Review Article

IgYs as an Alternative Approach to Antibiotics

Julia Zajac[1], Andreas Schubert[1] and Christopher Oelkrug[1,2*]

[1]Fraunhofer Institute for Cell Therapy and Immunology (IZI), Leipzig, Germany

[2]Fraunhofer Project Centre for Biomedical Engineering and Advanced Manufacturing, McMaster University, Hamilton, Canada

*Correspondence author: Christopher Oelkrug, Fraunhofer Institute for Cell Therapy and Immunology, Perlickstraße 1, 04103, Leipzig, Germany, Tel: 49 1739726390; E-mail: christopher.oelkrug@oelkrug-enterprises.com

Abstract

Misuse and overdose of antibiotics in agriculture and human disease lead to a worldwide problem of resistant bacteria. In 2012, 10,000 metric tons of antibiotics in the US and 8,000 in Europe were used in animals for the food industry. Resistance can be transferred within and between bacteria species, but also spread easily over to humans by contact with contaminated food, soil or infected animals and patients. To find a successful therapy against resistant microbes is extremely difficult due to the high cost of developing next generation antibiotics and its production, but also because of bacteria ability of developing new mechanisms against these drugs. Therefore, technology based on avian antibodies IgYs can be a strong alternative for antibiotic therapy. Advantages of IgYs include: low costs, effective and non-harmful method of production. But the strongest argument is that they cannot be affected by bacterial resistance. IgYs can be used not only as a preventative or therapeutic but also as growth promoters in food production. In this review we present the reasons for antibiotic resistance in bacteria and its consequences in the field of farming and human medicine, including molecular biology mechanisms and statistics. Furthermore we introduce IgYs as a promising and potential candidate for the replacement of antibiotics in the near future.

Antibiotics in Agriculture and Human Diseases

Antibiotics were first defined by Waksman more than 70 years ago as bacteriostatic, bactericidal and antifungal chemicals naturally synthesized by microorganisms [1]. Nowadays, the definition includes substances not only originated from natural products but also those produced synthetically [2]. In 1946, the New York Academy of Science's conference was focused on pharmacological properties and clinical applications of two 'wonder drugs': Alexander Fleming's penicillin and Selman Waksman's streptomycin. Their discovery revolutionized the scientific world and opened the antibiotic era in human and veterinary medicine giving new opportunities in the battle against microbes [2,3].

Antibiotics also play a very important role in agriculture and are widely used in livestock for growth control and promotion, disease prevention and treatment [4,5]. Even though the use of antibiotic growth promoters is banned in the European Union and has been relatively minimalized in the United States, they are still used extensively in other regions of the world [6,7]. Antibiotics may influence faster animal growth by eliminating harmful bacteria, which reduces immune stimulation and decreases a number of bacteria in the bowel which leads to smaller nutrition competition [4]. Even though only a few animals show symptoms of an infectious disease, the entire stock is treated shortly with high doses of antibiotics. For the prevention antimicrobials are administrated to animals orally, in low doses for several weeks [6].

The ubiquity of antibiotics is the result of the increasing worldwide need for meat. According to statistics, the USA is the largest meat consumer over the past thirty years with more than 100 kg meat per capita, followed by Brazil, EU, Russia and China [8]. During the last five decades in the US, the average number of chickens and cattle on the farms doubled and the number of pork from the animal producers more than twenty-two fold. Till now the biggest meat producer is China, with the amount of beef, pork and chicken almost double over the European production (2013), and at the same time it was estimated that half of the antibiotic consumption (150,000-200,000 metric tons per year) goes to livestock [8]. In 2012, 10,000 metric tons of antibiotics in the US and 8,000 in Europe were used in food animals [8].

Even though the use of antibiotic growth promoters is limited, they are still given to animals as prophylaxis or treatment. In cattle antibiotics are used mostly against the bovine shipping fever complex (pneumonia), mastitis and diarrhea [9]. In pigs antibiotics are given after the cut of the umbilical cord and the tail, castration, during ablactation and vaccination, for the treatment of pneumonia, bacterial enteritis, swine dysentery and ileitis [10,11].

In poultry antibiotics are used commonly against coccidiosis and *Clostridium perfringens,* which both lead to the very popular chicken's disease-necrotic enteritis [12,13]. There is a broad spectrum of antibiotics used in livestock production: tetracyclines, amphenicols, penicillins, cephalosporins, first, second, third and fourth-generation cephalosporins, sulfonamides, trimethoprim, macrolides, lincosamides, aminoglycosides, quinolones, polymyxins, pleuromutilins and ionophores [4,5]. Many of them are also used in human medicine [14]. Consequently, the increasing usage of antimicrobials leads to the resistance of bacteria which has become a major threat to public health in the XXI century.

Worldwide Problem with Multi-Resistant Bacteria

The resistance to critically important antibiotics for human medicine can have unpredictable impact on health care and clinical procedures. Antibiotic resistance has been associated with more frequent and longer hospitalization, longer illness, a higher risk of invasive infection and a twofold increase in the risk of death [15]. The main problem comprises drug resistant foodborne pathogens such as *E. coli, Salmonella, Campylobacter, Staphylococcus* (including MRSA-methicyllin-resistant *Staphylococcus aureus*), *Enterococcus* (including VRE - Vancomycin-Resistant *Enterococcus*), *Clostridium difficile, Pseudomonas aeruginosa, Acinetobacter, Shigella,* and respiratory pathogens *Streptococcus pneumoniae, Mycobacterium tuberculosis* and *Klebsiella pneumonia* [16-18].

