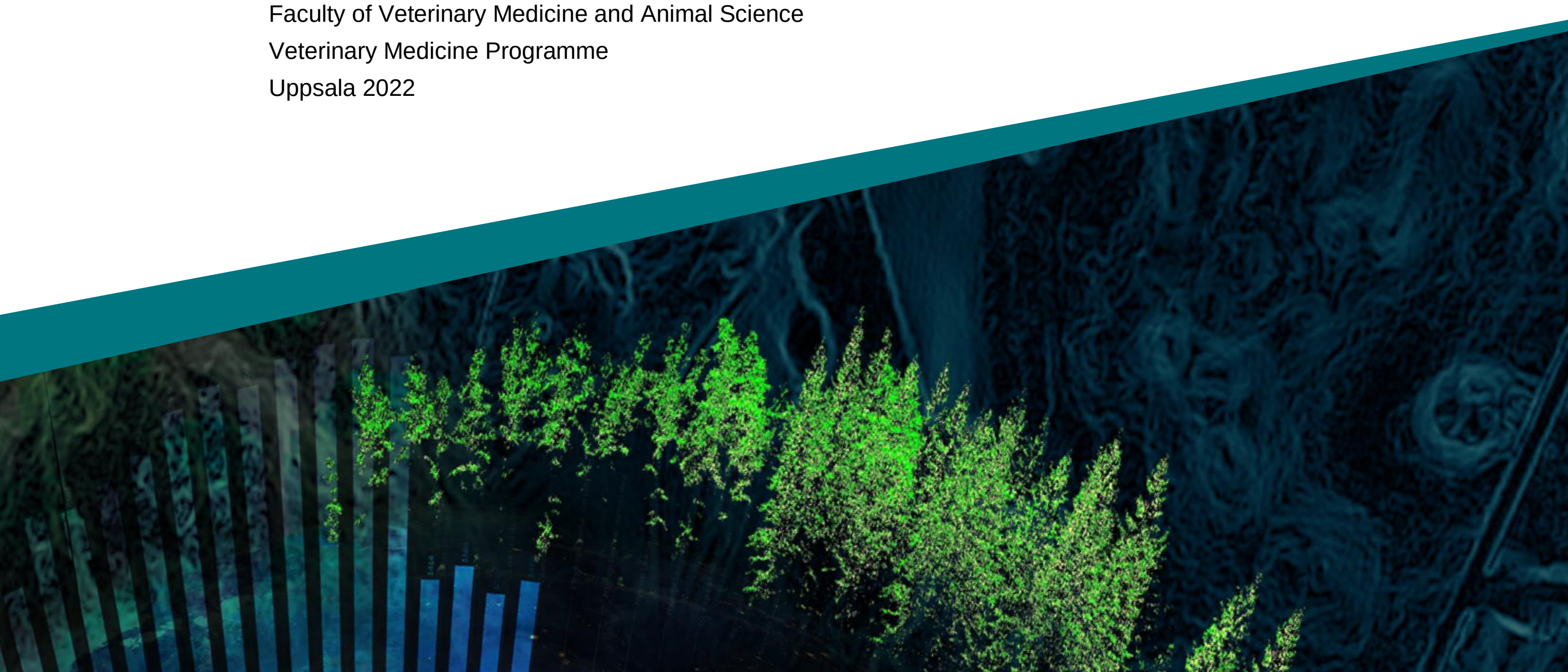

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Abstract

Antimicrobial resistance (AMR) is one of the biggest threats against public health in the world. Antimicrobial substances are used within all different sectors and contribute to development of AMR. Global action against irresponsible use of antibiotics and further development of AMR has been of great concern in the last years and risk factors are being pointed out.

Low- and middle-income countries (LMICs) have a precarious role in the matter. Insufficient health care systems, poor law enforcement and, high accessibility of over-the-counter drugs (OTCs) are contributing to the unregulated use of antibiotics. Poorly developed surveillance programmes make it hard to correctly analyse the situation of both antimicrobial use (AMU) and AMR.

Bangladesh, like its neighbouring countries, faces a lot of challenges regarding public health. One of the major concerns related to public health is access to safe food. Food products can be contaminated with toxins, chemical substances, and microbial organisms, including AMR-bacteria. Furthermore, national programmes for surveillance of AMU and AMR are inadequate.

In this study, data from previously done field studies by Bangladesh Livestock Research Institute (BLRI), Bangladesh Food Safety Authority (BFSA), International Livestock Research Institute (ILRI), and International Food Policy Research Institute (IFPRI) and newly collected information from interviews were put together to analyse the AMR situation in Bangladesh. Sampling of food products (tomato, chicken, fish) from traditional markets and supermarkets was done at three locations representing rural, peri-urban, and urban areas from November 2018 to June 2019. Samples were tested for prevalence of *Salmonella* spp, *Escherichia coli*, *Vibrio cholerae*. Samples positive for bacteria were tested for antimicrobial susceptibility through disc diffusion test. As a supplement to the analysis of samples, questionnaires to the vendors of the food products were made to provide background information. During 2020, statistical analysis of previously collected data and interviews with stakeholders working with AMR was made. The interviews aimed to serve as baseline information about current conditions regarding AMU and AMR.

320 cultivations of 1589 (20.1%) were positive for bacterial prevalence. 319 of these were tested for antimicrobial susceptibility where 203 (63.6%) were found to be multidrug-resistant (MDR) (resistant to three or more antibiotic groups). Furthermore, interviews with stakeholders stated that surveillance of AMU and AMR in Bangladesh is inadequate, especially within the animal and agriculture sector, and that a one health approach on a government level is needed to improve the situation. To be able to fully analyse the AMR situation in Bangladesh, a nation-wide study would need to be conducted, within all sectors, including both AMU and AMR testing.

Keywords: AMR, Bangladesh, AMU, multidrug-resistant (MDR), low-and middle-income countries

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Abbreviations

AGP	Animal growth promotors
AMR	Antimicrobial resistance
AMU	Antimicrobial use
BFSA	Bangladesh Food Safety Authority
BLRI	Bangladesh Livestock Research Institute
CAT	Chloramphenicol acetyltransferase
CFU	Colony forming units
CT	Cholerae toxin
DGHS	Directorate General of Health Services in Bangladesh
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteric inflammation *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ESBL	Extended-spectra beta-lactamases
HGT	Horizontal gene transfer
IFPRI	International Food Policy Research Institute
ILRI	International Livestock Research Institute
LMICs	Low- and middle-income countries
LPS	Lipopolysaccharide
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
MRL	Maximum residue level
MRSA	Methicillin-resistant *Staphylococcus aureus*
NTS	Non-typhoid *Salmonella* spp.
OTC	Over the counter (drugs)
PBP	Penicillin-binding places
SMX	Sulfamethoxazole
TCC	Total coliform count
TDR	Totally drug-resistant
TMP	Trimethoprim
THF	Tetrahydrofolate

1. Introduction

Antimicrobial resistance (AMR) is regarded as one of the major threats against public health in the world. Since the discovery of antibiotics, they have been used and misused in human medicine, veterinary medicine, as growth promotors, and agriculture. The antibiotics have made it possible for medicine to evolve and improved animal health and production, but hand in hand with the beneficial effects of antibiotics, development of resistance has taken place. The development of AMR is leading to a situation where antibiotics become less effective or even non-functional.

The antibiotics critical for human health care are the same ones used within the livestock sector (FAO 2016; WHO AGISAR & WHO 2017). The extent of antibiotic use varies between countries due to regulations in place, policies, knowledge, and income levels (Laxminarayan *et al.* 2013). Low- and middle- income countries (LMICs) have a precarious role in the matter. Insufficient health care systems and high accessibility of over-the-counter drugs (OTCs) are contributing to unregulated use of antibiotics (WHO 2015a). Additionally, lack of awareness about antimicrobials and poorly developed surveillance programmes make it hard to correctly analyse the situation of both antimicrobial use (AMU) and AMR (WHO 2015a; FAO 2016).

Bangladesh, a country with one of the world's most dense populations, share the problems of other LMICs. People tend to live close to their animals, creating an environment where zoonotic diseases and AMR can emerge (Ahmed *et al.* 2019). Food safety is also a major concern to public health in the country, which needs to be addressed (WHO 2014b). Within the country, there is little information about pathogens in foodstuff and no national covering surveillance of AMU and AMR. This leads to questions about current pathogens in foodstuff and their eventual AMR-pattern, including the risk to contract such pathogens. Even more, it raises questions of whether people see problems with AMU and AMR in Bangladesh, and what is needed to improve the situation.

In this report, data collected in Bangladesh during 2018-2019, interviews with key informants from 2020, and a literature review on AMU and AMR try to circle the problems and what should be done to try to improve the situation.

2. Literature review

2.1. Antibiotics

Antibiotics have long been regarded as the cure for infectious diseases. Since Fleming discovered penicillin in 1928, the medicine has been praised within health care and made it possible for medicine to evolve to what we see it as today. Infectious diseases previously thought to be deadly are now treatable, transplants are a possibility and advanced chemotherapy is an option – all of these are dependent of the work of antibiotics (Laxminarayan *et al.* 2013).

Antibiotics have been excessively used, not only within human health care, but also within veterinary medicine, agriculture, and as growth promotors, contributing to health advances for humans, but also affecting animal welfare. Alongside the use, microbes have been developing different strategies to avoid being targets for antibiotics, leading to antibiotics becoming less effectful and development of resistance (AMR) (Barton 2000; Laxminarayan *et al.* 2013).

The first reports of antibiotic resistance occurred after the introduction of antibiotics to clinical environments. When resistance arose, development of new antibiotic substances was prioritized, resulting in new antimicrobial drugs targeting other mechanisms in microbes. With the new drugs developed, antibiotics have continued to be a force to count on, up to recent years. Bacteria resistant to not only one, but three or more antibiotic groups, eg multi-drug resistant (MDR) have become more frequent (Alekshun & Levy 2007; Laxminarayan *et al.* 2013).

Development of antibiotics between the 1940s to 1960s are described as products from the golden era, but after the 1960s, not many new antibiotics have been introduced to the market until the early 2000s (Walsh 2003). Research targeting development of new antibiotics has decreased over the years and many of the substances under present development consist of minor modifications from already discovered molecules (Walsh 2003).

The decrease in development of new antibiotic classes might be dependent on the insecurity of markets, behavioural changes of prescription of antibiotics, and economical insecurities for investing companies (Gould & Bal 2013). To turn this trend around, Gould and Bal (2013) propose collaborative development between major drug companies, investing in research and economic stimuli to public-private partnerships.

2.1.1. Groups of antibiotics

Antibiotics are divided into different groups depending on their work of action and characteristics. Within these groups, multiple substances exist with antimicrobial spectra that slightly differ from each other. A few substances and their characteristics including spectra and resistance towards mentioned antibiotic groups, with connection to this study, are displayed in table 1 and table 2.

Table 1. Antimicrobial substances and spectra

	Antimicrobial spectra and specifics
Beta-lactams	
Penicillin G	Narrow spectra, used to treat infections with Streptococcus spp. Sensitive for penicillinase (Bush & Bradford 2016).
Amoxicillin + clavulanic acid	Improved antibacterial spectra against gram-negatives (Bush & Bradford 2016). The combination is effective against several gram-positive, aerobic bacteria and gram-negative bacteria (Williams 1999; Wright 1999).
Cefixime and Ceftriaxone	Cephalosporins of the third generation, effective against many beta-lactamases producing bacteria including *Escherichia coli* and *Klebsiella pneumoniae* (Paterson & Bonomo 2005). Spectra against gram-positive bacteria and extended spectra against some gram-negatives (Bui & Preuss 2020).
Macrolides	
Erythromycin	Broad-spectrum, mainly against gram-positive and intracellular bacteria such as *Mycoplasma* spp. and *Chlamydophila* spp. Target a couple of gram-negative bacteria (Fyfe *et al.* 2016; Dinos 2017).
Chlorampenicol	Broad-spectrum, effective against gram-positive and gram-negative bacteria, both aerobes and anaerobes. Also effective against *Chlamylodophila* spp, *Mycoplasma* spp and *Rickettsia* spp (Schwarz *et al*. 2004).
Aminoglycosides	
Streptomycin	Broad-spectrum, used against infections with tuberculosis (Vakulenko & Mobashery 2003).
Gentamicin	Broad-spectrum, mildly extended spectra combined to other aminoglycosides, often used in combination with beta-lactams to treat infections caused by gram negatives (Vakulenko & Mobashery 2003).
Tetracyclines	Broad-spectrum. Gram-negatives, gram positives and intracellular organisms such as *Chlamydophila* spp. and *Mycoplasma* spp. Also have action against protozoan organisms (Chopra & Roberts 2001).
Trimethoprim + Sulfamethoxazole	Broad-spectrum, including gram positives and gram-negatives where *Enterobacteriaceae*, *Streptococcus* spp. and *Staphylococcus* spp. have been described. Effectiveness against intracellular organisms is not clinically manifested (Huovinen *et al.* 1995; Huovinen & Eliopoulos 2001).
Quinolones	Broad-spectrum against gram negatives and gram positives (Aldred *et al.* 2014).
Nalidixic acid	Specifically used to treat uncomplicated infections caused by enteric bacteria (Aldred *et al.* 2014).

