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BIOMEDICAL SCIENCE: ANIMAL TESTING IN ANTIBIOTICS PRODUCTION

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Abstract

The purpose of this research paper is to explore, through the use of various sources, the potential, and importance of animal potency and safety testing as it relates to the discovery and production of both human and agricultural antibiotics. Through an in depth analysis into both current and past research, the study will show the benefits, as well as the needs of animal testing in today's global community in regards to vaccines, treatments, and as a deterrent to bioterrorism. In doing this, the production process of antibiotics, the importance of antibiotics, and the effects that antibiotics have on humans were examined. In conjunction with the aforementioned, global regulations and ethical arguments will be considered and analyzed. The use of animals in antibiotic discovery and production has complex variables, many that are associated with social and political issues, and can be evaluated on several levels; this study also touched on these concerns.

Keywords: antibiotics, vaccines, bioterrorism,

Introduction

Throughout history, antibiotics have proven to be the preferred drug of choice for the treatment of numerous evolving bacteria. One of the most notable occurrences can be traced back to the discovery of penicillin during World War II. With the increased need to combat different bacteria, also came the need to test and develop antibiotics using animals as test subjects. Along

with its success came the evolution of animal antibiotics being used in a high capacity within agriculture, and has raised various ethical issues. The use of animals in antibiotics production has complex variables that are associated with social and political issues that can be analyzed on several levels.

Phillips and Alves, authors of Immunization update: A 2011 retrospective on product changes and new recommendations (2012), wrote it best when they stated, "Vaccines are one of greatest achievements in biomedical science and health care" (p. 58). Vaccines help protect patients by giving them immunity to certain viruses. They have the ability to "induce active immunity by stimulating a primary immune response that results in the induction of B cells, production of antibodies, and sensitization of T cells" (Phillips & Alves, 2012). Vaccines have eradicated viruses such as polio, and have helped prevent innumerable outbreaks and epidemics, and continuously provide humans with immunity worldwide. Such a boosted immunity has become crucial as the risk of bioterrorism continues to increase. In that same aspect, vaccines also aid in preventing the spread of the viruses and bioterrorism agents to other populations. Stopping the spread of a virus can be vital in saving lives. Viral and disease treatments are good, but vaccines can establish immunity to a virus, the preferred method over treatment.

Production Process and Economics

Large amounts of antibiotics are distributed annually for agricultural use as non-therapeutic agents. The Centers for Disease Control (CDC) estimates that the United States produces 23 million kg of antibiotics during an average year, with approximately 8 million kg of those antibiotics being used for animals (Cromwell, 2002). This figure makes up only a small amount of how much antibiotics are actually used globally within agriculture. The U.S. uses antibiotics quite frequently for agricultural use, but several countries have scaled back in this capacity. For example, the European Union banned the use of many antibiotics that can be used as growth promotion agents (Besler & Essack, 2010). The ban in-turn resulted in the decline of overall antibiotic sales.

The production of antibiotics involves several steps, which have critical measurements that come along with them. The evolution of the production of antibiotics began in the 1940's and was labor intensive, but now the process is more computer-controlled (Besler & Essack, 2010). This is a direct result of the constant demand for antibiotics and the evolution of different organisms. According to Madehow, How Products are made (2012), the process of producing an antibiotic involves a number of steps that include:

> *Isolation of the antibiotic-producing organism and start a culture: Transfer the culture to the seed tanks that will provide an ideal environment for the microorganisms to grow. This process usually takes 24-48 hours.*

The fermentation process is needed so the microorganisms can grow at a highly efficient rate. This is a very important process in large-scale antibiotic production.

Isolate the useful antibiotic product from the waste compounds.

Refining the antibiotic involves making them into pills, powders, or topical ointments.

Presently, 80 percent of the total production of antibiotics within the United States is used in agriculture, and majority of this is used for non-therapeutic purposes of growth promotion (Smith, Harris, & Johnson, 2002). Agricultural antibiotics use may pose medical consequences to humans by producing resistant organisms. As a result, the focus has now shifted from the production of antibiotics that target a specific class, to targeting the resistant organism (Ratledge & Kristiansen, 2006). This has the potential to pose a direct economic threat to many nations due the possible declination in antibiotic production. This very real threat has led to some pharmaceutical companies questioning whether it is cost efficient to continue to proceed with massive antibiotic drug discovery efforts, and has even forced some companies to downsize (Ratledge & Kristiansen, 2006). If antibiotic discovery and production were to cease in a large capacity, the results could be catastrophic.

Animal potency testing for antibiotic and vaccine production around the world has become an enormous issue. While research advocates argue that animal testing is the best way to get pre-clinical safety and efficacy results prior to human testing, animal-rights campaigners argue that the rationale behind that is lacking, and animal testing, in most cases, can be and should be minimized or even altogether avoided. Several laws and guidances have been put into place by governments, which can assist researchers in regards to proper animal testing so they may conduct the necessary experiments ethically and successfully.