According to the Centers for Disease Control and Prevention (CDC) report from 2013 the annual number of infections in US caused by resistant *E. coli* and *Klebsiella* was 35,300 altogether, with 2,310 cases of death, for drug resistant *Salmonella*: 103,800 infected patients and ~40 deaths, for *Campylobacter*: 310,000 infections and 28 deaths, for MRSA: 80,000 infections and 11,000 deaths; for VRE 20,000 infections, 1,300 deaths, for *Clostridium difficile* 250,000 infections and 14,000 deaths, for *Pseudomonas aeruginosa*: 6,700 infections and 400 deaths, for *Acinetobacter* 7,300 infections and 500 deaths, for *Shigella* 27,000 infections and >5 deaths, for Streptococcus (with data regarding *Streptococcus pneumoniae*) 1,208,900 infections and 7,600 deaths; for drug resistant tuberculosis 1,042 infections and 50 deaths [16]. In Europe the situation does not look better.

Antibiotic-resistant bacteria	Annual number of infections	Annual number of deaths
E. coli + Klebsiella	35300	2310
Salmonella	103800	40
Campylobacter	310000	28
MRSA	80000	11000
VRE	20000	1300
Clostridium difficle	250000	14000
Pseudomonas aeruginosa	6700	400
Acinetobacter	7300	500
Shigella	27000	5
Streptococcus	1208900	7600
E. coli	32500	5100
Klebsiella pneumoniae	18900	2900
MRSA	171200	5400
VRE	18100	1500
Streptococcus pneumoniae	3500	Data not published
Pseudomonas aeruginosa	141900	10200

Table 1: Annual number of infections and deaths caused by resistant strains of bacteria in Europe and USA, reported by European Centre for Disease Prevention and Control (ECDC) in 2009 and Centers for Disease Control and Prevention (CDC) in 2013 [16,19].

According to the European Centre for Disease Prevention and Control/European Medicines Agency (ECDC/EMEA) Joint report from 2009 at least 25,000 people die every year in Europe due to antibiotic-resistant infection. The estimated number of annual infections of resistant *Staphylococcus aureus* (MRSA) was 171,200 with 5,400 deaths, for VRE 18,100 infections and 1,500 deaths, for resistant *Streptococcus pneumoniae* 3,500 infections (data on deaths not published), for resistant *Escherichia coli* 32,500 infections and 5,100 deaths; for resistant *Klebsiella pneumoniae* 18,900 infections and 2,900 deaths, for resistant *Pseudomonas aeruginosa* 141,900 infections and 10,200 deaths. [19] The annual infection and death rates coming from resistant bacteria are summarized in Table 1.

The EU Report on antimicrobial resistance of *Salmonella, Campylobacter, Escherichia coli* and *Staphylococcus aureus* isolates from humans, animals and food published in 2013 brings bothering analysis [20]. 36.1% of Salmonella isolates from human were resistant to ampicillin, 35.7% to sulfonamides and 34.5% to tetracyclines, whereas 31.8% showed multi-resistance.

Salmonella isolated from meat showed resistance to tetracyclines, ampicillin and sulfonamides in the range from moderate to extremely high. 56% of *Salmonella* isolates from broilers were multi-resistant, as well as 37.9% from pigs and 73% from turkeys. *Campylobacter* samples from patients showed very high resistance to the clinically important antibiotics: more than 50% of all isolates were resistant to ciproflaxin, 33.5% isolates of *Campylobacter jejuni* and 58.1% of *Campylobacter coli* were resistant to tetracycline, 13.4% of *Campylobacter coli* isolates were resistant to Erythromycin. *C. jejuni* and *C. coli* isolates from avian showed from high to extremely high resistance to ciproflaxin, nalidixic acid and tetracyclines, comparable to those from pigs and cattle.

Percentage of resistant *E. coli* isolates from broilers and pigs were in the range of 6.3%-64.7% and 17.7%-88.0% respectively [20]. *Escherichia coli* isolated from avian, pigs and cattle showed resistance in the range of moderate to very high to: Ampicillin (13%-58.6% of isolates), sulfonamides (20.2-48.6% of isolates), tetracycline (23.2%-52.8% of isolates), streptomycin (17.6%-50.4% of isolates) and nalidixic acid (55.4% of isolates, data referred only to broilers). *Enterococcus* isolates (*E. faecium* and *E. faecalis*) showed very high resistance to tetracyclines (from broilers 61.6%-87.0% of isolates, from pigs 45.6%-85.5% of isolates, from cattle 30.8%-85.5% of isolates) and erythromycin (from broilers 59.0%-60.6% of isolates). The resistance of *Enterococcus* to quinupristin/dalfopristin was extremely high among all types of tested animals, giving the number of 73.7%-94.7% of investigated isolates. Analysis of *Staphylococcus aureus* in meat from turkeys, pigs, broilers and cattle was narrowed down to MRSA, and the number of its infection increased from 2.2% in 2009 to 20.8% in 2013 [20]. This data might be supplemented by the ECDC Annual epidemiological report from 2014 which shows that MRSA is above 25% of isolates in 7 of 29 analyzed countries in UE/EEA. The percentage of aminoglycoside-resistant *Enterococcus faecalis* isolates is between 25%-50% among reporting countries. More than 50% of *Acinetobacter* spp. isolates were resistant to carbapenems, fluoroquinolones and aminoglycosides. Above the 10% of *Pseudomonas aeruginosa* isolates in 19 of 29 reporting countries were resistant to carbapenems, and 14% showed multi-resistance. Resistant to both penicillin and macrolides was 10% of *Streptococcus*

pneumoniae isolates within 10 from 28 reporting countries. Moreover, due to data from 2012 in EU/EEA countries 11.9% of *Escherichia coli* isolates were resistant to third-generation cephalosporines and 4.4% showed multi-resistance. For *Klebsiella pneumonia* the percentage of isolates resistant to third-generation cephalosporines was 25.6% and 18.2% was multi-resistant [21].