Beta-lactam antibiotics

Beta-lactams are a widely used category of antibiotics. Since the discovery of penicillin, new beta-lactam antibiotics have been developed to increase the spectrum of activity or to counteract specific resistance mechanisms developed by bacteria. All antibiotics classified as beta-lactams or derivates from beta-lactams, have the beta-lactam ring in common. The beta-lactam ring is the component of the molecule that is active in the bacteriostatic process (Bush & Bradford 2016).

The beta-lactam antibiotics act by inhibiting penicillin-binding proteins (PBPs) in the bacteria, which disrupts cell wall synthesis. PBP are members of a subgroup of enzymes, called transpeptidases, and are involved in bacterial synthesis of peptidoglycan. When beta-lactams bind to PBP, the real action of the enzymes is inhibited, resulting in incorporation of antibiotics in the cell wall. This results in a defective cell wall, which ultimately results in lysis and cell death (Bonomo 2017).

Beta-lactams can be combined with clavulanic acid with the purpose to inhibit beta-lactamases produced by bacteria. Alone, the clavulanic acid is not a bactericide, but in combination with some beta-lactams, a synergetic bactericide work of action is achieved.

There are four different mechanisms by which bacteria can become resistant to beta-lactams. One type of resistance is seen when speaking of methicillin-resistance within the *Staphylococcus* spp., for example, methicillin-resistant *Staphylococcus aureus* (MRSA). In this case, a different type of PBP called PBP2a is used, changing the binding place for antibiotics. Porins necessary for the transport of the antibiotic into the bacteria can also be altered, and for example, resistance towards carbapenems can be mediated this way. Multicomponent drug efflux pumps, *mex*, is a third mechanism that also is seen among gram-negative bacteria. Lastly, beta-lactamases, different enzymes, eg penicillinase, produced by bacteria that can make beta-lactams non-effective (Bonomo 2017).

Beta-lactamases work by hydrolyzing the beta-lactam ring and make the antibiotic noneffective. This mechanism is regarded as the most important resistance mechanism against beta-lactams and is especially an important trait among gram-negative bacteria (Bush & Bradford 2016). Production of beta-lactamases has been reported in a variety of different bacteria, such as among Enterobacteriaceae, within *Moraxella* spp., *Vibrio cholerae* and *Pseudomonas aeruginosa* (Williams 1999). One of the most known beta-lactamases is called extended-spectra beta-lactamases (ESBL), which is a rapidly evolving problem among Enterobacteriaceae that result in resistance towards beta-lactams with extended spectra (Paterson & Bonomo 2005).

Genes responsible for the rise of beta-lactamases can be chromosomally encoded or located on mobile elements. The innate encoded beta-lactams are specific to the bacteria, but if encoded for on a mobile element they might spread between different bacteria. Furthermore, transposons can potentially transfer genes from mobile elements to chromosomes, incorporating resistance traits within different bacterial families (Williams 1999; Bush & Bradford 2016).

Macrolides

Macrolides are a big group of antibiotics that act by interfering with bacterial protein synthesis. They consist of a macrocyclic lactone, which one or more deoxy-sugar or amino sugar residues are attached to.

By binding to the 23S rRNA in ribosomal subunit 50S, the group of antibiotics has its antimicrobial effect. Since ribosomal subunits are traits among all bacteria, this group of antibiotics is considered to be broad spectra. Since the development of the first macrolide, new different macrolides have been generated to improve bioavailability. With this development, the spectra have been altered (Fyfe *et al.* 2016; Dinos 2017).

When testing for resistance among macrolides usually erythromycin is used, and if resistance towards this antibiotic is found, the bacteria is regarded to be resistant to macrolides (Fyfe *et al.* 2016).

The group of macrolides is mainly affected by two different resistance mechanisms (Dinos 2017). Mutations in the 23S rRNA lead to production of an enzyme group named *erm*, which leads to less capability for the antibiotic to interfere with protein synthesis. There is a large group of different *erm*-enzymes, and they are regarded as one of the most important resistance mechanisms. This mechanism is seen among *Streptococcus* spp. and *Escherichia coli* (Fyfe *et al.* 2016). Efflux pumps are mediated by the *Mef*-family, which work as antiporters. This mechanism is found within gram-positive bacteria but also among some gram negatives. Other than these mechanisms, ribosomal protein mutations, phosphotransferases and a few more resistance mechanisms mediate the widespread resistance towards macrolides (Fyfe *et al.* 2016; Dinos 2017).

Chloramphenicol

Chloramphenicols were thought to be a new promising group of antibiotics when first discovered, but due to severe side effects including aplastic anemia and reversible bone-marrow suppression the substance never made a big impact. The side effects led to decreased clinical use within human medicine and a ban against use of the antibiotic in food-producing animals in the European Union.

The antibiotic group works their work of action by inhibiting bacterial protein synthesis. The molecule binds to a peptidyl transferase centre at 50S ribosomal unit of 70S ribosomes and prevents peptide chain elongation. The antibiotic is regarded to be bacteriostatic.

The most widespread resistance mechanism to chloramphenicols is enzymatic inactivation of the antibiotic by acetylation via different chloramphenicol acetyl-transferases (CATs) (Schwarz *et al.* 2004; Alekshun & Levy 2007). CAT-genes have been identified both on chromosomal location and on plasmids, and the genes are found at both gram-negative and gram positives. In addition to this, mechanisms such as resistance due to efflux pumps, inactivation by phosphotransferases, and mutations of target sites are described (Schwarz *et al.* 2004).

Aminoglycosides

Aminoglycosides bind to the ribosome of bacteria and affect bacterial protein synthesis. The antibiotic bind to the 30S subunit of the ribosome and interfere with translation of genetic material. This interference results in cell lysis and amino-glycosides are regarded as bactericides.

Aminoglycosides have a broad spectrum against bacteria in vitro, including a variety of gram-negative bacteria and some gram positives. It is most sufficient when the concentration is at a specific level over MIC and combined with beta-lactams or vancomycin, it has a synergetic effect against Enterococci and other bacterial species. Because of its synergistic properties, aminoglycosides are used to treat a variety of diseases, often in combination with other antibiotic groups (Vakulenko & Mobashery 2003).

Many of the resistance mechanisms towards aminoglycosides are often found on integrons and other mobile genetic elements (Alekshun & Levy 2007). Enzymatic modification of the antibiotic is the mechanism most frequent, which includes methylation of the amino or hydroxyl groups of bacteria, resulting in poor binding qualities of antibiotic (Vakulenko & Mobashery 2003).

Tetracycline

Tetracyclines are a group of antibiotics widely used in the world due to their broad spectra against microbes, low price, and few side effects. They have been used excessively within human medicine, veterinary medicine and as growth promotors within livestock production (Chopra & Roberts 2001).

The antibiotics work their action by interfering with bacterial protein synthesis. They interact with molecules associated with ribosomes in a reversible way, describing the bacteriostatic properties within the class. The inhibiting activity is not fully understood, and different suggestions such as a special binding place on ribosomal units are considered (Schnappinger & Hillen 1996; Chopra & Roberts 2001). Over the years, new tetracyclines have reached the market, but a decrease in use has been seen since development of resistance towards the group (Chopra & Roberts 2001).

Mechanisms such as energy-dependent efflux systems, alterations of the target site at ribosomes, increased permeability of the cell envelope and production of enzymes inactivating the antibiotic result in resistance to the antibiotic. The different mechanisms are widespread among bacteria of all genres (Schnappinger & Hillen 1996; Roberts 2005). According to Roberts (2005), 29 *tet* genes (responsible for coding of resistance) and three *otr* genes (resistance to oxytetracycline) have been identified. Of these genes, a number is responsible for the rise of efflux pumps, ribosomal protection proteins and enzymatic inactivation of the antibiotic (Chopra & Roberts 2001; Roberts 2005).

Trimethoprim and Sulfamethoxazole

Trimethoprim (TMP) and sulfamethoxazole (SMX) are two bacteriostatic components that together act as a bactericide due to jointly interaction. They work by interfering with different steps of the biosynthetic pathway for tetrahydrofolate (THF), affecting the bacteria's ability to produce RNA and DNA (Minato *et al.* 2018).

The combination of the two antibiotics has been used to treat urinary tract infections, pneumonia, and other diseases. Resistance to sulphonamide, the substance that sulfamethoxazole is made of, has been reported since the first introduction in the 1930s, and the more recent substance trimethoprim is also a subject to resistance, two factors contributing to a decreased use of the drugs (Huovinen *et al.* 1995; Sköld 2001).

Resistance mechanisms to the two different components are mediated similarly. Changes in the permeability of membranes and efflux pumps are traits that can affect both TMP and SMX, while mutational changes in bacterial genome mainly affect one or the other antimicrobial substance. These changes can occur on mobile elements, contributing to the spread of resistance towards TMP and SMX (Huovinen *et al.* 1995; Huovinen & Eliopoulos 2001; Sköld 2001).

Quinolones

Quinolones are a group of antimicrobials frequently prescribed in the world. They were introduced on the market as late as the 1960s and are today regarded as one of the most important antibiotics which are active against a variety of diseases. The medication is used against urinary tract infections, intra-abdominal infections, skin infections, and a variety of other illnesses because of its broad spectrum against both gram-negative and gram-positive bacteria.

The antimicrobial substance works its action by converting gyrase and topoisomerase IV to toxic enzymes, which fragment the bacterial chromosome, leading to loss of function in the cell and lysis. Except for acting as a poison for chromosomes, the substance also acts as a catalytic inhibitor in the cell.

Over the years different kinds of quinolones have been developed, but as for every class of antibiotics, the use of quinolones is now threatened because of rising resistance. There are three different groups of mechanisms responsible for resistance to quinolones which are due to specific mutations in gyrase or topoisomerase IV, or more general alterations resulting in mechanisms counteracting the concentrations of antibiotics (Aldred *et al.* 2014).

Table 2. Resistance mechanisms

	Resistance mechanisms of importance towards different antibiotics
Beta-lactams	Change of active sites at bacteria, i.e. change of the appearance of PBP, alteration of transport porines, efflux pumps, production of enzymes that hydrolyzes antibiotics, i.e. beta-lactamases (Bonomo 2017).
Macrolides	Reduced binding affinity by the drug due to changes in binding site at antibiotic molecule or at bacterial ribosome, production of enzymes making interaction with protein synthesis harder, efflux pumps, other mutations in the genome (Fyfe *et al.* 2016; Dinos 2017).
Chloramphenicol	Enzymatic inactivation of the antibiotic, efflux pumps, change in binding site at bacteria (Schwarz *et al.* 2004).
Aminoglycosides	Decreased antibiotic uptake, modification of ribosomal target, efflux pumps, enzymatic modification of ribosomal target (Vakulenko & Mobashery 2003; Alekshun & Levy 2007).
Tetracyclines	Efflux pumps, alteration of target sites, increased permeability, production of enzymes (Schnappinger & Hillen 1996; Chopra & Roberts 2001).
Trimethoprim + Sulfamethoxazole	Efflux pumps, changes in permeability, regulation of target enzymes (Huovinen *et al*. 1995; Huovinen & Eliopoulos 2001).
Quinolones	Target-mediated resistance, production of proteins and enzymes acting against antibiotics, efflux systems and changes in permeability (Aldred *et al.* 2014).

2.2. Bacteria

2.2.1. *Salmonella* spp.

Salmonella spp. are a group of gram-negative, facultatively anaerobe bacteria within the family *Enterobacteriaceae*. Within the species, there are more than 1800 different serovars which are assigned to one of nine serogroups, categorizing bacteria and gives important epidemiologic information.

Like all gram-negative bacteria, *Salmonella* spp. has a cell envelope with lipo-polysaccharide (LPS) that may function as an endotoxin, which also has an important role when determining the pathogen's virulence and characteristics, explaining how *Salmonella* spp. pathogens can cause different diseases.

The most known disease caused by *Salmonella* spp. might be typhoid fever and secondly gastroenteritis. Typhoid fever is caused by *S. enterica* ser. Typhi and is mainly transmitted from person-to-person, while non-typhoid *Salmonella* spp. (NTS) cause gastroenteritis, which often is a result of consumption of contaminated food. The bacteria have an enormous animal reservoir, including chickens, pigs and cows, which make it prone to act as a zoonotic disease (Giannella 1996; Ibarra & Steele-Mortimer 2009).