In 1985, the United States Interagency Research Animal Committee released the Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. These principles were adopted into Public Health Service policy the next year, and have provided a frame for U. S. Government Agencies involved in experimental research to build off of every year since. In addition to "The Principles…," Good Laboratory Practice (GLP) Regulations (2007 edition) was released by the United States Department of Health and Human Services in conjunction with the Food and Drug Administration and Office of Regulatory Affairs. The GLP offers companies and researchers guidelines pertaining to clinical and non-clinical laboratory procedures and protocols, testing facilities, test and control articles, personnel, etc. (pp. 1-14). These regulations should be consulted and properly used alongside the Animal Welfare Act to ensure that companies are maintaining and utilizing test animal subjects properly.

Like the United States, countries around the world have adopted similar regulations involving animal safety testing in antibiotic discovery and production. The German Animal Welfare Act requires that all biomedical research to be conducted on animal test subjects must first be authorized, and then licensed before commencing. Only experimental designs that contain an

ethically acceptable plan for animal testing in relation to the study, and that are deemed indispensable will receive this license (Sauer, 2006). In 2010, members of the European Council, Parliament, Representatives of the European Commission, compromised on a directive that would not only protect the animals in experimental testing, but also would please and protect the researcher as well. Surprisingly, the final directive decided upon included that basic research using primates and endangered species will now be allowed, although research on great apes has be banned, and animals will not have to be destroyed immediately after research procedures that cause "moderate" discomfort (European Commission; Abbot, 2010). In addition, a minimum cage size has been introduced, hen's eggs can no longer be used in the production of vaccines, the forms of research that cause extreme or prolonged pain, etc.

In Japan, a similar cessation of the ban on biomedical research studies using primates has occurred, but unlike the EU, national policy does not allow for "the life-long care of retired laboratory chimpanzees," (Morimura, Idani, & Matsuzawa, 2011). In 2007, Japan established the Chimpanzee Sanctuary Uto (CSU), which acts as refuge for laboratory chimpanzees that are no longer being used (Morimura, Idani, & Matsuzawa, 2011). The introduction and utilization of the 3Rs concept, which includes the reduction, refinement, and replacement of non-humans in experimental testing is taking place all over the world, to include Canada, the United States, Europe, and Asia (Isbrucker, Sontakke, & Smith, 2011) (Shin, Lei, Conrad, Knezevic, & Wood, 2011). Although animal experimentation for the discovery and production of antibiotics is still heavily utilized globally, governments are making a conscious effort to reduce any over and/or improper usage.

Current Uses for Animal Testing in Antibiotic Production

Antibiotic production and manufacturing entails numerous trials before the antibiotics can be marketed and sold to consumers. After the drugs are licensed as experimental drugs, they must undergo preclinical animal testing before the antibiotics can enter into the clinical phases of testing. Antibiotics cannot reach the FDA approval process without first being deemed successful through animal testing. Much can be learned from the use of animal models to test antibiotics including possible side effects, possible/probable associated human immune system reactions. According to Clemens and Stevens, authors of Animal models of Aspergillus infection in preclinical trials, diagnostics, and pharmacodynamics: What can we learn from them? (n.d.), suggest, "Other laboratories have used inhalational murine models, and pulmonary or systemic models in rabbits and guinea pigs, as well as birds and insects," (p.44). Different animal models exhibit reactions to antibiotics that are similar human reactions. The animal models chosen are based on a set of desired reactions that are similar, or may mimic, human reactions. Researchers have found that the effectiveness of an antibiotic in a mouse will have presumably the same effectiveness in a human. The goals in animal testing are to test the efficacy and safety of the antibiotics being developed. If the drugs are deemed fatal or do not produce an effective reaction during the preclinical animal testing phase, the development of the drug is either stopped or

modified so as to attempt to prevent any death or like adverse reactions from occurring in humans.

One current and noteworthy use for animal testing in antibiotic production is bioterrorism. The threat of biological warfare is prevalent, and researchers are doing everything in their power to help protect against an attack. Scientists are now using mice and monkeys to develop antiviral medications to combat monkeypox and smallpox, which are both acute, contagious viral diseases. Testing for antiviral medications developed to combat bioterrorism, namely CMX001 and ST-246 for smallpox and monkeypox found through animal testing, that both have efficacy in the C57BL/6 mousepox models of smallpox and monkeypox. (Scott et al, n.d.). Using animal testing, scientists have found ways to vaccinate and treat deadly viruses such as the aforementioned. Both bioterrorism and other unforeseen outbreaks can cripple a nation, but if used correctly and ethically, vaccines and antibiotics can help save lives.