Reasons for Antibiotic Resistance

Researchers, authorities from health organizations and medical doctors together agree that the antibiotic resistance is a consequence of overuse and misuse of antibiotics in human and veterinary medicine [9,15,17,22-24]. Bacteria which are known for their adaptive capabilities have developed several mechanisms to fight against antimicrobial drugs. Even shortly after first use of ampicillin in the middle of the XX century it was noticed that bacteria are able to destroy its activity and survive [3]. Since, then different molecular mechanisms of antibiotic resistance were described: Drug target modification, compound chemical modification, active efflux or molecular bypass [25]. The first option is based either on genetic point mutations (e.g. mutation in DNA gyrase leads to resistance to synthetic fluoroquinolone antibiotics-ciprofloxacin) or efficient and selective enzymatic activity within microorganisms (e.g. in ribosome methyltransferases).

The result in both cases is a molecular change in the bacterial target of the antibiotic. In chemical modification antibiotics are inactivated by enzymatic catalysis (e.g. β-lactamases destroy β-lactams by hydrolyzing their four atom ring). The mechanism of active efflux, supported by membrane proteins, helps bacteria actively remove antibiotics from inside. Molecular bypass is a process of replacement of antibiotic sensitive targets by the resistant ones, for example vancomycin resistance is a result of replacement of an amide with an ester [25,26].

Antibiotic resistance has its origins in gene mutations or in genetic elements exchange (such as plasmids, transposons, gene cassettes, integrons) between bacteria strains through the horizontal transfer. This includes three mechanisms: Transformation (uptake of naked DNA), conjugation (direct cell-to-cell transfer of genes), and transduction (bacteriophage plays the role of a DNA vector) [27,28].

Additionally, interactions between bacterial cells can lead to the development of a biofilm community, which increases the resistance to environmental stress influenced by antimicrobial agents. The antibiotic resistance of bacteria in a biofilm can be 1000 times higher than in planktonic cells, which enhances the risk of failure of antibiotic treatment even more [26]. Resistance can be spread easily in many different routes linking human population with agriculture. The net of correlations is shown in Figure 1.

Animals, humans, agriculture products and environment (soil) can be the source of resistant pathogens. Bacteria can be transferred between all of them through contaminated vegetables, meat, sewage, manure or by direct contact with infected animals or people. [17,28-30]. Therefore, farms and hospitals should be considered as places of increased risk. Due to the effect of 'global village' and increased number of travelers, the epidemiology of antibiotic-resistance can get out of control by dissemination across borders [31,32].

All of these facts show how incredibly important it is to find an alternative way to fight against pathogens overcoming their increasing resistance to antibiotics. A strong candidate is technology using IgY antibodies.
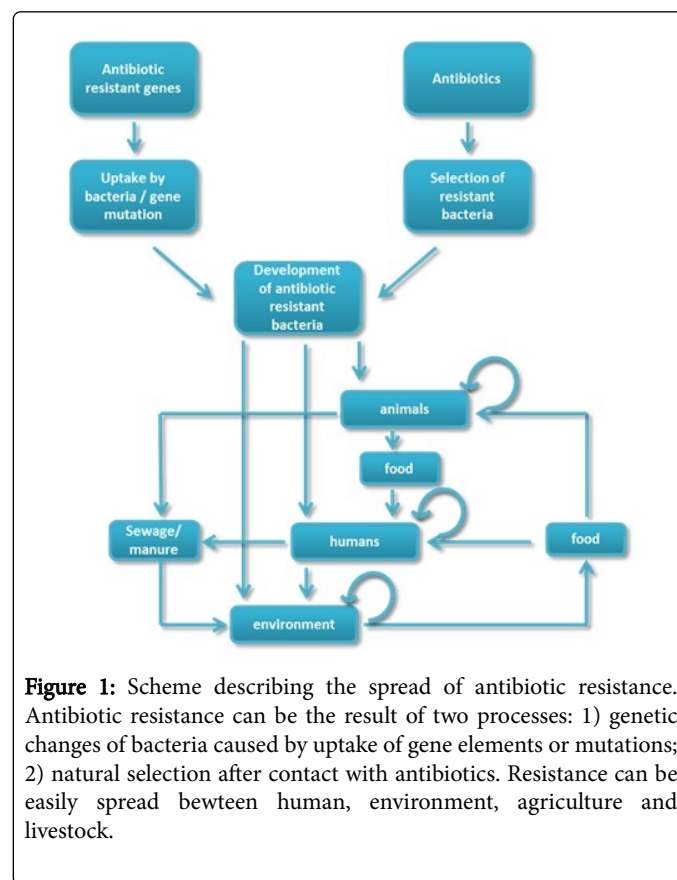
Figure 1: Scheme describing the spread of antibiotic resistance. Antibiotic resistance can be the result of two processes: 1) genetic changes of bacteria caused by uptake of gene elements or mutations; 2) natural selection after contact with antibiotics. Resistance can be easily spread bewteen human, environment, agriculture and livestock.