Salmonella in the world

Around the world, *Salmonella* spp. is a frequently studied bacteria, mainly because of its high prevalence in animals and connection to foodborne illness. The bacteria are of importance when investigating the occurrence of resistance mechanism to antibiotics (Parisi *et al.* 2018). In an estimate by the World Health Organization (WHO) done in 2010, *Salmonella enterica* was the cause of foodborne diarrheal disease that resulted in the biggest disease burden, mainly in Africa and Southeast Asia. Children were more affected than grown persons. The study also concluded that NTS was one of the pathogens causing highest number of infections, with a total number of ca 153 million reported illnesses in total, where ca 78.5 million were of foodborne cause (Kirk *et al*. 2015).

Reported resistance among Salmonella spp.

Salmonella spp. have been frequently studied for several years. Before the introduce.tion of antibiotics, isolates were susceptible to most antibiotics classes (van den Bogaard & Stobberingh 2000). After the introduction of antibiotics, resistance towards different antibiotic classes occurred. In recent years, more and more MDR strains of Salmonella are being reported from multiple sources. Resistance patterns include resistance to tetracyclines, betalactams, cephalosporins, trimethoprim-sulfamethoxazole, quinolones. (Panhotra *et al.* 2004; Kumar *et al.* 2009; Michael & Schwarz 2016; Cameron-Veas *et al.* 2018; Parisi *et al.* 2018). With the emerging resistance to quinolones and other last choice antibiotics, a concern of failure of treatment of serious illnesses has arisen (WHO 2014a).

2.2.2. *Escherichia coli*

Escherichia coli is a gram-negative bacterium of the family *Enterobacteriaceae*. It is a facultative anaerobe bacteria that colonize the lower gut of most vertebrates but causes opportunistic infections in some cases.

E coli causes infections in both humans and animals of variable severity. The bacteria cause enteric disease, urinary tract infections and septicemia in humans and can cause similar diseases in animals, as well as mastitis. Dependent on the characteristics of the bacteria, *Escherichia coli* can be classified as EPEC (entero-pathogenic), ETEC (enterotoxigenic), EHEC (enterohemorrhagic) and EIEC (causing enteric inflammation) (Evans & Evans 1996).

The route of infection is fecal-oral and foodborne illness is frequently reported. Illness can occur among all age categories, but mortality is most common among infants, especially in developing countries (Baron 1996; Kirk *et al.* 2015).

Escherichia coli in the world

ETEC, a serogroup of *Escherichia coli*, is one of the pathogens resulting in most reported cases of illness in the world, around 240 million. Of these millions, 86.5 million were reported to be foodborne (Kirk *et al.* 2015). The bacteria are frequently studied due to their status as an indicator bacteria, which makes it possible to track resistance patterns and prevalence (van den Bogaard & Stobberingh 2000).

Reported resistance among Escherichia coli

Already during the 1950s, resistance against tetracyclines was detected among *E coli*, and after that resistance has become more and more widespread. It has been described how animals in groups previously treated with aminoglycosides and sulphonamides are carriers of bacteria with several resistance genes (Barton 2000).

Among *E coli*, MDR strains have become more frequent around the world, which can be explained by the transmittance of plasmids carrying resistance mechanisms. Therapeutic treatment of *E coli* infections is threatened due to the emerging resistance (Da Silva & Mendonça 2012; Allocati *et al*. 2013).

2.2.3. Vibrio cholerae

Vibrio cholerae is a gram-negative facultatively anaerobe bacterium shaped as slightly curved rods. Optimal growth is reached under aerobe conditions, but the bacterial numbers can increase in anaerobe settings.

The bacteria have around 200 serogroups, classified by somatic antigens, O-antigens. Serogroups O1 and O139 are known for causing endemic disease among humans (Lipp *et al*. 2002). These serogroups can further be divided into serotypes and biotypes based on biochemical properties (Faruque *et al.* 1998). A common trait within serogroups O1 and O139 is the production of cholerae toxin (CT) (Lipp *et al.* 2002). Apart from CT, some serogroups of cholerae produce other toxins such as shiga-like toxins, further complicating the illness (Kaper *et al.* 1995).

The bacteria are transmitted through water and foodstuff, with a fecal-oral infection route, resulting in foodborne illness. The sickness leads to hypovolemic shock and metabolic acidosis in severe cases, with potentially lethal outcomes. Outbreaks of the disease are often explosive and affect groups of humans (Kaper *et al*. 1995).

Vibrio cholerae in the world

Vibrio is almost endemic in areas with poor sanitation, with areas most affecting include Africa, South America, and Asia. Historically, it is known for being the cause of eight major pandemic outbreaks around the world (Faruque *et al.* 1998). An estimate done in 2010 shows that around 3 million illnesses caused by *Vibrio cholerae* were reported, of which 760 thousand were foodborne (drinking water not included). Around 100 thousand of the total reported cases resulted in death. The mortality rates were highest among children (Kirk *et al.* 2015).

Reported resistance among Vibrio cholerae

The antimicrobial drug of choice for cholera has been tetracyclines, but resistance towards the drug is reported to be widespread. Other possible therapies include chloramphenicol and trimethoprim-sulfamethoxazole (Kaper *et al*. 1995).

Studies done in India and Bangladesh suggest that resistance to quinolones and trimethoprim-sulfamethoxazole are appearing within different strains of the bacteria, but notable is that grade of resistance is dependent on geographical isolation and isolation time (Faruque *et al.* 1998).

2.2.4. Food-borne illness

Food as a source of infection with pathogenic bacteria has long been known. The risk of enteric bacteria spreading AMR has been pointed at for many years (Swann *et al.* 1969). With the rising development of AMR in bacteria known for causing food-borne illness (Butaye *et al*. 2006), the concern is of importance, and the high number of diseases caused by foodborne pathogens, and their consequences in society are big (Kirk *et al*. 2015). Commensal bacteria present in both animals and humans represent an important fraction for understanding epidemiological aspects of AMR. Commensals can act as a reservoir of resistance genes, which could be spread to pathogens. By monitoring commensal bacteria, it is possible to estimate which resistance mechanisms could be expected in pathogenic bacteria, and get a glimpse of how bacteria spread between animals and humans (van den Bogaard & Stobberingh 2000)

2.3. Antimicrobial resistance

Antimicrobial resistance (AMR) is a rapidly growing problem in the world with the potential to affect health care, economic and socioeconomic standards (Laxminarayan *et al.* 2013). The problem is not new, shortly after penicillin was introduced to clinical setting during the 1940s, resistance was reported (Ventola 2015), but during the last years, the situation has become more urgent due to the increasing number of MDR bacteria (Levy & Marshall 2004). MDR is often defined as bacteria resistant to three or more antibiotic groups (Magiorakos *et al*. 2012) and was early reported among enteric bacteria like *Escherichia coli*, *Salmonella* spp. and *Shigella* spp (Levy & Marshall 2004). Among gram negatives, MDR is getting more widespread to the extent of being totally drug-resistant (TDR). Some bacteria within the family of Enterobacteriaceae are displaying ESBL, which makes them significantly harder to treat (Levy & Marshall 2004).

MDR bacteria are seen in the whole world (WHO 2014a) and the growing movement of humans, animals, and food around the world is complicating the matter (WHO 2015a). Identified as important for managing the growing number of AMR bacteria are information, hygiene practice, guidelines for antibiotic use, and surveillance of AMR prevalence within different sectors (FAO 2016; OIE 2016).

2.3.1. Development of resistance

Resistance towards antimicrobial agents is due to AMU within all sectors; human medicine, veterinary medicine, within livestock production as AGPs, within aquatic environments, and within agricultural sectors (FAO 2016). In the presence of antibiotics, bacteria with resistance qualities may survive and reproduce, creating a bigger population of bacteria carrying resistance genes. Another important contributor to increased resistance is subtherapeutic concentration of antibiotics, poor quality of drugs, and time under antibiotic treatment (Levy & Marshall 2004).

Intrinsic and acquired resistance mechanisms

Naturally occurring genes situated on the host´s chromosome which result in AMR among bacteria are classified as intrinsic resistance mechanisms. Among these mechanisms, production of beta-lactamases and specific efflux systems are being described. Mutations in genes targeted by antimicrobial agents and resistance spread on mobile elements such as plasmids, bacteriophages, and transposons are regarded to be acquired resistance mechanisms (Alekshun & Levy 2007).

Transfer of resistance genes

Genetically mobile elements are one of the contributing factors to the spread of resistance among bacteria. These elements can carry both resistance and virulence mechanisms, resulting in a possibly devastating combination where new, more virulent bacteria that also are resistant to antibiotic could emerge (Laxminarayan *et al.* 2013). Among these mobile elements responsible for transfer of resistance genes, plasmids, bacteriophages, transposons, and naked DNA are of importance (Levy & Marshall 2004). A bacterium can receive multiple plasmids from different bacteria, carrying different genes that are coding for different resistance mechanisms. Genes from plasmids like this can get incorporated into the genome of the recipient bacteria (Alekshun & Levy 2007). This process is called horizontal gene transfer (HGT) and can occur through transformation (incorporation of chromosomal DNA or plasmids into another chromosome), transduction (by the act of bacteriophages), or conjugation (by plasmids or transposons) (Blair *et al.* 2015). HGT has been described to occur not only within the same species of bacteria, but also between completely different families, and even between gram-negative and gram-positive bacteria (Alekshun & Levy 2007).

Loss of resistance

Studies have shown that bacteria that have developed resistance can undergo loss of resistance genes (Barbosa & Levy 2000), but this is a time-consuming process and when antibiotics once again are introduced into the setting, resistance rapidly increases again (Levy & Marshall 2004).

2.3.2. AMR in livestock production and aquatic environments

Antibiotics are being used for treatment and non-therapeutical purposes (animal growth promotors – AGP) within terrestrial, aquatic and plant production.

AMU used as a preventive method at a herd level (Van Boeckel *et al.* 2015), contributes to maintaining healthy animals in environments with poor biosecurity and husbandry (Page & Gautier 2012). It can also, in some circumstances, provide economic benefits for the farmer, even if that statement depends on other variable factors (Laxminarayan *et al.* 2015).

Globally, the demand for animal products for consumption is rising, which has led to the start of more intensive farming with big units. This type of farming depends on use of antibiotics to maintain good health among animals and a high production rate (Tiseo *et al.* 2020). Correlation between AMU in livestock and presence of AMR bacteria have been concluded (Chantziaras *et al.* 2014) and studies have also shown same resistance patterns among bacteria in animals and farmers (Katsunuma *et al.* 2007). AMR has also been seen to spread both through direct contact (Smith *et al*. 2013) and the environment (Chee-Sanford *et al*. 2009; Graham *et al*. 2009), even if the latter is dependent on many factors (Chee-Sanford *et al.* 2009). Poor surveillance of AMU and AMR within livestock production (OIE 2017) contributes to a concern for the global situation, and estimates of annual consumption vary due to lack of proper surveillance (FAO 2016).

In an analysis done by OIE 2015, 74% of the member countries did not authorise antimicrobials agents as AGPs (Moulin *et al.* 2016). However, in many LMICs AMU and AMR is largely undocumented (WHO 2015b; Schar *et al.* 2018), in contrast to many high-income countries, where AGPs are banned or restricted and surveillance of AMU and AMR within veterinary practice is present (Maron *et al*. 2013; WHO 2015b; OIE 2017).

A large amount of AMU is in animal production (Van Boeckel *et al*. 2015). An estimate made in 2017 establishes that the global consumption of veterinary anti-microbials was 93,309 tonnes and that an increase of 11.5% could be expected by 2030 (104,079 tonnes). In Asia, the projected antimicrobial use by 2030 is 68% of the total antimicrobial consumption in 2017. Europe, Oceania, and North America are predicted to have the lowest rise in AMU in 2030, and Africa is predicted to have the highest rise (Tiseo *et al.* 2020).

Furthermore, a high number of reports suggest that antibiotic use in aquaculture affects the presence of AMR bacteria in other niches, as well as providing a route for reaching human population through ingestion of the substance. This can cause both anaphylactic reactions and contribute to development of AMR in humans (Cabello 2006). Aquatic production is a rapidly growing food section (Henriksson *et al*. 2018) and needs to be included in studies.

2.3.3. AMR situation in LMICs

The AMR situation in low- and middle-income countries (LMICs) is hard to assess due to lacking surveillance of both AMU and AMR, especially within the veterinary sector (FAO 2016; Schar *et al.* 2018). Laxminarayan *et al.* (2013) state that use of antibiotics increases with economical advances, number hospitalized within human sector, and prevalence of infectious diseases and Levy & Marshall (2004) say that emerging enteric diseases caused by resistant strains of *Salmonella enteriditis*, *Shigella flexneri* and *Vibrio cholerae* is threatening public health in many LMICs.

Factors contributing to the problem of AMR in LMICs are the availability of proper health care within human and veterinary sectors, lack of education about the topic, low biosecurity, challenges regarding safe food, and access to over-the-counter drugs (OTC) (WHO 2015b; FAO 2016). In many cases, antibiotics available are also of poor quality, further complicating the matter (WHO 2015b).

Within the veterinary sector, there is a need for support among OIE Member Countries to develop policies, legislation and use of quality veterinary medicines, including antibiotics (OIE 2016). This area is also discussed by Schar *et al.* (2018) who notice that many LMICs have banned AGPs, but regulatory structures are often insufficient to monitor and enforce the bans.

