STUDY OF THE ROLE OF ANTIMICROBIAL RESIDUE CONTAMINATION IN CHICKEN MEAT

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Abstract: The investigation aimed to assess bacterial contamination in 120 chicken meat samples collected from various retail marketplaces in Uzbekistan. Samples were analyzed microbiologically by isolating them on Agar medium, performing Gram's staining and enzymatic tests for confirmation. Antibacterial compounds were tested using the STAF procedure. The suspected Bacillus subtilis colonies exhibited specific characteristics like yellowish-brown color, smooth margins, and white curly growth, further confirmed by sheep blood agar growth and Gram staining. Biochemical tests confirmed Bacillus subtilis through catalase, gelatin hydrolysis, glucose fermentation, citrate utilization, and nitrate reduction tests. Microscopy revealed green spores and red bacterial cells. The study revealed significant antimicrobial drug residue contamination in chicken meat, likely due to antibiotic misuse or insufficient withdrawal periods. The presence of Bacillus subtilis in poultry meat indicates potential quality degradation and risks to human health. The research aims to enhance food safety policies by identifying contamination sources and antibiotic misuse, ultimately improving poultry product safety. This study not only benefits scientific understanding of microbial dynamics in poultry production but also contributes to reducing foodborne illnesses linked to contaminated meat.

Keywords: Bacillus subtilis, chicken meat, food safety, public health.

ИЗУЧЕНИЕ РОЛИ ЗАГРЯЗНЕНИЕ ОСТАТКАМИ ПРОТИВОМИКРОБНЫХ ПРЕПАРАТОВ В КУРИНОМ МЯСЕ

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Аннотация: Исследование было направлено на оценку бактериального загрязнения в 120 образцах куриного мяса, собранных на различных розничных рынках в Узбекистане. Образцы были проанализированы микробиологически путем выделения их на среде агара, проведения окрашивания по Граму и ферментативных тестов для подтверждения. Антибактериальные соединения были протестированы с помощью процедуры STAF. Предполагаемые колонии Bacillus subtilis демонстрировали специфические характеристики, такие как желтовато-коричневый цвет, гладкие края и белый курчавый рост, что дополнительно подтверждается ростом агара в крови овец и окрашиванием по Граму. Биохимические тесты подтвердили Bacillus subtilis с помощью каталазы, гидролиза желатина, ферментации глюкозы, утилизации цитратов и тестов на снижение нитратов. Микроскопия выявила зеленые споры и эритроциты. Исследование выявило значительное загрязнение остатков противомикробных препаратов в курином мясе, вероятно, из-за неправильного использования антибиотиков или недостаточных периодов отмены. Присутствие Bacillus subtilis в мясе птицы указывает на потенциальное ухудшение качества и риск для здоровья человека. Исследование направлено на совершенствование политики безопасности пищевых продуктов путем выявления источников загрязнения и злоупотребления антибиотиками, что в конечном итоге повышает безопасность продуктов птицеводства. Это исследование не только способствует научному пониманию микробной

динамики в птицеводстве, но и способствует снижению случаев пищевого отравления, связанных с зараженным мясом.

Ключевые слова: Bacillus subtilis, куриное мясо, пищевая безопасность, здоровье населения.

INTRODUCTION

In Uzbekistan, the chicken industry plays a pivotal role in agriculture, as well as in bridging the price and quantity gap on a business scale.. The government is paying special attention to this sector as to include it in the chain of food processing. It is considered to have paid main role to fulfill the animal-based protein deficiency in Uzbekistan. The consumption and high demand of white meat leads to establish large commercial production of the poultry meat [2]. Broiler meat is widely consumed globally, but the increasing demand for chicken poses significant challenges for the farming industry in preserving hygiene and nutritional quality [1]. The industry faces issues related to infection control and the lifespan of food animals, which are at a higher risk of being infected with harmful pathogens and residues of preventive medications . Nearly 95% of foodborne illnesses are associated with the consumption of tainted poultry, seafood, and animal products [3]. Human beings have employed various strategies to combat disease-causing microbes, utilizing medicinal plant extracts, pure compounds, and antibiotics. The use of antibiotics has significantly reduced morbidity and mortality rates attributed to pathogenic microbes. While antibiotics are commonly utilized as growth promoters in the meat production industry to meet commercial demands, this practice can contribute to the rise of antimicrobial resistance. Consequently, the European Union has banned the use of antibiotics as growth promoters in chicken farms [4]. The mechanism behind antibiotics as growth promoters involves their interaction with intestinal microbes, leading to the displacement of microbial variants and the colonization of beneficial bacteria in the intestine. This process can enhance resource utilization and promote gastrointestinal epithelial repair through the production of volatile fatty acids [5] The increasing demand for poultry products has compelled farmers to intensify poultry meat production within shorter time frames. Various approaches, including the utilization of antibiotics, have been adopted to achieve this objective. Antibiotics, which were developed in the 1940s to address microbial challenges, are substances capable of inhibiting or killing microorganisms. Within the category of antibiotics, antibacterials play a critical role. Antibiotics can be classified based on their chemical structure, function, mode of action, source of antibacterial agents, and range of activity [7]. Functionally, antibiotics are categorized as inhibitors of cell wall synthesis, membrane function, protein synthesis, and nucleic acid synthesis [6]. In the poultry industry, the use of antibiotics is commonly divided into three primary categories: therapeutic (targeting bacterial pathogens), prophylactic (prevention against pathogens), and growth promotion (to stimulate rapid growth in a short period). Therapeutically, antibiotics are administered for a specific duration from the onset of symptoms until the patient fully recovers.