Specific IgY Antibodies against Pathogens-Structure and Function

Increasing interest for IgY technology has been observed, especially in the field of immunodiagnostics and immunotherapy, but also in livestock farming. IgY antibodies can be produced against many different antigens including pathogens, which is why they can be easily used as a specific immunotherapeutic agent e.g. as a passive immunization against bacteria. This is a very attractive approach especially because of the emerging antibiotic resistant microorganisms. Passively administered antibodies might provide rapid and fast protection against diseases unresponsive to antibiotic therapies or for patients with immune system defections who are not able to use conventional treatment or vaccinations.

IgY antibodies are immunoglobulins produced in avian species by plasma cells against an immunogen, to provide an immunogenic response to newly hatched chicks. IgYs are collected from egg yolk, where it is accumulated through blood transport from mother to egg (parental immunization) in high doses (1 ml contains up to 25 mg of IgYs).

There are many advantages of using avian IgY antibodies: 1) Easy purification (only one class of IgY antibodies in egg yolk and high IgY concentration), 2) They are polyclonal, so its specificity to antigen will not decrease significantly after the change of single epitopes, 3) Can be produced against conserved mammalian proteins, 4) Recognition of different epitopes than mammalian, 5) Method of production is not

harmful to animals (collecting eggs instead of blood), 6) Can be used in passive immunization, as a ready and specific antibodies for direct delivery into organism [33-36].

Advantages of IgYs are as follows:

- Specific antibody ready to be delivered into patient/organism of choice
- Do not provoke allergic reactions
- Cost-effective and convenient production
- High yield (up to 25 mg of IgYs per ml of egg yolk)
- High stability (pH 3, 5-11, 30-70°C)
- High avidity
- Low cross reactivity (in comparison to other polyclonal antibodies)
- Method of production is not harmful for animals
- Can be produced against mammalian proteins

Three classes of immunoglobulins in birds are known: IgY, IgM and IgA. During egg formation IgYs are selectively transferred from serum to yolk, whereas IgMs and IgAs are stored in the egg white [33]. In previous studies, IgY has been called IgG, due to its function and serum concentration in comparison with the mammalian IgG.

Nowadays, it has become clear that this is not a correct term, due to clear differences in molecular structure (e.g. IgYs do not contain a hinge region, possesses four constant domains instead of three in addition to the variable domain, IgYs are more hydrophobic comparing to IgGs because of its lower isoelectric point) and weight (IgY-180 kDa, IgG-159 kDa) [37,38]. There are also differences in biological properties: IgYs compared to IgGs do not provoke the mammalian complement system, do not interact with mammalian Fc receptors, do not bind to rheumatoid factor, protein A and G [39]. Additionally, IgYs show high stability at temperatures ranging between 30-70°C, and pH 3.5-11, which can be fine-tuned by additives (e.g. sorbitol) [34].

The technology of producing specific IgYs is relatively cheap, effective and simple. The first step includes the immunization of chickens with chosen antigens, which can be administrated to the animal orally. IgYs produced by avian are transported then with the blood to the egg, and accumulate in egg yolk in high quantities. Purified IgYs can be directly administrated to the host organism/ patient where they will generate an adaptive response against a specific microorganism. The entire procedure is shown in Figure 2a. The choice of antigen depends on the characteristics of the pathogen and therapy strategy, as described in Figure 2b.

IgYs can be generated against colonization factors (outer membrane proteins, fimbriae/pili and lipopolysaccharides), flagella, mucosal receptors, enzymes and toxins important for bacterial survival [40,41].

Wherefore we can distinguish several strategies for using IgYs in host protection: 1) agglutination of bacteria, 2) inhibition of bacterial adhesion, 3) suppression of virulence factors, 4) toxin neutralization, 5) enzyme inactivation [42]. The process of chicken immunization can be influenced by the following factors: antigenicity of the immunogen, type of adjuvant, route of antigen delivery, frequency of administration, avian properties (breed, age, egg lying capacity). One hen is able to produce ~22.5 grams of IgYs per year (with up to 10% specific antibodies), which is equivalent to one year production of antibodies by 4.3 rabbits [42,43].
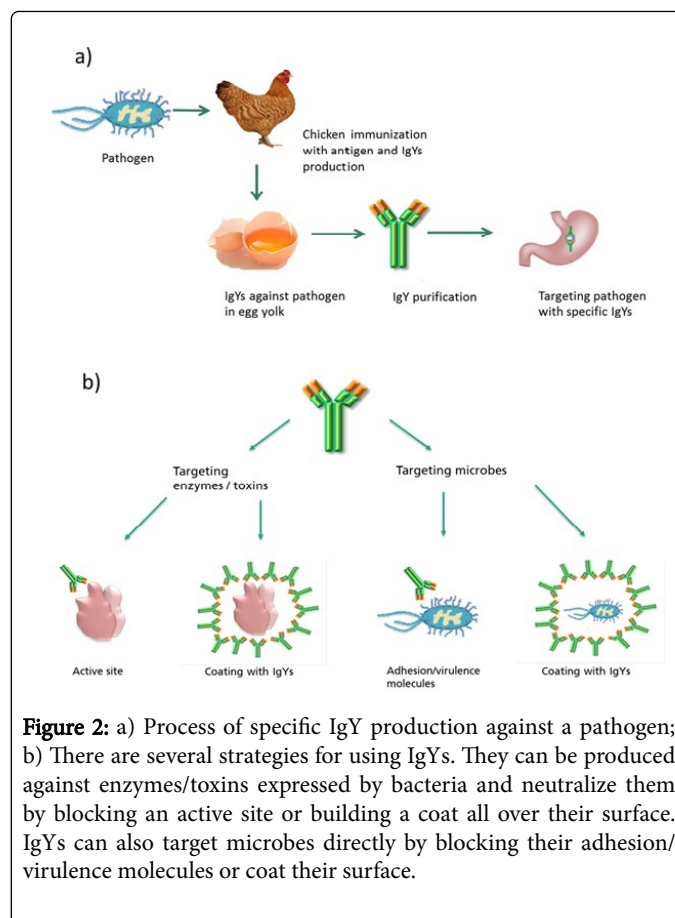
Figure 2: a) Process of specific IgY production against a pathogen; b) There are several strategies for using IgYs. They can be produced against enzymes/toxins expressed by bacteria and neutralize them by blocking an active site or building a coat all over their surface. IgYs can also target microbes directly by blocking their adhesion/ virulence molecules or coat their surface.