2.3.4. Global work against AMR

In 2015 the World Health Organization issued a global action plan (GAP) on antimicrobial resistance. This plan comes with five major bullet points: 1) to improve awareness and understanding of antimicrobial resistance through effective communication, education and training; 2) to strengthen the knowledge and evidence base through surveillance and research; 3) to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; 4) to optimize the use of antimicrobial medicines in human and animal health; and 5) to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions. These five bullet points, and the GAP itself, was made to serve as a template for countries to develop national action plans. (WHO 2015a).

Both Food and Agriculture Organization (FAO) and World Organisation for Animal Health (OIE) have contributed with their own action plans within their specific area to help the One Health concept. In these ones, specific approaches as development of surveillance of both AMU and AMR in the food and agriculture sectors (FAO 2016), and try to ensure that veterinary services have the ability to work according to OIE standards (OIE 2016) are mentioned.

2.4. Bangladesh

Bangladesh is a country in south Asia situated between India and Myanmar. The country has one of the world's most dense populations and faces a lot of challenges regarding health care and public health. One of the major concerns related to public health is access to safe food. Food products are at risk of being contaminated with microbial organisms, toxins, and chemical substances (WHO 2014b).

Big parts of the country are rural, and have a large scale of agricultural features (Orubu *et al.* 2020), many people live in close contact with animals and this in combination with a widespread problem of AMR make the risk for emergence of zoonotic disease and AMR transmission high (Ahmed *et al.* 2019; Orubu *et al*. 2020).

2.4.1. Antimicrobial use in Bangladesh within the food- and agricultural sectors

Present policies

Bangladesh, like many of its neighbouring countries in Asia, faces the problem with unregulated AMU (Hoque *et al.* 2020). A national action plan (BNAP) for containment of AMR was endorsed in 2017 which discusses the country's policies for improving AMU and AMR by multisectoral measures like implementing rational AMU, surveillance of AMR, and enforcing present laws (WHO, 2022). This plan is aligned to the Global action plan issued by WHO in 2015 and is complemented by a road map.

A study by Orubu *et al*. (2020) has evaluated the BNAP by comparing it to the GAP. In this study, BNAP was found to align well with the GAP, but a few policy gaps regarding financing modality, specifications for AMR stewardship in the veterinary sector, and frameworks for monitoring and evaluation were found. Furthermore, they concluded that these gaps need to be addressed for successful veterinary AMR containment.

Another study made by Hoque *et al.* (2020) states that several policies, guidelines and laws regulating the use of antibiotics, and thereby are connected to the resistance situation, are in place.

The government of Bangladesh has developed policy documents related to AMR in the human sector, and the Ministry of Fisheries and Livestock has formed a National Livestock Development Policy where it is highlighted that inadequate veterinary services and weak implementation of regulatory frameworks are barriers to address AMR in this sector. Laws banning AGPs and guidelines for waste management are present since a couple of years back. However, the author discusses that the policies and strategies are at an early stage of development, poor awareness among policymakers and practitioners, inadequate veterinary service and that there are challenges regarding implementation of these regulations.

Access to antibiotics

In Bangladesh, the animal health care system is inadequate, which results in informal health care providers being contacted when animals get sick (Roess *et al.* 2015). The health care system provides different categorisations, where "village doctors" or quacks are among categories with no formal training (also mentioned as paraveterinarians) and veterinary graduates classify as formally trained. Also, employees in pharmacies range from persons with no training to graduated pharmacists (Rousham *et al*. 2019). A study has shown that people went to the local bazaar to get medicines for their animal, or to what they called a practitioner. If the practitioner was licensed or not however was unclear (Lucas *et al.* 2019). Poor infrastructure was mentioned as one of the reasons for not getting adequate help from a government veterinarian, but it was also discussed that villagers rather contact a drug seller or pharmacy than a government veterinarian to get medicine for their animals since the veterinarian would charge them more. It was also described that animals get antibiotic treatment without a previous prescription, contributing to the overall use of antibiotics (Roess *et al.* 2015).

In a study made by Lucas *et al.* (2019) it is suggested that household members pursue antibiotics from five different sources: drug shops, private clinics, government/charity hospitals, community/family planning clinics and specialized/private hospitals. Antibiotics purchased from these actors were made by a family member with, or most often without, prescription. Antibiotics were bought for both animals and people. The study suggests that the availability and prices were the two things that had an effect on which actor was chosen for the purchase.

The availability of over-the-counter antibiotics in LMICs is a factor contributing to the development of antibiotic resistance (FAO 2016). This route of distribution also occur in Bangladesh, and studies have shown that both prescription of antibiotics, and use without a prescription, is inappropriate in many cases (Biswas *et al.* 2014; Mohiuddin *et al*. 2015). Further complicating the situation in Bangladesh is the number of drug sellers and unlicensed drug sellers (SIAPS 2015). Practically all retailers distribute antibiotics, but only a few have educated staff (Lucas *et al.* 2019).

2.4.2. Antimicrobial resistance in Bangladesh within the veterinary sector

AMR surveillance within the veterinary sector in Bangladesh is considered to be inadequate (Hoque *et al.* 2020) and the surveillance that has been made is not standardized, which implements problems while interpreting data (Ahmed *et al*. 2019).

The issue of AMR in domestic animals, in wildlife, from food sources and the environment are discussed in a review article from 2020. 45 articles were included and 15 described resistance patterns among *Escherichia coli*, and twelve among *Salmonella* spp. Samples were divided into groups, where the highest prevalence of E coli was from food sources but was still over 50% among domestic animals. Regarding *Salmonella*, prevalence varied between 10% to 91% between the different groups. Resistance patterns differed between the two bacteria where among *Escherichia coli*, the highest percentage resistance in the food group was towards tetracyclines, followed by aminopenicillins and among *Salmonella* spp. the highest percentage among food samples was to aminoglycosides, followed by tetracyclines (Khan *et al*. 2020).

Another review article discusses various short-term studies within the areas of food animals, fisheries, and environmental issues regarding AMR. It is found that residues of antimicrobial substances as tetracycline, ciprofloxacin, enrofloxacin and amoxicillin were present in poultry and eggs made for human consumption. Manure from poultry farms is used in aquatic environments, risking spreading antibiotic residues in water and with that increasing the risk for AMR bacteria. Presence of AMR bacteria in water can further affect humans. From a study made in Chittagong, among a number of samples positive for *Salmonella* spp. all categorised as MDR (Hoque *et al.* 2020).

The AMR situation in Bangladesh, especially within the veterinary sector, is still much unknown (Orubu *et al.* 2020), and further development of surveillance, preferable long-term surveillance is of importance in the matter (Hoque *et al.* 2020).

3. Material and methods

3.1. Literature review

The literature review was based on searches on the databases Web of Science and PubMed. Keywords were related to the different topics: antibiotics, resistance, bacteria, and antibiotic use/resistance in LMICs. Within the topics, words such as AMR, antimicrobial resistance, antibiotics, LMICs, were used in different combinations.

For charting the present situation in Bangladesh regarding AMR, articles published between 2014-2020 were included, preferably made in Bangladesh or countries similar to Bangladesh regarding socioeconomic standards, way of living and structure of health care and drug stores. Both review articles and primary sources were included. Articles were to discuss topics affecting central parts of the AMU and AMR situation in Bangladesh, where examples as to how people seek advice for care for both humans and animals, and also how pharmacies work and consider their responsibility in the matter. Relevant articles from lists of references were also included.

3.2. Analyses of AMR in Bangladesh food products

This was a cross-sectional study of antimicrobial prevalence, charting of AMR-pattern and interviews made with vendors based on previously made fieldwork done in Bangladesh from November 2018 to June 2019. This fieldwork, including laboratory work, was done by Bangladesh Livestock Research Institute (BLRI), Bangladesh Food Safety Authority (BFSA), International Livestock Research Institute (ILRI), and International Food Policy Research Institute (IFPRI).

Complementary interviews, descriptive and statistical analysis of data from previously collected material mentioned above were done during November and December 2020 as a part of this thesis.

3.2.1. Sampling and analysis

All sampling and analyses of samples were previously made by actors from BLRI, BFSA, ILRI and IFPRI.

Food products taken for cultivation of microbes were collected from different markets in Bangladesh, i.e rural, peri-urban, and urban. The samples of food products were of three sources: tomato, fish, and chicken. Food products were chosen of importance as a food source in the country, where fish and chicken (poultry) are common sources of protein from animals and tomatoes represent a common vegetable commonly consumed raw. All food samples taken were fresh and raw when sampled, see table 3 for specification of amount of food sampled.

Table 3. Food products sampled

Fresh fish (Pangash)	Chicken (poultry)	Tomato
~300g edible portion per vendor, *"several smaller fish (if <3-6 cm wide), one whole fish, or small pieces of multiple larger fish"*	>300g broiler per vendor *"one small whole chicken, or ½ of a larger chicken"*	~ 150-300 g tomatoes per vendor, *"2-4 tomatoes, depending on size"*

Fish were preferable of Pangash type, but other kinds of fish were included. Preferably fish came from aquaculture, but the vendors were not asked to verify this upon sampling. Chicken came from different kinds of housing, but vendors were not asked to specify this during sampling.

A total number of 976 samples were collected from 853 different vendors in Dhaka city (urban), Savar (peri-urban) and Netrokona (rural) district, displayed in figure 1. In Dhaka, samples came from both traditional markets and supermarkets and in Savar and Netrokona districts samples came from traditional markets. The number of samples to be taken from each outlet depended on the census, and at markets with multiple vendors selection of samples was based on systematic random method. An estimate of the total number of stalls was made and divided by the number of samples needed to get the proportion of stalls that should be sampled, i.e, if the total number of stalls were 40 and ten samples were needed, every fourth stall was included. The stall nearest the market entry was to be the first stall sampled. Samples are regarded to be unique if being from different vendors, except when a vendor sold more than one product of interest, where multiple samples from different food sources could be taken at the same stall.

Figure 1. Map over Bangladesh including sites for sampling.

Of the samples, 250 were fish, 366 tomatoes and 360 chicken. Of the total number of 976 samples, 974 were included in the study due to missing data in two cases. Only 249 samples of fish were analysed for *Vibrio cholerae* and *Escherichia coli* vs 359 samples of chicken for *Salmonella* spp. Samples were to be held cool during transport to BLRI for analysis.

Isolation and identification of *Escherichia coli*, *Salmonella* spp. and *Vibrio cholerae* were carried out through conventional culture method, where fish were analysed for prevalence of *Vibrio cholerae* and *Escherichia coli*, chicken for *Salmonella* and tomato for *Salmonella* and *Escherichia coli*. For a subgroup of samples positive for bacterial cultivation, i.e. positive samples from rural and urban settings, quantitative data of the logarithm of the colony-forming units (LogCFU) for total coliform count, coliform count and *E. coli* count were also available.

Samples with positive cultivation were tested for antibiotic susceptibility (AST) against eleven different antibiotics, including beta-lactam with clavulanic acid, penicillin, tetracyclines, aminoglycosides, chloramphenicol, cephalosporins, folate pathway inhibitors, macrolides, and quinolones through standard disc diffusion. The AST panel was chosen at the laboratory due to current routines and in accordance with CLSI 2016 and VITEK-2. One sample (from tomato) positive for *E. coli* was not tested for antibiotic susceptibility. All laboratory analyses were done at BLRI.

3.2.2. Questionnaires

Interviews made with the vendors of products chosen for sampling was held in connection with the sampling. The interviews included questions about the type of stall, knowledge about how to handle foodstuff, and hygiene routines. These questionnaires are included as a supplement for background information (Annex 1).

3.3. Mapping of factors related to AMR

During December 2020, complementary key informant interviews (KII) were held with key persons working with AMR in Bangladesh. A questionnaire and invite to a discussion over zoom were sent out to 15 persons. Seven persons participated through interviews via zoom where the questionnaire was filled in subsequently, and eight persons did not respond to the invite because of unknown reasons. Interviews were held in English, with possibility to clarify questions in Bangla when needed. All interviews were held with the same interviewer, all participants were assured to be anonymous in this thesis.

Key persons were chosen based on their work assignment and insight of the current situation regarding AMR in Bangladesh. Persons from different work categories such as government workers from both human health and animal health departments, other specialized departments connected to animal health and food security, pharmaceutical companies, universities, laboratories carrying out analysis for AMR and epidemiologists were asked to participate. The seven persons that participated were connected to import and sales of antibiotics, specialist knowledge of poultry and fish, connected to governmental work regarding human health and animal health and laboratory work within the livestock sector.

Participants were presented with 9-21 questions dependent on which work assignments they had. Answers from the interview were inserted into a template to get an overview of similarities and differences between the answers, trying to identify shared opinions about AMU and AMR.