MATERIALS AND METHODS

Isolation of Bacillus subtilis Ten soil samples were gathered and then diluted before being inoculated onto nutrient agar plates containing 0.4% dextrose with a pH of 7.4. Following the inoculation of the soil samples, the plates were placed in an incubator for 24 hours at 37°C. Subsequently, colonies displaying a range of morphological, cultural, and biochemical characteristics were analyzed individually.

Sample collection One hundred and fifty poultry meat samples, comprising 75 kidney samples and 75 liver samples, were obtained from various retail markets in Andijan city, Uzbekistan. All samples were collected aseptically and maintained under refrigerated conditions. Subsequently, these samples underwent testing to detect the presence of antibiotic residues. Sterilization of materials used To ensure proper sterilization, a hot air oven was employed to sterilize glassware such as glass test tubes and Petri plates using dry heat. The recommended temperature for the hot air oven is 160°C. Glassware should be sterilized at this temperature for a duration ranging from 50 to 65 minutes. It is crucial not to remove materials from the hot air oven immediately; they should only be taken out once the temperature has gradually decreased to 50°C. This gradual cooling process is essential to prevent the glassware from cracking and to minimize the risk of external air carrying potential microorganisms entering the hot air oven [8]. Sterilization of glass petri plates Begin by setting the hot air oven to 160°C. Prepare a mixture of antiseptic dish soap and water. Thoroughly clean and wash the Petri plates. Rinse the plates with sterile water and dry them using a soft cloth, ensuring all debris is removed. Wrap the plates in paper Uzair et al. 209 and place them in the hot air oven. Set the timer for 40 to 60 minutes and turn off the oven after 45 to 60 minutes. Allow the oven to cool before removing the glassware. Wear sterile gloves to take out the Petri plates from the oven, ensuring that no unsterile material touches the sterilized plates.

RESULTS

Growth on nutrient agar Out of the 120 poultry samples (60 kidneys and 60 livers) inoculated on Nutrient Agar, 50 liver samples and 40 kidney samples tested positive. The colonies exhibited a yellowish-brown color with smooth margins, and after 24 to 48 hours, white curly growth was observed. Additionally, 10 soil samples were collected, with 5 of them testing positive for Bacillus subtilis.

Citrate utilization test Bacillus subtilis utilized the alkaline bicarbonate and carbonate byproducts of citrate metabolism to elevate the pH above 7.5, causing the bromothymol blue indicator to shift from a blue hue to a bottle green color. In cases where there was no color change, the medium retained a deep green shade. Bacteria capable of utilizing citrate as an energy and carbon source could grow on Simmons citrate. The test bacterium Bacillus subtilis was introduced onto Mueller-Hinton agar using a sterile swab and then left to grow for 18-24 hours at 37°C. Sterile cotton swabs were immersed in chicken meat samples from the Pure Appl. Biol.,14(2):206-223,June, 2025 http://dx.doi.org/10.19045/bspab.2025.140021 218 kidney and liver for 5 minutes. These swabs were then transferred to separate plates, each containing a Neomycin filter paper disk as a positive control, along with 11 different antibiotics to detect any residual presence in the samples.

DISCUSSION

Poultry industry is one of the most promising sectors for Uzbekistan. This industry provides various opportunities to increase GDP growth rate plus equitable distribution through arranging food security as well as ensuring self -employment, creating purchasing power and reducing poverty at a large scale [7]. About 44 per cent of daily human intake of animal protein comes from livestock products. The poultry sector is an important and vibrant segment of agriculture in Uzbekistan with a significant contribution to the national GDP (1.3%) [5]. Commercial poultry production in Uzbekistan started in the 1960's and has been providing a significant portion of daily proteins to the Uzbek population ever since. During its evolution the industry enjoyed promotional policies of the Government but has faced several challenges such as disease outbreaks and retail

price fluctuations [6]. Despite its important role in the country's economy, not a single scientific study is available on its evolutionary history. The data available in this regard are scattered and lack reliability Uzair et al. 221 [7]. The poultry industry started to stabilize in the late 1990s by gaining better profit margins on poultry products. New investors entered into the industry and adopted modern technologies such as environmentally controlled housing [8]. Keeping in view this need, the present study was designed for the microbiological assessment of antibiotic residues in poultry meat samples. In present study, a total of 120 poultry meat samples were collected from various retail markets of Faisalabad. For further analysis, samples were inoculated on Nutrient agar and incubated at 37oC for 48 hours. These suspected samples were also grown on sheep blood agar and after 48 incubation at 37oC, growth was observed. Then microscopy (Gram staining) was performed and Bacillus subtilis appeared as purple coloured rod shape bacteria.Further confirmation was done based upon biochemical tests (catalase, indole, methyl red, Voges Proskauer, gelatin hydrolysis, glucose fermentation, citrate utilization, and nitrate reduction).Among these biochemical tests, Bacillus subtilis was positive for catalase test, gelatin hydrolysis test, glucose fermentation (with no gas production), citrate utilization test and nitrate reduction test while other tests were negative for Bacillus subtilis. The spores of Bacillus subtilis were also stained which appeared as green and bacterial cells appeared red during microscopy.

CONCLUSION

The study concluded that antibiotic residues have been commonly associated with poultry meat and is a source of serious threat for mankind. These high incidences of antibiotic residues are mainly due to irrational, repetitive and overuse of antibiotics. The use of probiotics as feed additive and use of antibiotics for sake of increase production of meat and growth promoter are also leading cause of antibiotic residue in poultry meat. So, in order to control antibiotic residues in meat strict policies should be made by government and regular monitoring and surveillance program should be adopted in order to minimize the issue.

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