IgY technology is an effective way to provide immunity against a wide range of pathogens, which might be able to reduce or possibly replace the use of antibiotics in clinics and industry, and provide successful prevention, treatment, or growth enhancement overtaking the problem of increasing antibiotic resistance. Increased number of reported IgYs against a wide range of bacteria has been observed. Generated IgYs against anti-cell-associated glucosyltransferase (anti-CA-GTAase) of *Streptococcus mutans* can selectively suppress oral colonization of those microbes [44-46]. Specific IgYs inhibit the adherence of *S. mutans* by 59%, but only 8% when IgYs from non-immunized hens were used, which shows that anti *S. mutans* IgYs can be used as a prevention against dental plaque in humans [46]. Anti-*P. gingivalis*-IgYs can be also used as prevention against periodontitis [47,48]. In vitro studies on human intestinal epithelial cell culture Caco-2, show that *S. enteritidis* incubated previously with specific IgYs, lose their adherence to human cells which inhibits the bacterial infection [49]. Nilsson et al. made a 12 year study on prophylactic oral IgY treatment against *Pseudomonas aeruginosa* on 17 patients and reported in most cases prevention of its colonization, which indicates the high potential of IgYs as a preventive therapy against respiratory infections caused by this bacteria [50]. Therapeutic strategies based on IgYs generated against bacterial enzymes/toxins also bring promising results. LeClaire et al. and Trott et al. reported that IgYs specific to enterotoxin B and botulinum type A, neutralize their activity and therefore can prevent and treat infections of *Staphylococcus aureus* and *Clostridium spp.*, respectively [51,52]. Hirai et al. passively immunized mice with 3 different types of IgYs specific to *Vibrio cholera*: anti-01, O139 and anti-cholera toxin B and reported effective

prevention of cholera infection [53]. Another example is antiUreC-IgY, which was generated against one of the subunits of Urease enzyme and successfully prevented and eradicated antibiotic resistant *Helicobacter pylori* infections causing gastritis, gastric ulcer and gastric cancer [40,54]. Added as a supplement to food, for example in yogurt, IgYs can be used as a widely available prevention and treatment for humans and animals [6,55,56]. IgYs against *Escherichia coli* 0157:H7 also have high potential to be a food additive for passive immunization protecting humans from harmful results of its infections: diarrhea, hemorrhagic colitis and hemolytic uremic syndrome [57].

IgYs can be used as well in farm animals as an alternative treatment and prevention in a form of food additive against common bacterial livestock diseases. Li et al. reported Anti-K88+ IgYs successfully prevent *E. coli* infection in pigs [58]. The growth of *E. coli* in cattle could be also inhibited by Anti-O111 IgYs and increased uptake by macrophages was observed [59].

Yokoyama et al. orally administered specific IgYs against *Salmonella typhimurium* and *Salmonella Dublin* to neonatal calves, which prevented salmonellosis in animals [60]. Gurtler et al. reduced salmonellosis of eggs from previously infected chickens by oral therapy with anti-*Salmonella enteritidis*-IgYs [61]. Moreover, Zhen et al. studied the efficacy of IgYs against bovine mastitis caused by *Staphylococcus aureus* and compared it with antibiotic therapy and reported that usage of IgYs for clinical and experimental bovine mastitis showed cure rates of 50% and 83.3%, respectively. In comparison to penicillin the cure rates were 33.3% and 66.7%, respectively [62]. Another example is research conducted by Tsubokura et al. on IgYs against *Campylobacter jejuni* as a prevention and therapy for infected chickens. Prophylactic efficacy was observed as a 99% decrease of number of bacteria, and therapeutic efficacy as an 80-95% decrease number of faecal bacterial counts [63]. IgYs generated against different bacterial strains are summarized in Table 2.

Pathogen	Target	IgY therapy	Reference
Streptococcus mutans	Humans	Inhibition of bacterial adherence and prevention of dental plaque	[46]
Helicobacter pylori	Humans	Anti-UreC-IgYs inactivate Urease and eradicate *H. pylori* colonization	[40]
Escherichia coli	Humans	IgY Inhibits the growth of *E. coli* O157:H7 strain	[57]
	pigs	Anti-K88+-IgYs protect against *E. coli* and enhance weight	[58]
	Cattle	Anti-O111-IgYs inhibit growth of *E. coli* and activate uptake by macrophages	[59]
Salmonella spp.	Cattle	IgY against *S. typhimurium* and S. Dublin protects neonatal calves from infection and lethal effect	[60]
	Humans	IgYs generated against *Salmonella enteritidis* protects infected cultured human intestinal epithelial cells by inhibiting the bacterial adhesion	[49]
	Chickens	Anti-*Salmonella enteritidis* IgYs reduce contamination of eggs from previously infected chickens	[61]
Pseudomonas aeruginosa	Humans	Anti-*Pseudomonas aeruginosa* IgYs prevents infections in cystic fibrosis patients	[50]
Staphylococcus aureus	Humans	Anti-enerotoxin B-IgYs protected monkeys from lethal effect of *S. aureus* toxin	[51]
	Cattle	Anti-Staphylococcus areus IgYs reduced symptoms of mastitis caused by *S. aureus*	[62]
Clostridium spp.	Humans	IgYs generated against enterotoxin A block its activity in vivo and IgYs against *Clostridium perfringens* inhibit growth of its vegetative cells or spores	[52,64]
Campylobacter jejuni	Chickens	*Campylobacter jejuni* specific IgYs protects chickens from infection and decreases already existing *Campylobacter jejuni* infections	[63]
Vibrio cholerae	Humans	Anti-01-, O139 and anti-cholera toxin B subunit protected mice from cholera infection	[53]