4. Results

4.1. Survey results

Traditional markets were located at all different study sites, while supermarkets, called “supershops”, were found in urban areas. Total number of samples from urban area was 467 samples, where 242 samples were collected in supershops and 225 at traditional markets. The number of samples from peri-urban area was 255 and from rural 252. The 974 samples were taken in stalls of 853 different vendors. The vendors, i.e respondents, filled in a form of background questions, which products were sold in the stall and how the products were stored in the stall, see table 4.

The majority of respondents were men, only 1.8% consisted of women. Slightly more than half of the respondents were in the age group 20-35 (50.3%), but statistical analysis showed no significant difference in age of men and women.

Primary school was stated as highest completed education in 38.2% of answers, followed by 25.6 % that had completed class 5-10. Highest education completed differed between men and woman, where 33.3% of the women had completed graduation and above, in contrast to 7.3% of men in the same category. Of vendors working in supershops, almost all women had reported “graduation and above” as highest education completed, even though no statistical significance was detected. Among women working in traditional markets, 7/9 had no education, which was a significantly higher proportion compared to men ($p=0.034$).

Most of the samples and participants came from traditional markets. However, among the females, there were only slightly more participants from traditional (60.0%) than from supershops (40.0%), while among men the vast majority were from traditional markets (traditional 86.3% and supershop 13.7%). Overall, it was significantly more common that a supershop worker was female than in traditional markets ($p=0.004$). The distribution of men and women at different study sites showed no significant difference in statistical analysis.

Only men sold fish, and the rest of the men sold tomatoes (37.0%) and chicken (37.6%). Most women, 86.7%, sold tomato, and 13.3% sold chicken. There was a significant difference ($p<0.001$) between products sold by women or men. The odds ratio (OR) for the vendor being female was 6.6 times higher (95% CI 1.5-29.5, p<0.013) for tomatoes than for chicken.

Table 4. Responders in the study divided by gender, age, highest education completed, study site, market type, type of product sold, cooling of product

Total number of respondents in each category	**Female (% of female)** 15 (100)	**Male (% of male)** 838 (100)	**Total (% of total)** 853 (100)
Age of respondents			
<20	1 (6.67)	30 (3.58)	31 (3.63)
20-35	9 (60.00)	420 (50.12)	429 (50.29)
36-50	5 (33.33)	357 (42.60)	362 (42.44)
>51	0	30 (3.58)	30 (3.52)
N/A	0	1 (0.12)	1 (0.12)
Highest education completed			
None	7 (46.67)	192 (22.91)	199 (22.51)
Primary	1 (6.67)	325 (38.78)	326 (38.22)
Class 5–10	2 (13.33)	216 (25.78)	218 (25.56)
High school	0	44 (5.25)	44 (5.16)
Graduation and above	5 (33.33)	61 (7.28)	66 (7.74)
Study site			
Rural	2 (13.33)	250 (29.83)	252 (29.54)
Peri-urban	5 (33.33)	250 (29.83)	255 (29.89)
Urban	8 (53.33)	338 (40.33)	346 (40.56)
Market type			
Supershop	6 (0.40)	115 (13.72)	121 (14.19)
Traditional	9 (0.60)	723 (86.28)	732 (85.81)
Type of product sold			
Chicken	2 (13.33)	315 (37.59)	317 (37.16)
Fish	0	213 (25.42)	213 (24.97)
Tomato	13 (86.67)	310 (36.99)	323 (37.87)
Is the product cooled			
No	14 (93.33)	697 (83.17)	711 (83.35)
Yes, unspecified	0	5 (0.60)	5 (0.59)
Yes, in cool box	0	52 (6.21)	52 (6.10)
Yes, open to environment but on ice	1 (6.67)	84 (10.02)	85 (9.96)

Of the products sold, 711 (83.4%) were not on ice in the stall, and 142 (16.7%) were somehow cooled (Table 1). If the product were cooled or not did not depend on gender, however, there was a significant difference (p<0.001) in cooling between products. Fish was more likely to be on cooling compared to chicken, OR 3.6 (95% CI 2.39-5.53, p<0.001), and tomatoes were less likely to be cold than chicken OR 0.3 (95% CI 0.19-0.60, p<0.001). What more affected if the product were cold was the type of shop, where supershops were more likely to have cold products compared to traditional markets, OR 31.5 (95% CI 19.4-51.0, p<0.001).

Data showed that in supershops, 73/74 fish were held in cold. Study site did also affect cooling, where, compared to rural, shops in peri-urban area were less likely to have cold products (OR 0.1, 95% CI 0.05-0.32, p<0.001) and shops in urban area were more likely to have cold products (OR 2.6, 95% CI 1.69-3.97, p <0.001).

4.2. Prevalence of bacteria

Of the 974 samples taken, 615 were analysed for two different bacteria (*Escherichia coli* and *Vibrio cholerae*) and 359 for one bacterium (*Salmonella* spp.). Of the total number of cultivations, 320 (20.1%) were positive.

Table 5. Number analysed samples and number positive samples (%)

	Number positives /number analysed (%)	**Samples positive per study site, including percent of samples taken (%)**		
		Rural	**Peri-urban**	**Urban**
Tomato				
Salmonella spp.	27/366 (7.38)	15/96 (15.63)	2/96 (2.08)	10 /174 (5.75)
Escherichia coli	52/366 (14.21)	10/96 (10.42)	29/96 (30.21)	13/174 (7.47)
Chicken				
Salmonella spp.	62/359 (17.27)	25/95 (26.32)	14/94 (14.89)	23/170 (13.53)
Fish				
Escherichia coli	67/249 (26.91)	27/61 (44.26)	22/65 (33.85)	18/123 (14.63)
Vibrio cholerae	112/249 (44.98)	24/61 (39.34)	26/65 (40.00)	62/123 (50.41)
Total number	320/1589 (20.14)	101/409 (24.69)	93/416 (22.36)	126/764 (16.49)

Positive samples from cultivation are displayed in table 5. In total, 89 samples were positive for *Salmonella* spp., representing 12.3% of total analyses done for the bacterium. Of the analyses made for *Escherichia coli,* 119 (19.4%) were positive and corresponding number for *Vibrio cholerae* was 112 (45.0%). All areas had positive samples, but there were differences between them, with peri-urban having higher proportion tomatoes positive for *E coli* and rural had a higher proportion chicken positive for *Salmonella* (table 5).

Samples were less likely to be positive for *Salmonella* spp. if they came from peri-urban and urban area, compared to rural (OR 0.3, 95% CI 0.18-0.63, p=0.001 resp. OR 0.4, 95% CI 0.24-0.65, p<0.001), and if the product were tomato compared to chicken (OR =0.4, 95% CI 0.23-0.61, p<0.001). For *Escherichia coli*, the odds ratio for a positive cultivation was 3.6 times higher (OR 3.5, 95% CI 1.56-8.08, p=0.003) if the sample came from a traditional market. If the sample came from peri-urban area, it was also more likely to be positive for *E coli* (OR 2.4, 95%

CI 1.37-4.31, p=0.002) and if the sample was from fish, the odds ratio for being positive was 2.5 times higher (OR 2.5, 95% CI 1.64-3.83, p<0.001).

There was no significant difference in positive respectively negative cultivations for *Salmonella* spp. or *E coli* dependent on if the product were held cold.

4.2.1. Quantification of bacteria

Logarithmic values for colony forming units (LogCFU) of coliform count (CC) and total coliform count (TCC) were performed on a subgroup of samples positive for bacterial prevalence. Subgroups of samples positive for *Escherichia coli* had the total amount of *E. coli* colonies counted (TEC) in addition to these values. The logarithmic values for coliform count had a span from 0 (not detected) – TNTC (too numerous to count). The values for total coliform count were 0 - TNTC and for total Escherichia coli count 0 - TNTC. The values classified as TNTC has been given the number 8 in statistical analysis in this study. Figure 2 shows the distribution for LogCFU CC.

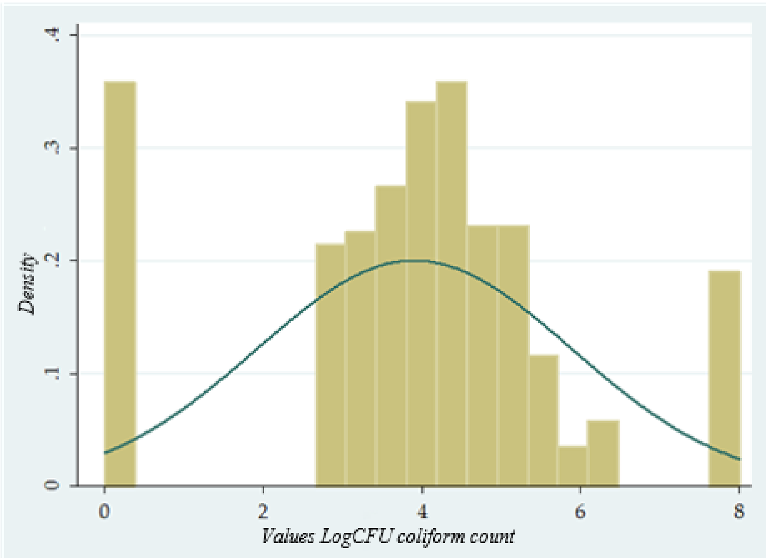

Figure 2. Bell curve for coliform count.

Logarithmic values for total coliforms were significantly higher in traditional markets compared to supershops (3.9 and 3.2 respectively, p=0.0001) and in products not being cooled (3.8 and 3.3 respectively, p=0.017).

Among tomato, the logarithmic values for total coliform count were significantly higher than for fish (3.8 resp. 3.4, p=0.037), but no statistical difference was detected for logarithmic values for coliform count.

4.3. Antimicrobial resistance

A total of 319 samples were tested for antimicrobial susceptibility through standard disc diffusion. Susceptibility for Amoxicillin + clavulanic acid (AMC), Cefixime (CFM), Ceftriaxone (CRO), Chloramphenicol (C), Erythromycin (E), Gentamicin (CN), Streptomycin (S), Penicillin G (P), Tetracycline (TET), Sulfamethoxazole + trimethoprim (SXT) and Nalidixic acid (NA) was tested in the panel. CFM and CRO, as well as CN and S, were counted as having the same antimicrobial spectra, i.e being the same group. All the other substances were counted as separate groups. Cut-off values are displayed in table 6.

Table 6. Cut off values for disc diffusion test for antimicrobial susceptibility. Unit µg/mm (except for P; units/mm)

	S	**I**	**R**
AMC	≥18	14–17	≤13
CFM	≥19	16–18	≤15
CRO	≥23	20–22	≤19
C	≥18	13–17	≤12
E	≥23	14–22	≤13
CN	≥15	13–14	≤12
S	≥15	12–14	≤11
P	≥29	-	≤28
TET	≥15	12–14	≤11
SXT	≥16	11–15	≤10
NA	≥19	14–18	≤13

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

The 319 samples were categorised as sensitive (S), intermediate (I), and resistant (R) to each of the tested substances in the panel. Categorisation was made based on disc diffusion values for every sample. The total number of samples were placed in groups due to product and prevalence of bacteria, displayed in table 7, table 8, table 9, table 10 and table 11.