Ethical Considerations of Animal Testing

There are several arguments present against the use of animal potency and safety testing. Animal-activists argue that such test animals undergo extreme pain and suffering, cruel living conditions, etc. To combat this, the 3Rs (reduce, refine, replace) have been implemented as methods for vaccine safety testing, and have been amended into regulations globally in an effort to minimize improper and over usage (Kulpa-Eddy & Srinivas, 2011). Ethically, this employment has made much progress towards the "improved well-being of laboratory animals," (Carbone, 2011). According to Larry Carbone, Chief Veterinarian at the University of California , San Francisco, and author of Pain in Laboratory Animals: The Ethical and Regulatory Imperitives (2011), "Current public policy in most countries calls for treatment or prevention of laboratory animal pain whenever possible," (p. e21578). Unfortunately, however, most regulations state that laboratory animals can suffer moderate pain when scientifically necessary in the success of the study. Carbone goes on to argue that such clauses that contain the statement, "when scientifically necessary," when added to polices and mandates fail to refine procedures to alleviate stress and pain in such laboratory animals, nor do they reduce animal use or replace the animals with other experimentation models. Animal activists also offer the argument that facilities, handlers, and experimentation techniques are too cruel and suggest painkillers for animals that must undergo such painful procedures. However, as aforementioned, governments have made note of such claims and have ratified regulations to include guidances that aid in avoiding such unethical practices.

While arguments for the complete ban of animal testing are still being offered, some, like Rainer Nobiling of the University of Heidelberg in Germany, who has collaboratively contributed over 160 scientific articles, assert that it would be more unethical to ban animal testing in biomedical research. This claim is based on the idea a ban like this could potentially encourage more experimental testing in humans, which would be contrary to what is published in the Nuremberg Code of Research Ethics, in 1949. In that same regard, many maintain that such a ban would

hinder the success of some studies, suggesting that it would "clash with…a duty to carry out research, as enshrined in the Lisbon Treaty, adopted in December 2009", which too would be unethical (Abbot, 2010). In an answer to providing painkillers, as stated in the United States Department of Agriculture's 1971 designation of ''Category E'' painful procedures, painkillers can interfere immensely to an experiment (Carbone, 2011), which also would be unethical.

The Effects of Antibiotics on Humans

The antibiotics used for agricultural purposes involving animals can affect human health in many ways. The most controversial topic discussed is whether the antibiotics that humans consume in their daily diets have an effect on recent cases of antibiotic resistance. Many scientists and medical professionals believe that the consumption of various types of animal meats that contain antibiotics can result in human resistance. However, many disagree. Nevertheless, over the years, the interest in uncovering the truth has increased.

Given the up rise in the number of global antibiotic resistant cases, we must begin to analyze the potential root cause of this trend. This leads us to livestock. It has been proven that enteric bacteria from an animal can colonize to a human gut under experimental conditions. However, the topic in question is whether the crossover of antibiotic resistance between bacteria of these animals to occur (Dorn, Tsutakawa, & Fein, 1975). This was the premise of a study that was conducted at the University of Missouri. A group of scientists conducted a study on the antibiotic resistance of E.coli. Using 61 human volunteers from 14 different farms. Volunteers were compared to 10 percent of the animal population at their farms. The resistance pattern of the fecal samples that were taken from humans and the farm animals were both tested for the resistance of tetracycline, neomycin, sulfathiazole, kanamycin, cephalothin, ampicillin, and chloramphenicol (Dorn et al., 2005). The results concluded that the combination of E.coli infected meat with human consumption is a significant pathway for the transfer of antimicrobial resistance from animal to human.

To get a better understanding of how antibiotic resistance can be passed from animal to human we must first describe the methods these bacteria can be transferred. The two most recognized pathways are indirect and direct. There have been three indirect pathways recognized in which resistance acquisitions can be sorted and they are transformation, conjugation and transduction (Besler & Essack, 2010). All three pathways involve different mechanisms of actions. Transformation involves dying bacterium releasing its plasmids and a healthy bacterium cell acquires this material. Conjugation involves the transmission of plasmids and DNA from cell to cell with the use of specific enzymes. Transduction involves an injection of genetic material into the host cell after it attaches to another cell. Direct pathways are recognized as gene mutations in the gene encoding resistance against mechanisms of specific antibodies (Besler & Essack, 2010).

Conclusion

The interactions that are involved with animals and antibiotics are controversial and dynamic. The issue associated with antibiotics production and animals will continue to be a major topic of discussion, but the future need for antibiotics may continue to fluctuate. On a global level, antibiotic production is declining but there are still some countries that produce substantial amounts. The evolution of modern medicine, with the help of antibiotics, has made it possible to fight various bacteria, and we can only hope that they continue to keep their effectiveness.

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