Table 2: Examples of IgYs generated against different antibiotic resistant bacterial strains.

IgYs might be also used alternatively to antibiotics not only to protect animals from bacterial infections or as a treatment, but as well can successfully be used as growth enhancers. Owusu-Asiedu et al. compared the influence of IgYs oral therapy and antibiotic carbadox on 10-24 days old pigs infected with enterotoxigenic *Escherichia coli* K88. Both of them increased animal health performance in comparison to untreated controls. After the examination of factors such as weight, food intake ratios and mortality, no significant

differences were noticed between ETEC K88 specific IgYs and carbadox treatment [65]. Results are summarized in Table 3.

Parameter	Control	IgY	Carbadox
Weight gain, g/d	100.9	151.2	152.6
Feed intake, g/d	141	208.1	222.4
Feed conversion	1.39	1.38	1.45
Scour score	2.7	1.3	1.1
Mortality, %	40	6.6	13.3
Villus height, m	355	564	570
Crypt depth, m	204	183	204
Villous height/crypt depth	1.7	3.1	2.8

Table 3: Comparison between orally delivered IgY and antibiotics (Carbadox) on the intestinal morphology and performance of 10-24 day old pigs.

Discussion

Increasing attention of the scientific community on building a new strategy against resistant bacteria based on IgY technology, is due to its advantageous properties. The best evidence for its attraction is shown by comparison between factors describing the usage of antibiotics and IgY antibodies in Table 4.

Factor	IgY	Antibiotics
Specificity	↑	↓
Bacterial resistance	↓	↑
Broad effect on bacteria (also commensal)	↓	↑
Negative influence on environment & food industry	↓	↑
Risk of overdose	↓	↑
Safe prophylaxis	↑	↓
Safe food additive	↑	↓
Easy production	↑	↓

Table 4: Comparison of IgYs and antibiotics.

IgY antibodies are characterized by high specificity, due to their biological function and structure as antibodies, described in the previous section. Based on the strategy of production they can be directed specifically to a concrete strain of bacteria by overlapping them (IgY coat determined by chicken immunization with cell lysate) or targeting adhesion/virulence molecules or enzymes (chicken immunization with antigens). It brings the opportunity to design a specific and an individualized therapy, in contrast to antibiotics which can affect broad spectrum bacteria with similar phenotypes including commensals, which can lead to very negative consequences. Moreover, the usage of antibiotics cannot guarantee successful therapy due to the risk that microbes can become. With the strategy based on IgY technology this problem might be resolved. Even though some changes in epitopes can occur, the specificity of IgY antibody to antigen will not

change significantly because of their polyclonal nature. Another argument for IgY is their safety-as it is a naturally occurred molecule in eggs and it is also present in our everyday diet. The use of IgYs avoids the delivery of chemicals to the organism, which can have a negative influence on metabolism and gut microbial balance, like antibiotics. IgYs can be safely used as a food additive, not only against bacterial infections or as prevention but also as a growth factor in animal farming, without toxic effects and risk of overdosing. In contrast to antibiotics which have an impact on the host microbiome even after short time usage, IgY can be used regularly. Additionally IgY does not provoke allergic reactions: do not activate mammalian complement system, do not interact with Fc receptors mediating an inflammatory response and the method of purification separates IgY from other egg derived allergens. Also the technology of IgY is easier and more effective. However, the strongest argument for IgY technology is that it does not bring the risk of environmental contamination. They are biological molecules occurring in nature and to be effective they require a homogenous IgY microenvironment against the same antigens. Even small amounts of antibiotics transferred to the environment, including water, soil or animal/plants farms can be a selection factor supporting drug resistance development. Combined with effective genetic material exchange between bacterial strains and the possible routes of bacterial spread mentioned in the previous section, increasing antibiotic resistance can lead to serious and unpredictable consequences.

Conflicts of Interest

None of the authors have any competing interests.