Table 7. Antimicrobial susceptibility test for samples of tomato positive for Salmonella spp

	S		I		R		
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**
AMC	16	59.26%	1	3.70%	10	37.04%	27
CFM	27	100.00%	0	0%	0	0%	27
CRO	27	100.00%	0	0%	0	0%	27
C	19	70.37%	4	14.81%	4	14.81%	27
E	10	37.04%	0	0.00%	17	62.96%	27
CN	22	81.48%	0	0%	5	18.52%	27
S	16	59.26%	4	14.81%	7	25.93%	27
P	7	25.93%			20	74.07%	27
TET	10	37.04%	0	0%	17	62.96%	27
SXT	18	66.67%	1	3.70%	8	29.63%	27
NA	19	70.37%	2	7.41%	6	22.22%	27

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

Table 8. Antimicrobial susceptibility test for samples of tomato positive for Escherichia coli

	S		I		R		
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**
AMC	22	43.14%	18	35.29%	11	21.57%	51
CFM	41	80.39%	4	7.84%	6	11.76%	51
CRO	42	82.35%	5	9.80%	4	7.84%	51
C	30	58.82%	6	11.76%	15	29.41%	51
E	13	25.49%	3	5.88%	35	68.63%	51
CN	40	78.43%	7	13.73%	4	7.84%	51
S	7	13.73%	4	7.84%	40	78.43%	51
P	0	0%			51	100.00%	51
TET	16	31.37%	0	0%	35	68.63%	51
SXT	25	49.02%	2	3.92%	24	47.06%	51
NA	19	37.25%	18	35.29%	14	27.45%	51

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

Table 9. Antimicrobial susceptibility test for samples of chicken positive for Salmonella spp

	S		I		R		
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**
AMC	29	46.77%	10	16.13%	23	37.10%	62
CFM	58	93.55%	1	1.61%	3	4.84%	62
CRO	51	82.26%	7	11.29%	4	6.45%	62
C	44	70.97%	5	8.06%	13	20.97%	62
E	7	11.29%	1	1.61%	54	87.10%	62
CN	44	70.97%	5	8.06%	13	20.97%	62
S	24	38.71%	5	8.06%	33	53.23%	62
P	2	3.23%			60	96.77%	62
TET	4	6.45%	0	0%	58	93.55%	62
SXT	11	17.74%	7	11.29%	44	70.97%	62
NA	24	38.71%	7	11.29%	31	50.00%	62

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

Table 10. Antimicrobial susceptibility test for samples of fish positive for Escherichia coli

	S		I		R		Total
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**
AMC	24	35.82%	33	49.25%	10	14.93%	67
CFM	47	70.15%	6	8.96%	14	20.90%	67
CRO	44	65.67%	16	23.88%	7	10.45%	67
C	35	52.24%	6	8.96%	26	38.81%	67
E	28	41.79%	9	13.43%	30	44.78%	67
CN	49	73.13%	6	8.96%	12	17.91%	67
S	20	29.85%	3	4.48%	44	65.67%	67
P	0	0%			67	100.00%	67
TET	20	29.85%	1	1.49%	46	68.66%	67
SXT	32	47.76%	1	1.49%	34	50.75%	67
NA	20	29.85%	23	34.33%	24	35.82%	67

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

Table 11. Antimicrobial susceptibility test for samples of fish positive for Vibrio cholerae

	S		I		R		Total
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**
AMC	65	58.04%	22	19.64%	25	22.32%	112
CFM	76	67.86%	13	11.61%	23	20.54%	112
CRO	72	64.29%	16	14.29%	24	21.43%	112
C	98	87.50%	5	4.46%	9	8.04%	112
E	36	32.14%	40	35.71%	36	32.14%	112
CN	75	66.96%	10	8.93%	27	24.11%	112
S	56	50.00%	17	15.18%	39	34.82%	112
P	0	0%	.	.	112	100.00%	112
TET	89	79.46%	7	6.25%	16	14.29%	112
SXT	75	66.96%	13	11.61%	24	21.43%	112
NA	71	63.39%	16	14.29%	25	22.32%	112

* AMC: amoxicillin + clavulanic acid, CFM: cefixime, CRO: ceftriaxone, C: chloramphenicol, E: erythromycin, CN: gentamicin, S: streptomycin, P: penicillin G, TET: tetracycline, SXT: sulfamethoxazole + trimethoprim, NA: nalidixic acid

Among tomatoes positive for *Salmonella*, the highest percentage of resistance was seen for Penicillin G (74.1%), followed by Erythromycin (63.0%) and Tetracycline (63.0%). Tomato positive for *E coli* had a similar pattern: 100% resistance to Penicillin G and 68.6% for Erythromycin and Tetracycline. However, second highest percentage of resistance was found against Streptomycin (78.4%). Chicken positive for *Salmonella* showed highest values of resistance for Penicillin G (96.8%), followed by Tetracycline (93.6%) and Erythromycin (87.1%), but were also high for Sulfamethoxazole+trimethoprim (71.0%). Regarding samples from fish, highest percentage of resistance was found to Penicillin G (100%) for both *Vibrio* and *E coli*, followed by 65.7% resistance to Streptomycin and 68.7% to Tetracycline for *E coli*, and to Streptomycin and Erythromycin (34.8% resp. 32.1%) for *Vibrio*. Fish samples positive for *Vibrio* had only one category, penicillin, where the proportion resistant exceeded 50%.

Table 12. Number of samples classified as MDR

	Positive samples from cultivation/total samples analysed	Number MDR of positive samples (%)	Number MDR of total amount samples (%)
Tomato			
Salmonella spp.	27/366	12/27 (44.44)	12/366 (3.28)
Escherichia coli	51/366	41/51 (80.39)	41/366 (11.20)
Chicken			
Salmonella spp.	62/359	53/62 (85.48)	53/359 (14.76)
Fish			
Escherichia coli	67/249	47/67 (70.15)	47/249 (18.88)
Vibrio cholerae	112/249	50/112 (44.64)	50/249 (20.08)
Total	319/1589	203/319 (63.64)	203/1589 (12.78)

Samples that were resistant to three or more substance groups were classified as being MDR. Number of samples classified in this group are displayed in table 12. *Salmonella* spp. and *Escherichia coli* are considered to be naturally resistant to Penicillin G, and resistance to this antibiotic is not included in the MDR classification.

Of the 89 samples positive for *Salmonella* spp., 65 tested for antimicrobial susceptibility classified as MDR, representing 73.0% of the total number positive samples for the bacterium. Corresponding numbers for *Escherichia coli* and *Vibrio cholerae* were 88 out of 118 (74.6%) and 50 out of 112 (44.6%) respectively.

In total, 63.6% of the total number positive cultivations classified as being MDR, where the largest number was found among samples from chicken positive for *Salmonella* spp (85.5% of positive cultivations classified as MDR). The second largest observation was made among tomato analysed for prevalence of *Escherichia coli*, where 80.4% of positive cultivations classified as MDR. For fish samples analysed for both *Vibrio cholerae* and *Escherichia coli*, the percentage classified as MDR of positive samples was 44.6% respectively 70.2%. This represents a lower proportion than among tomatoes and chicken analysed, however in relationship to the total number of fish samples taken, more than 20% of fish samples had MDR.

For *Vibrio cholerae*, there was a significant difference ($p<0.001$) in MDR between samples from different study sites, and also from supershop versus traditional market ($p=0.007$). The same result regarding different study sites was not found among samples positive for *Salmonella* and *Escherichia coli*. Samples of tomato, positive for *E coli* however, had a significant difference in MDR between supershops and traditional markets ($p=0.007$). For samples positive of *Salmonella*, there was a significant difference ($p<0.001$) in prevalence of MDR, where numbers were higher in chicken than tomato.

4.4. Key informant interviews

Interviews made with the aim to pinpoint problems around AMR in Bangladesh highlighted a number of opinions on the matter. All key informants (KI) classified AMR as a national problem in Bangladesh. Many of the participating persons pointed at things like “lack of education”, “not enough enforcement of laws”, “not enough cooperation between different actors” and “unregulated pharmacies” as major problems both regarding AMU and AMR. Other aspects as “not enough of laboratory facilities” and “lack of knowledge for how to use laboratory facilities” were also pointed out as contributing factors to both the AMU and lack of national long-term surveillance of AMR in Bangladesh.

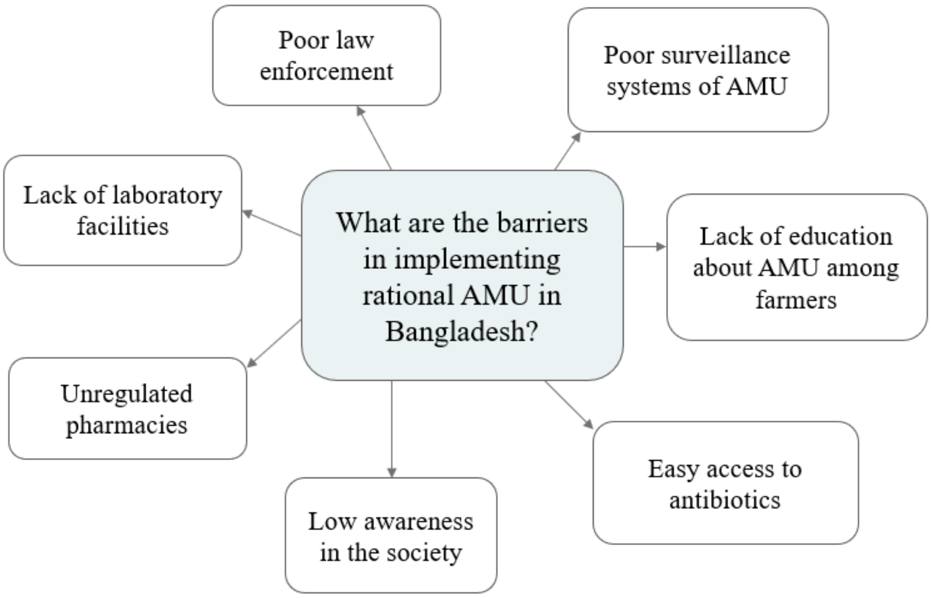

Figure 3. Frequent answers to the question "What are the barriers in implementing rational AMU in Bangladesh?".

Everyone putting AMR as a nationwide problem thought that the situation could be improved, and all respondents also agreed that better and more sufficient surveillance programmes needed to be developed. In this matter, people came with opinions like "the infrastructure and logistics can be hard" and "the veterinary coverage is not good enough". Other stakeholders put out cooperation and working in a one health perspective as the solution to putting together nationwide surveillance, preferably for both human and animal health. When asked questions about current guidelines for AMU, the answers varied from "do not know if there is for both human and animals, but for humans, we are developing some now" to "I think there is, but no one can follow that".

Among people interviewed, the government was frequently pointed out as an instance that was believed to make the first steps for enforcing laws and putting together new guidelines or surveillance programmes. Many of the stakeholders particularly added that also private companies had a responsibility to set an example. Another interviewee said that "professors interested in creating publications care about this, but what difference does it make", meaning that a lot of studies are being made, without changing the actual situation. Others listed that regarding facilities for testing for AMR, there was a lack of management within the public sector which led to facilities not operating.

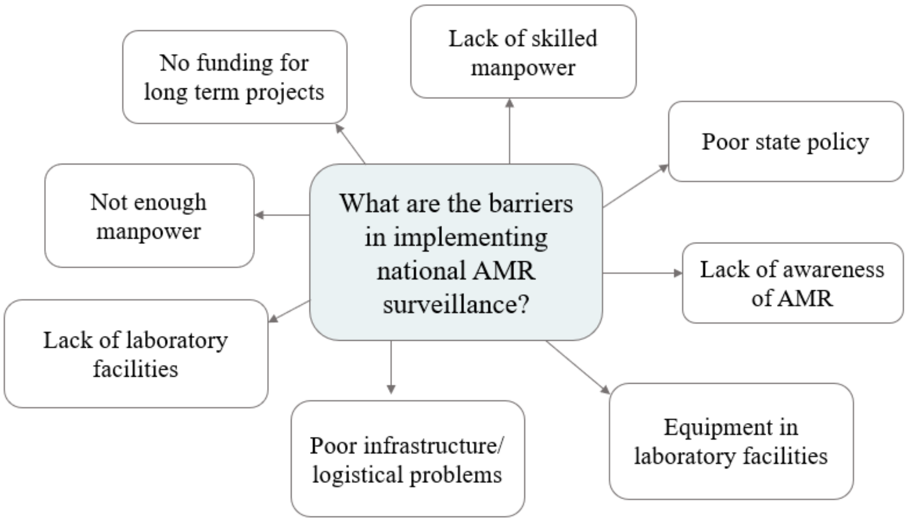

Figure 4. Frequent answers to the question "What are the barriers in implementing national AMR surveillance?"

Standing out was one of the interviewees who repeatedly took up the question about preventive work at farms as a solution for decreasing the AMU. This interviewee was of the opinion that antibiotics are necessary and should not be ruled out, but that preventive work should be the major interest. Another interviewee pointed at more testing, and above all, always testing before change of antibiotic in case of failure of treatment as important.

Overall, the common picture among KII was that AMU and AMR are problems in Bangladesh, and that action against these problems need to be taken. Many of the respondents spoke about current laws in place for regulating AMU, which states that no antibiotics can be bought without a prescription and that AGP is regulated. They also spoke about laws regulating maximum residue levels (MRL) of antibiotics in food products. Other things pointed out were drugs of poor quality and incorrect dosage and treatment lengths when antibiotics were used. All of these statements and facts leading back to two of the most frequent opinions “raise awareness” and “better law enforcement”. The goal should, according to one of the respondents, be “no prescription, no antibiotics” within all sectors.

5. Discussion

Antimicrobial resistance is a rapidly evolving problem of great concern to public health around the world (Laxminarayan *et al.* 2013). MDR bacteria are reported from multiple countries (WHO 2014a), and the increasing movement of people, animals, and food products around the world complicates the scenario (WHO 2015a; b). Reports of resistance genes spreading over world, creating a pandemic situation are present (Butaye *et al.* 2006; Cantón & Coque 2006). Some enteric bacteria have been well documented over a long time, and studies indicate low resistance even before antimicrobials were introduced (Datta & Hughes 1983), while more recent reports indicate a broad resistance (Panhotra *et al*. 2004; Butaye *et al.* 2006; Kumar *et al*. 2009). Overall, the problem requires a solution, which preferably not only consists of more prudent use of existent antibiotics, but also development of new ones (Cars *et al.* 2008).