References

1. Waksman SA, Woodruff HB (1942) Selective antibiotic action of various substances of microbial origin. J Bacteriol 44: 373-384.
2. Bush K (2010) The coming of age of antibiotics: Discovery and therapeutic value. Ann N Y Acad Sci 1213: 1-4.
3. Aminov RI (2010) A brief history of the antibiotic era: Lessons Learned and challenges for the future. Front Microbiol 1: 134.
4. Economou V, Gousia P (2015) Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect Drug Resist 8: 49-61.
5. Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24: 718-733.
6. http://www.fao.org/fileadmin/user_upload/animalwelfare/antibiotics_in_animal_farming.pdf
7. Casewell M, Friis C, Marco E, McMullin P, Phillips I (2003) The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52: 159-161.
8. http://www.cgdev.org/sites/default/files/CGD-Policy-Paper-59-Elliott-Antibiotics-Farm-Agriculture-Drug-Resistance.pdf
9. McEwen SA, Fedorka-Cray PJ (2002) Antimicrobial use and resistance in animals. Antimicrob Use Resist Animals 34: 93-106.
10. Dewey CE, Cox BD, Straw BE (1999) Use of antimicrobials in swine feeds in the United States. Swine Health Prod 7: 19-25.
11. Dunlop RH, McEwen SA, Meek AH, Friendship RA, Clarke RC, et al. (1998) Antimicrobial drug use and related management practices among Ontario swine producers. Can Vet J 39: 87-96.
12. Islam MN, Rashid SMH, Juli MSB, Hoque MF, Akter MR (2009) Necrotic enteritis in chickens: Pathological, bacteriological and therapeutical investigation. Int J Sustain Crop Prod 4: 1-7.
13. Van Immerseel F, De Buck J, Pasmans F, Huyghebaert G, Haesebrouck F et al. (2004) Clostridium perfringens in poultry: An emerging threat for animal and public health. Avian Pathol 33: 537-549.

14. Shryock TR, Richwine A (2010) The interface between veterinary and human antibiotic use. Ann N Y Acad Sci 1213: 92-105.

15. http://www.euro.who.int/__data/assets/pdf_file/0005/136454/e94889.pdf

16. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

17. Woolhouse M, Ward M, Van Bunnik B, Farrar J (2015) Antimicrobial resistance in humans, livestock and the wider environment. Philos Trans R Soc Lond B Biol Sci 370.

18. Llor C, Bjerrum L (2014) Antimicrobial resistance: Risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf 5: 229-241.

19. http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

20. http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-zoonotic-bacteria-humans-animals-food-EU-summary-report-2013.pdf

21. http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-annual-epidemiological-report.pdf

22. http://www.bezpecnostpotravin.cz/UserFiles/File/Kvasnickova/EFSA_BIOHAZ_rezistence.pdf

23. Akinyandenu O, Akinyandenu A (2014) Irrational use and non-prescription sale of antibiotics in Nigeria: A need for change. J Scientific Innovat Res 3: 251-257.

24. Shallcross LJ, Davies DS (2014) Antibiotic overuse: A key driver of antimicrobial resistance. Br J Gen Pract 64: 604-605.

25. Wright GD (2011) Molecular mechanisms of antibiotic resistance. Chem Commun 47: 4055-4061.

26. Penesyan A, Gillings M, Paulsen IT (2015) Antibiotic discovery: Combatting bacterial resistance in cells and in biofilm communities. Molecules 20: 5286-5298.

27. Davison J (1999) Genetic exchange between bacteria in the environment. Plasmid 42: 73-91.

28. Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, et al. (2013) Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect 121: 993-1001.

29. Smith DL, Dushoff J, Morris JG (2005) Agricultural antibiotics and human health. PLoS Med 2: 731-735.

30. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP (2015) Antibiotics in agriculture and the risk to human health: How worried should we be? Evolut Applicat 8: 240-247.

31. Okeke IN, Edelmann R (2001) Dissemination of antibiotic resistant bacteria across geographic borders. Clin Infect Dis 33: 364-369.

32. Wiklund S, Fagerberg I, Örtqvist Å, Vading M, Giske CG, et al. (2015) Knowledge and understanding of antibiotic resistance and the risk of becoming a carrier when travelling abroad: A qualitative study of Swedish travellers. Scand J Public health 43: 302-308.

33. Kovacs-Nolan J, Mine Y (2012) Egg yolk antibodies for passive immunity. Annu Rev Food Sci Technol 3: 163-182.

34. Rahman S, Van Nguyen S, Icatlo FC, Umeda K, Kodama Y (2013) Oral passive IgY-based immunotherapeutics: A novel solution for prevention and treatment of alimentary tract diseases. Hum Vaccin Immunother 9: 1039-1048.

35. Mine Y, Kovacs-Nolan J (2004) Chicken egg yolk antibodies as therapeutics in enteric infectious disease: A review. J Medici Food 5: 159-169.

36. Müller S, Schubert A, Zajac J, Dyck T, Oelkrug C (2015) IgY antibodies in human tuntrition for disease prevention. Nutr J 14: 109.

37. Warr GW, Magor KE, Higgins DA (1995) IgY: Clues to the origins of modern antibodies. Immunol Today 16: 392-398.

38. Dávalos-Pantoja L, Ortega-Vinuesa JL, Bastos-González D, Hidalgo-Alvarez R (2000) A comparative study between the adsorption of IgY and IgG on latex particles. J Biomater Sci Polym Ed 11: 657-673.

39. Carlander D, Kollberg H, Wejåker P, Larsson A (2000) Peroral immunotheraphy with yolk antibodies for the prevention and treatment of enteric infections. Immunol Res 21: 1-6.

40. Malekshahi ZV, Gargari SL, Rasooli I, Ebrahimizadeh W (2011) Treatment of Helicobacter pylori infection in mice with oral administration of egg yolk-driven anti-UreC immunoglobulin. Microb Pathog 51: 366-372.

41. Jin LZ, Baidoo SK, Marquardt RR, Frohlich AA (1998) In vitro inhibition of adhesion of enterotoxigenic Escherichia coli K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunol Med Microbiol 21: 313-321.

42. Xu Y, Li X, Jin L, Zhen Y, Lu Y, et al. (2011) Application of chicken egg yolk immunoglobulins in the control of terrestrial and aquatic animal diseases: A review. Biotech Adv 29: 860-868.

43. Schade R, Calzado EG, Sarmiento R, Chacana PA, Porankiewicz-Asplund J, et al. (2005) Chicken egg yolk antibodies (IgY-technology): A review of progress in production and use in research and human and veterinary medicine. Altern Lab Anim 33: 129-154.

44. Nguyen SV, Icatlo FC Jr., Nakano T, Isogai E, Hirose K, et al. (2011) Anti-cell-associated glucosyltransferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults. J Am Dent Assoc 142: 943-949.

45. Krüger C, Pearson SK, Kodama Y, Vacca SA, Bowen WH (2004) The effects of egg-derived antibodies to glucosyltransferases on dental caries in rats. Caries Res 38: 9–14.

46. Hatta H, Tsuda K, Ozeki M, Kim M, Yamamoto T, et al. (1997) Passive immunization against dental plaque formation in humans: Effect of a mouth rinse containing egg yolk antibodies (IgY) specific to Streptococcus mutans. Caries Res 31: 268-274.

47. Yokoyama K, Sugano N, Shimada T, Shofiqur RA, Ibrahim S, et al. (2007) Effects of egg yolk antibody against Porphyromonas gingivalis gingipains in periodontitis patients. J Oral Sci 49: 201-206.

48. Sugano N (2009) Adjunctive effects of anti-Porphyromonas gingivalis egg yolk antibody with scaling and root planning: A randomized, placebo-controlled clinical trial. J Periodontol 80: 1901-1903.

49. Sugita-Konishi Y, Shibata K, Yun SS, Hara-Kudo Y, Yamaguchi K, et al. (1996) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. Biosci Biotechnol Biochem 60: 886-888.

50. Nilsson E, Larsson A, Olesen HV, Wejåker PE, Kollberg H (2008) Good effect of IgY against Pseudomonas aeruginosa infections in cystic fibrosis patients. Pediatr Pulmonol 43: 892-899.

51. LeClaire RD, Hunt RE, Bavari S (2002) Protection against bacterial superantigen staphylococcal enterotoxin b by passive vaccination. Infect Immun 70: 2278-2281.

52. Trott DL, Yang M, Gonzalez J, Larson AE, Tepp WH, et al. (2009) Egg yolk antibodies for detection and neutralization of Clostridium botulinum type a neurotoxin. J Food Prot 5: 1005-1011.

53. Hirai K, Arimitsu H, Umeda K, Yokota K, Shen L, et al. (2010) Passive oral immunization by egg yolk immunoglobulin (IgY) to Vibrio cholerae effectively prevents cholera. Acta Med 64: 163-170.

54. Shin JH, Yang M, Nam SW, Kim JT, Myung NH, et al. (2002) Use of egg yolk-derived immunoglobulin as an alternative to antibiotic treatment for control of Helicobacter pylori infection. Clin Vaccine Immunol 9: 1061-1066.

55. Suzuki H, Nomura S, Masaoka T, Goshima H, Kamata N, et al. (2004) Effect of dietary anti-Helicobacter pylori-urease immunoglobulin Y on Helicobacter pylori infection. Aliment Pharmacol Ther 20: 185-192.

56. Horie K, Horie N, Abdou AM, Yang JO, Yun SS, et al. (2004) Suppressive effect of functional drinking yogurt containing specific egg yolk immunoglobulin on Helicobacter pylori in humans. J Dairy Sci 87: 4073-4079.

57. Sunwoo HH, Lee EN, Menninen K, Suresh MR, Sim JS (2002) Growth inhibitory effect of chicken egg yolk antibody (IgY) on Escherichia coli O157: H7. J Food Sci 67: 1486-1494.

58. Li XY, Jin LJ, Uzonna JE, Li SY, Liu JJ, et al. (2009) Chitosan-alginate microcapsules for oral delivery of egg yolk immunoglobulin (IgY): In vivo evaluation in a pig model of enteric colibacillosis. Vet Immunol Immunopathol 129: 132-136.

59. Zhen YH, Guo J, Jin LJ, Xu YP (2008) Characterization of specific egg yolk immunoglobulin (IgY) against mastitis-causing Escherichia coli. Vet Microbiol 130: 126-133.

60. Yokoyama H, Peralta RC, Umeda K, Hashi T, Icatlo FC, et al. (1998) Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk Salmonella-specific antibodies. Am J Vet Res 59: 416-420.

61. Gürtler M, Methner U, Kobilke H, Fehlhaber K (2004) Effect of orally administered egg yolk antibodies on Salmonella enteritidis contamination of hen's eggs. J Vet Med B Infect Dis Vet Public Health 51: 129-134.

62. Zhen YH, Jin LJ, Li XY, Guo J, Li Z, et al. (2009) Efficacy of specific egg yolk immunoglobulin (IgY) to bovine mastitis caused by Staphylococcus aureus. Vet Microbiol 133: 317-322.

63. Tsubokura K, Berndtson E, Bogstedt A, Kaijser B, Kim M, et al. (1997) Oral administration of antibodies as prophylaxis and therapy in Campylobacter jejuni-infected chickens. Clin Exp Immunol 108: 451-455.

64. Song MS, Kim CJ, Cho WI, Sunwoo HH (2009) Growth inhibition of Clostridium perfringens vegetative cells and spores using chicken immunoglobulin Y. J Food Saf 29: 511-520.

65. Owusu-Asiedu A, Nyachoti CM, Baidoo S, Marquardt R, Yang X (2003) Response of early-weaned pigs to an enterotoxigenic Esherichia coli (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. J Anim Sci 81: 1781-1789.