In this study, interviews with key informants in Bangladesh regarding present AMU and AMR clarified specifics about the country correlating with studies made of other LMICs, such as a high proportion of OTC sales of antibiotics, poor surveillance and lack of awareness (Morgan *et al*. 2011; WHO 2015b). Apart from this, also other studies (Biswas *et al.* 2014; Mohiuddin *et al.* 2015) concluded that inappropriate sales of antibiotics occurred in Bangladesh. All these are factors contributing to both AMU and indirectly AMR within the country. Within the area of OTC, several studies have been conducted trying to clarify why this route of administration is so common, where knowledge about AMR, the pharmacists' education and that pharmacies often were first point of contact was listed as different explanations (Darj *et al.* 2019; Rousham *et al.* 2019). At least two of these three eventual contributors to high OTC sales were also mentioned in KII.

In present day there are laws and regulations about AMU in Bangladesh (WHO 2022; Hoque *et al.* 2020; Orubu *et al.* 2020), which also are mentioned by KI, but there is a failure to follow these among society. The key informants stated that this could be due to disregard, lack of knowledge or because of logistical difficulties. Poor infrastructure and inadequate health care are regarded to be factors contributing to difficulties in the matter also in other LMICs (WHO 2015b). Within the country, there is also a lack of national ongoing surveillance programmes for both AMR and AMU, especially within the veterinary sector. Few short-term studies have been made, but no one scoping between sectors, lasting for a long time (Hoque *et al.* 2020). Guidelines are under development for human health care according to one key informant, but for the animal sector none of the key informants could recall anything about guidelines. This indicates that present guidelines might be unknown among the population. The WHO has stated both of these matters as important for a better AMR situation through better AMU, as well as that smaller studies can give a hinge about AMR situation (WHO 2015b). When speaking of this matter, it is also of importance to mention funding of projects and availability of manpower and facilities. All of these matters were below desired levels according to the key informants, further complicating the situation.

Another aspect, apart from trying to implement a prudent use of antibiotics within all sectors, to be considered is the production of antibiotics. A large scale of the production of antibiotics takes place in neighbouring countries to Bangladesh. Waste from this kind of production, i.e. antibiotic residues, could also be a source of resistance that complicates the situation in Bangladesh. This kind of aspect points out that the resistance problem knows no borders and that countries might have a situation that can not be controlled or improved just by implementing restrictions within the own country.

Also included in this study was a cross-sectional analysis of food products at markets in three different areas. The food products chosen in this study aimed to correlate to the importance of the most common source of food in Bangladesh, but also food that has their origin in environments where antibiotics could have been used excessively and resulted in AMR. Both products meant to be cooked and to be eaten raw were included, where products meant to be cooked could result in lower bacterial burden for the consumer. Even so, the handling of the product itself could still be a source of coming in contact with bacteria.

Within this study, prevalence of three different bacteria and their antimicrobial susceptibility was studied. Out of the 974 samples, 320 cultivations (of 1589 cultivations made) were positive for bacteria. The majority of positive cultivations (64.3 %) classified as MDR. Since food products and environmental aspects have been discussed as sources for transmitting resistance genes (Barton 2000; Lester *et al.* 2006; Marshall & Levy 2011; Van Boeckel *et al.* 2015; Michael & Schwarz 2016), findings of MDR in animal food products is a concern for further strengthening of this theories. Among all positive samples (not depending on food type or bacteria), MDR bacteria was over 55%, except for fish samples positive for *Vibrio cholerae*. In this category, only around 45% of positive samples were MDR.

The panel of antibiotics to be included in sensitivity testing were selected by the national lab, based on their standard procedures. What is worth noticing is that among the eleven antibiotics used in screening for resistance in this study, four different antibiotic substances could be classified within two groups (Cefixime and ceftriaxone as cephalosporins within the beta-lactams, and gentamicin and strepto-mycin within the aminoglycosides). This study considered resistance to both antibiotics in one group as the same when calculating MDR, which means that studies not classifying the same way may show higher rates of MDR. Also, Penicillin G was included in the study, even though two of the bacteria are considered to be naturally resistant to this antibiotic. Overall, if the study were to be made again, a different panel of antibiotics with a more wide range and higher relevance for chosen bacteria would be of importance.

Not only food products from animals were included in the study, but also tomato. Among tomatoes, the majority of positive samples classified as MDR. Without knowing the exact cause for this, factors such as contamination from environment, other products or due to poor hygiene among vendors could contribute to positive cultivations among tomatoes. This could also be the source of positive samples among chicken and fish, and could possibly vary between different study sites since movement of people, number of people, facilities for keeping good hygiene, and movement of live animals probably vary significantly between the sites.

Logarithmic values for colony forming units (LogCFU) of total coliform count (TCC) and *E. coli* count of were calculated for a subgroup of samples. There was a wide range of values, from zero to too numerous to count. Samples with positive cultivation, as for these ones included in the subgroups, are unlikely to have a coliform count of zero, since coliform bacteria is one of the most common indicator bacteria, often present at cultivations from food and food surroundings (Kennedy *et al.* 2005; Teramura *et al.* 2017). Instead, samples with the value zero are suspected to be a source of error. Values that were too numerous to count were given a specific value in analysis, which could interfere with the result. Even so, the TCC was significantly higher at traditional markets than at supershops, and also in products not being cooled at the vendors. This could indicate that cooling of product leads to less TCC, independent of a positive sample. Approximately 83% of the samples were not cooled, which were statistically correlated to type of food. Fish were more likely to be kept cold than chicken, and chicken was more likely to be cold than tomato. It was also seen that traditional markets were less likely to have cooling of products than rural. Surprisingly, peri-urban areas were less likely to have cooling than rural areas, which could explain the relatively high prevalence of bacteria within peri-urban samples. TCC was also significantly higher for tomato than fish, which might be explained by the fact that tomato was less likely to be cooled than fish. Worth mentioning is, that most likely, not only cooling of products affected the bacterial prevalence or logarithmic numbers, but also factors as how the products became contaminated and in which extent that contamination took place in contributes.

Another area where statistical difference was found was among the distribution of vendors, where women were more likely to sell tomatoes and only men sold fish. However, no significant difference in prevalence of bacteria was connected to gender, and this kind of distribution could be due to socioeconomic standards, traditions, or other variables within the country.

In total, the results from bacterial analysis agree with other studies within different fields discussed in an article by Ahmed *et al.* (2019), and it also correlates with a high proportion of reported AMR in LMICs (WHO 2015b).

The bacterial prevalence at the foodstuff sampled in this study could be from multiple origins. Possible origins could be contamination from surroundings by other foodstuffs, the vendor, surfaces, the sampler, or residues from slaughter. It is not possible to say which origin or where bacteria possibly carrying AMR actually become present at the sampled material. To further evaluate the origin, studies through the whole value chain would have to be made. These could also be complemented by studies of bacterial flora in humans that have consumed previously sampled products. Bacterial cultivation is somehow a more uncertain method to determine bacterial prevalence than PCR, which also could be discussed. Things as storage of samples before analysis and transport can also affect the result, and even if the goal was to transport all samples in a cooling box, there is always a slight possibility of being delayed to the laboratory or having a malfunctioning box.

The situation in Bangladesh regarding AMR is not fully evaluated when it is a difficult task to do without complete, national, long term surveillance. AMU and its unregulated nature within the country, both within human, veterinary and agricultural sectors, contribute to the AMR situation. Even though it is not possible to say what is the biggest contributing part to the situation in Bangladesh, it is likely that all these different components interact with each other and creates the situation. Furthermore, the matter of neighbouring countries with antibiotic production that by its waste might contribute to the overall AMR picture, the situation becomes even more difficult.

In order to get a better understanding of the total picture in Bangladesh, rational AMU would have to be implemented and national AMR surveillance started. In addition, a higher common knowledge about antibiotics and AMR could hopefully contribute to change the situation. Complementary to this, further global work to achieve a more prudent use of antibiotics is needed since the problem knows no borders.

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Populärvetenskaplig sammanfattning

Bakterier resistenta mot antibiotika klassas av WHO som ett hot mot den globala folkhälsan. En bakterie som är resistent mot antibiotika är inte längre känslig för medicinens verkan, vilket innebär att den kan bli svår att bli av med och att sjukdomen orsakad av bakterien kan bli svår eller omöjlig att bota.

När antibiotikumet penicillin upptäcktes ansågs det att botemedlet för infektionssjukdomar hade upptäckts. Människor som tidigare skulle avlidit av bakterieorsakade sjukdomar botades, och i takt med att fler antibiotikum uppfanns kunde fler bakteriellt orsakade sjukdomar behandlas. Genom tiden har fler och fler antibiotikum upptäckts, vilket har hjälpt sjukvården att utvecklats till det den är idag. Organtransplantation, cancerbehandling och andra procedurer har blivit möjliga att genomföra tack vare antibiotikans upptäckt. Upptäckten av nya antibiotikum har dock avtagit med åren, och väldigt få nya preparat har presenterats sedan 1960-talet. Parallellt med antibiotikans positiva egenskaper har ett växande problem tillkommit, bakterier har utvecklat egenskaper som gör dem motståndskraftiga, resistenta, mot antibiotika.

Resistens hos bakterier är något som kan finnas naturligt eller som förvärvats. Naturlig resistens innebär att bakteriens gener kodar för egenskaper som gör den okänslig för en eller flera grupper av antibiotika. Det kan handla om mekanismer som produktion av vissa ämnen, eller om att sakna de specifika egenskaper som antibiotikan angriper. Förvärvad resistens är när bakterier på något sätt tar upp genetiskt material från en annan bakterie, vilken innehåller resistensegenskaper. Upptag av genetiskt material från en annan bakterie sker genom att bakterierna för rörliga bakteriedelar mellan sig.

I närvaro av antibiotika sker ett selektionstryck på resistenta bakterier. Bakterierna som överlever antibiotikumets verkan kan öka i antal och riskerar att sprida vidare sina resistensgener. Genom åren har antibiotika använts inom flera olika sektorer; humanmedicin; veterinärmedicin; som hjälp för tillväxt inom boskapsbranschen; inom jordbruk. Den höga användningen, och framför allt icke optimal användning av antibiotika, har gjort att utvecklingen av resistens har haft en snabb framfart. Saker som påverkar hur resistensdrivande användningen av antibiotika är, är behandlingsdos, om rätt antibiotika används, att den inte används i för låga doser, längd av behandling och vetskap om att det är en bakteriell sjukdom som behandlas. Utvecklingen av resistens har under de senaste åren nått den grad att det finns bakterier som är så kallat multiresistenta, resistenta mot tre eller flera grupper av antibiotikum.

Den negativa bilden antibiotikaanvändningen haft på resistensutveckling hos bakterier har gjort att viss användning och vissa antibiotika har förbjudits inom vissa sektorer. Inom EU och i många andra delar av välden är antibiotika inte längre tillåtet som hjälp för tillväxt inom boskapsbranschen, eller inom jordbruk. I många delar av världen saknas dock de rätta förhållandena för att detta ska uppfyllas.

I många låg- och medelinkomstländer råder socioekonomiska förhållanden som gör att rådande regler och lagar inte åtföljs. I många av dessa länder är sjukvård och infrastruktur under en önskvärd nivå, och antibiotika kan köpas på apotek utan recept. Detta är faktorer som komplicerar länders arbete med att få en hållbar användning av antibiotika och därmed kunna påverka resistensläget. Utöver bristande regler, är det många av dessa länder som saknar övervakningssystem av antibiotikaanvändning och bakteriell resistens.

Bangladesh, ett av världens mest tätbefolkade länder, delar dessa problem med många andra länder i bland annat Sydostasien och Afrika. Tillgången på antibiotika utan recept är hög, sjukvården har stora utmaningar med folkhälsan och livsmedelssäkerheten är låg. Människor bor nära sina djur, vilket skapar en situation där zoonoser kan uppstå och resistenta bakterier kan överföras från djur till människa. Aspekter likt dessa väcker frågor om hur resistensläget bland bakterier ser ut i Bangladesh, vilka riskfaktorer som finns och vilka förbättringar som skulle krävas för att få en sund antibiotikaanvändning.

I detta examensarbete gjordes en analys av tidigare insamlade prover från Bangladesh i syfte att kontrollera bakterieförekomst i mat, samt analysera antimikrobiellt resistensmönster hos de prover där bakterier kunde upptäckas. Proverna samlades in från tre olika provinser i Bangladesh under november 2018 till juni 2019. De tre olika provinserna klassades som stad, förort och landsbygd. Totalt samlades 976 prover in, av vilka 974 analyserades för bakterieförekomst. 366 tomater testades för förekomst av *Salmonella* spp. och *Escherichia coli*, 359 prover från kyckling analyserades för *Salmonella* spp. och 249 prover från fisk analyserades för förekomst av *Vibrio cholearae* och *E. coli*. Proverna som var positiva för bakterieförekomst kontrollerades för känslighet för elva olika typer av antibiotika, för att undersöka om resistensmönster fanns. Utöver detta intervjuades sju personer med insyn i Bangladesh rutiner kring användning av antibiotika samt antibiotikaresistens under december 2020.

De tre bakterierna som analyserades är utvalda för sina olika egenskaper: *Salmonella* spp. är en vanligt förekommande bakterie hos både människa och djur, känd för att vara orsak till mag- och tarmbesvär. *Escherichia coli* är också en vanlig bakterie hos både människa och djur och brukar användas för att konstatera fekal förorening. *Vibrio cholerae* är upphovsmakaren till kolera, och är en sjukdomsframkallande bakterie som kan ha stor påverkan på många människor.

Resultaten från denna studie visade att 27 av de 366 proverna från tomater innehöll *Salmonella* spp, av vilka 44.4 % bar på multiresistenta bakterier. Motsvarande siffra av positiva tomater för *Escherichia coli* var 51/366, varav 80.4 % var multiresistenta. Kycklingen som analyserades för *Salmonella* spp. hade 62 positiva analyser av de 359 som gjordes, varav 85.5 % bar på multiresistenta bakterier. För fisken som testades för *Escherichia coli* var 67 av 249 prover positiva och 70.2 % av dessa bar på multiresistenta bakterier. Av de 249 proverna från fisk som analyserades för *Vibrio cholerae* var 112 av 249 positiva, och 44.6 % av dessa multiresistenta. Resultaten från de olika analyserna var jämnt fördelade mellan olika insamlingsplatser.

Intervjuerna med de sju nyckelpersonerna med insyn i Bangladeshs rutiner kring antibiotikaanvändning och rådande resistensläge belyste ett antal punkter som förbättringsområden. Frekvent i intervjuerna nämndes att kunskapen om antibiotika och resistenta bakterier behövde öka, både hos yrkesverksamma grupper och gemene man. Samtliga ansåg att antibiotikaresistens är ett stort problem i Bangladesh. Det påtalades också att Bangladesh har lagar och regler för användning av antibiotika, men att dessa sällan efterlevs. Tillgången på antibiotika utan recept nämndes av samtliga deltagare i intervjuerna, och ansågs bidra till ökad användning av antibiotika. Samtliga ansåg också att det hade varit bra med ett nationellt övervakningsprogram för antibiotikaresistens. Utöver detta pekade olika personer ut andra saker som ansågs viktiga för att påverka användningen av antibiotika och därmed utvecklingen av resistens. Bland dessa saker nämndes ”ökad biosäkerhet – förebyggande åtgärder för att förhindra att antibiotika måste användas” och ”bättre logistik och ekonomiska tillgångar behövs”.

Sammantaget kan det konstateras att hos de prover som var positiva för bakterier i denna studie var andelen multiresistenta bakterier hög. Dessa prover var insamlade från olika områden, och ingen signifikant skillnad sågs på antalet positiva prover från de olika områdena. Trots det förhållandevis låga antalet provet sett i förhållande till ett helt land stärker dessa resultat tidigare gjorda studier som visar på en utbredd resistensproblematik i Bangladesh. Intervjuerna med nyckelpersonerna pekade också på en gemensam bild av att antibiotikaresistens i Bangladesh är ett problem, och att åtgärder måste vidtas gällande användning av antibiotika samt att övervakning av resistensläget är av yttersta vikt. Det är tveklöst många olika aspekter som berörs av ämnet, men likväl kvarstår att det är av yttersta vikt att föra frågan vidare och att genom ytterligare studier och åtgärder försöka förbättra läget både i Bangladesh och andra länder – för den globala folkhälsan.

Appendix 1

Vendor Questionnaire – Bangladesh Food Safety

☐ Consent provided to participate in study (*attach signed consent form*).

Name and contact information for the respondent

	Name of interviewer			Date	
	Town			Upazila	
	District		1.6	Market	
1.7	GPS North		1.8	East	
1.9	Name of the respondent		1.10	Phone	

Basic demography

	Gender of respondent (1=male, 2=female)			Age (years)	
	Type of product(s) sold by respondent				
	Years of selling in markets				
	Highest education (0=none, 1=primary, 2=class 5-10, 3=Higher secondary (11-12), 4=Graduation and above)				
	Are you member of a traders association (1=Yes, 0=No)?				
2.6a	(*If yes*) Give the name of the traders association:				

Sales

	Complete all that apply for this vendor:	a. Egg	b. Cucumber	c. Fish Frozen/Fresh (*circle 1*)
3.1	How much of these products did you sell yesterday?		Kg/day	Kg/day
3.2	What time in the morning do you usually procure the raw products?			
3.3	What time do you usually sell the last?			

3.4	What do you do with products that you cannot sell?			

3.5	Do you think it is possible to get sick from eating this kind of product? (1=Yes, 0=No)	
3.5a	(*If yes*) Please mention the diseases you can get:	
3.6	Do you sell any ready-to-eat products? (1=Yes, 0=No)	
3.6a	(*If yes)* Please describe which:	

Vendor knowledge and attitude towards food safety

	Do you agree with the following:	agree=1,/ disagree=0/ Don't know=99
4.1	The longer a raw product has been at the market, the lower the quality.	
4.2	It does not matter how old the food is if you cook it well.	
4.3	I can tell by the look and smell of food if it is safe to eat.	
4.4	I worry more about chemicals in the food than germs.	
4.5	I sometimes eat raw food without cooking it first.	

Vendor Observation

5.1	Is food uncovered, open to flies and dirt? (1=Yes, 0=No)	
5.2	Is display of food products on ground or table? (1=table, 0=ground)	
5.3	Is there any cooling? (0=No, 1 = refrigerator, 2= cool box, 3= other way_________)	
5.4	Are there flies on the products? (0=No, 1 <10, 2>10, 3> innumerable)	
5.5	Do the products look clean? (1=Yes, 0=No, there is visible dirt)	
5.6	Are products rinsed, sprayed or wiped before sale? (1=Yes, 0=No)	
5.7	(*Produce only*) Are the products visibly wet? (1=Yes, 0=No)	
5.8	Do utensils look clean (knife, cutting board, scale)? (1=Yes, 0=No)	

Market Observation Checklist – Bangladesh Food Safety

1.1	Name of interviewer		1.2	Date	

1.3	Town		1.4	Upazila	
1.5	District		1.6	Market	
1.7	GPS North		1.8	East	

6.1	Type of food market (0=traditional uncovered, 1=traditional covered roadside shop, 2=traditional municipal market, 3=modern supermarket, 4=modern convenience store)	
6.2	*Within the market*, is there a water source available to vendors? (1=yes, 0=no)	
6.3	*Within the market*, Is a drainage system visible? (1=yes, 0=no)	
6.4	*Within the market*, are live animals present? (1=yes, 0=no)	
6.5	Is there any evidence of food inspection by government officials? (1=yes, 0=no)	

Customer Questionnaire – Bangladesh Food Safety

☐ Consent provided to participate in study (*attach signed consent form*).

1.1	Name of interviewer		1.2	Date	
1.3	Town		1.4	Upazila	
1.5	District		1.6	Market	
1.7	GPS North		1.8	East	

Name and contact information for the customer responding

7.1	Name of the customer		7.2	Phone	
7.3	Gender of customer (1=male, 2=female)		7.4	Age	
7.5	Highest education (0=none, 1=primary, 2=class 5-10, 3=Higher secondary (11-12), 4=Graduation and above)				

Consumption

8.1	How many people in the household consumed this product yesterday (or most recently)?	people
8.2	How much was consumed in total (grams) yesterday/most recently?	g
8.3	How often do you consume this product?	/week or /month

Food safety practices

9.1	For the most recent consumption, how long was the product kept in your house before preparing? (*enumerator to indicate whether hours or days)*	
9.2	Where/how was it stored?	
9.3	Did you wash the product before preparation? (1=Yes, 0=No)	
9.4	Did you chop or cut the product before preparation? (1=Yes, 0=No)	
9.5	Did you cook the product? (1=Yes, 0=No)	
9.5a	(*If yes)* How did you cook it and how long (minutes)?	min
9.6	Which dishes do you often cook from this product? (*specify all that apply:* roast, fry, grill, boiled, soup, other)	
9.7	Did you use the same knives/cutting boards for both raw and cooked products? (1=Yes, 0=No)	
9.8	Which market/retail type do you often go to buy this product? (0=traditional uncovered, 1=traditional covered roadside shop, 2=traditional municipal market, 3=modern supermarket, 4=modern convenience store)	

Attitudes towards food safety and the market

	Do you agree with the following:	agree=1,/ disagree=0/ Don't know=99
10.1	The longer uncooked food has been at the market, the lower the quality.	
10.2	Improper handling of food by the seller could make me sick.	
10.3	Germs are on the skin, nose and mouth of everyone.	
10.4	The cleanliness of sellers is important when I choose where to buy food.	
10.5	Diarrhea can be transmitted through food.	
10.6	I worry more about chemicals in the food than germs.	

11. Food safety and the home: storage and cooking

	Do you agree with the following:	agree=1,/ disagree=0/ Don't know=99
11.1	Improper food storage is dangerous to health.	
11.2	Raw fish, egg, and meat should be stored in a cold place.	

11.3	Germs can get into food from insects and pests.	
11.4	I sometimes eat raw food without cooking it first.	
11.5	I can tell by the look and smell of food if it is safe to eat.	
11.6	High temperature or freezing is a safe method to destroy germs.	

12. Food safety and the home: hygiene/handwashing

	Do you agree with the following:	agree=1,/ disagree=0/ Don't know=99
12.1	Hand washing before handling food reduces the risk of contamination.	

13. Food safety and the home: consumption of leftovers

	Do you agree with the following:	agree=1,/ disagree=0/ Don't know=99
13.1	Contaminated food always has some change in color, odor or taste.	
13.2	I sometimes worry about eating leftovers because they can make me sick.	
13.3	I always cook leftovers again before eating them.	

Appendix 2

Questions to stakeholders regarding antimicrobial use and antimicrobial resistance in Bangladesh.

Questions to be answered by different categories:
Only white: epidemiologists or similar role
White and yellow: practitioners/pharmaceuticals
White and blue: stakeholders carrying out tests for AMR
Answer all the questions that you have knowledge about, even if it differs from the categories listed above. If you have an interview planned, the questions will be a part of the interview.

1. What is your occupation? Include designation.
2. What is your work responsibility?
3. How long have you worked in this role?

For practitioners/pharmaceuticals:

4. Do you prescribe/recommend antibiotic use in your profession, or sell antibiotics directly to the user?
5. Do you ever prescribe or sell antibiotics without seeing the sick animal? Do you ever recommend or sell antibiotics to animals that are not sick?
6. To which types of animal do you most often prescribe antibiotics?
7. How do the drugs reach the farmer?

	Very common	Not so common
Leave them at the farm		
Farmer buy at pharmacy		
Farmers get them from reseller		
Other:		
Other:		

8. Which is the most common disease to treat with antibiotics?

Please fill in the table below.

List diseases	Which antibiotics do you treat the disease with commonly?	Do you ever need to change to another medicine for this?	Which medicine do you change to commonly?

9. Do you know if there any national guidelines for ab use?

Answer 9a and b if yes, answer 9c if no.

a. If yes, do you know what they say?

b. If yes, do you think people know about them?

c. If no, do you think there should be guidelines, and what should there be in them?

10. Do you often se failure of treatment when antibiotics are used?

11. Do you think antimicrobial resistance (AMR) is a problem in Bangladesh?

12. If yes, is it possible to change the situation?

13. What needs to be done to improve the situation? *Put number 1 to 5 behind every alternative where; 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree*

Improvements	Number 1-5 below
Education of farmers	
Education of stakeholders	
Better guidelines	
Surveillance	
Improve biosecurity	
Other:	
Other:	

14. Do you have any responsibility for monitoring the AMR-situation in your profession?

15. Do you know if surveillance and testing regarding AMR are carried out in Bangladesh?

If yes; is it enough/does it have to be further developed? If no; would it be good?

For stakeholders carrying out testing for AMR:

16. Which kinds of antibiotics are tested?

Please put a cross behind every group of antibiotics tested and specify name of antibiotic is possible.

Groups of antibiotics	Cross if tested	If possible, specify name of antibiotic
Benzylpenicillin		
Cephalosporins of first generation		
Cephalosporins of third generation		

Macrolides		
Aminoglycosides		
Chloramphenicol		
Tetracyclines		
Quinolones		
Trimethoprim-sulphonamides		
Other:		

17. What are the organisms you tested to identify the AMR trend?
18. How is the situation based on available tests done?
19. Do you see resistance patterns?
20. Do you see signs of multidrug resistance? *(In this form, MDR is defined as bacteria resistant to 3 or more antibiotic groups).*
21. Are findings shared with other stakeholders?
22. Is testing done at regular basis? If no, what are the barrier of regular testing?
23. Would it be possible to do more testing? (In terms om enough capacity at labs etc)
24. Which are the biggest challenges in implementing a/improving a surveillance programme? *Put number 1 to 5 behind every alternative where; 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree*

Alternatives:	Numbers 1-5 below
Capacity for analysis	
Money	
Hard to get samples	
No meaning	
Other:	
Other:	

Please also give your opinion on these two overarching questions:

What are the barriers in implementing rational antibiotic use?

What are the barriers in implementing AMR surveillance?