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A STUDY OF ISOLATION OF ANTIBIOTIC RESISTANT BACTERIA FROM POULTRY WASTE

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ABSTRACT

On the other hand, bacteria from poultry waste were isolated for antibiotic susceptibility test using disc diffusion method. From the susceptible test results, the multiple antibiotic susceptible strains were tested against the probiotic bacteria isolated from food source. The well cut method was used to determine the antimicrobial of bacteriocin against indicator strain (antibiotic resistant bacteria from poultry waste). To improve the antagonistic substance production of probiotic bacteria against the antibiotic resistant bacteria, various parameters which includes incubation time, temperature and nutrient composition were studied. In addition, the stability of the antagonistic substance was also studied at different temperatures. Campylobacteriosis is at present the most frequent zoonosis in humans and its main source is poultry meat contaminated with Campylobacter jejuni. An alternative and effective approach to antibiotic administration in livestock to reduce bacterial contamination is the use of probiotics, which can help improve the natural defence of animals against pathogenic bacteria. B.longum PCB 133, possessing probiotic properties and a marked anti-Campylobacter (both in vitro and in vivo) are two excellent strains being employed as additives to poultry feed for the reduction of foodborne campylobacteriosis in humans. Antibiotics were extensively used to counteract bacterial infections during World War II. Since then, antibiotic utilization has steadily increased. Antibiotics are compounds, synthesized naturally or synthetically, which usually exert a negative effect on a specific population of bacteria by intervening in its metabolic pathway. They are mainly used in health care for the treatment and prevention of bacterial infections.

KEYWORDS: Isolation, Antibiotic Resistant, Poultry Waste, antibiotic susceptibility test, bacterial infections

INTRODUCTION

Campylobacteriosis is at present the most frequent zoonosis in humans and its main source is poultry meat contaminated with Campylobacter jejuni. An alternative and effective approach to antibiotic administration in livestock to reduce bacterial contamination is the use of probiotics, which can help improve the natural defence of animals against pathogenic bacteria. B.longum PCB 133, possessing probiotic properties and a marked anti-Campylobacter (both in vitro and in vivo) are two excellent strains being employed as additives to poultry feed for the reduction of foodborne campylobacteriosis in humans (Santini et al., 2010). The high impact of antibiotic use on the environmental microbiome is via poultry waste disposal. Application of litter onto open fields can impact the soil microbiome locally or regionally through run-off and air-borne drift. Most of the antibiotics in feeds pass largely unchanged through the broiler gut into excreta (Kumar et al., 2005). Some drugs (including oxytetracycline and fluoroquinoline analogs) can persist in the soil environment with half-lives as long as 150–250 days with undiminished potency (Kumar et al., 2005; Chee-Sanford, 2009). The impact of agricultural practices on soil and aquatic resistomes has been reviewed, but the ecological effects on bacterial communities from application of animal wastes to soils have not been clearly studied. This is because the interaction of naturally occurring and poultry waste derived antimicrobials on the environmental microbiome is probably highly complex and dynamic (Davis et al., 2011).

PROBIOTICS IN POULTRY

Patterson and Burkholder (2003), reported that the application of probiotics in poultry has gained considerable interest during the last few years because antibiotic growth promoters (AGPs), added to animal feed for high growth and to decrease the incidence of diseases leave harmful residues in meat. The most commonly used organisms in probiotic preparations are lactic acid producing bacteria such as Lactobacilli, Streptococci, Bifidobacteria and yeasts like Sacharomycescerevisiae, Sacharomycesboulardii and fungi Aspergillusoryzae. However, lactic acid bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (O’Sullivan et al., 1992). Among lactic acid bacteria, Lactobacilli are the most important and it mainly compose crop and ileum flora of poultry.

Poultry

Tilman et al., (2002) reported that poultry farming has always involved anthropogenic modifications of the natural ecosystem to generate protein for human consumption. Nutrient availability and on-farm microbial diversity further impacted the dynamics of host and environmental microbial communities. Poultry production has become highly concentrated geographically and each production operation now produces more animals and animal wastes than any time in the past. The number of large farms (i.e. with over 125,000 broilers) has increased over 10-fold between 1982 and 2002 in U.S. (GAO, 2008).

Antimicrobial resistant bacteria in poultry

In 1997, WHO reported that the use of antimicrobials in broiler chicken production increased the risk of food-borne infections caused by antimicrobial-resistant pathogens. The normal flora namely Staphylococci and Enterococci of poultry may serve as a reservoir of antimicrobial resistant bacteria and resistance genes (Lu et al., 2003), Research has shown that the use of antimicrobials in poultry production is associated with the occurrence of antimicrobial resistant bacteria and resistance genes in samples of fresh poultry litter (Nandi et al., 2004).

Schroeder et al.,(2004) showed that antimicrobial resistant E. coli have been isolated from various foods and a majority of them from traditional retail meats and poultry. This does not necessarily mean that the prevalence and/or levels of antimicrobial resistant E. coli are higher in these than other foods; it may simply reflect the fact that retail meat and poultry have been most extensively examined for presence of resistant strains. Nevertheless, the preponderance of data indicates that most E. coli recovered in meat and poultry are antimicrobial resistant.

Ferreira et al., (2013) reported that the genus Arcobacter is an emerging pathogen associated with several clinical symptoms and it is widely distributed in poultry. Arcobacterbutzleri strains isolated from poultry and the environment of a Portuguese slaughter house were characterized by pulsed field gel electrophoresis (PFGE) and assessed for antimicrobial susceptibility. PFGE patterns obtained using restriction enzymes SmaI and SacII revealed high genetic diversity, with 32 distinct PFGE patterns. Most of A. butzleri isolates showed multiple antimicrobial resistances, exhibiting four different resistance profiles.

Furtula et al., (2010) demonstrated antimicrobial chemical residues and antimicrobial resistant E. coli in poultry litter which is related to the feeding of these antimicrobials in commercial flocks and as part of a controlled trial that compared antimicrobial treated and untreated flocks. Litter type, age, and reuse (not cleaning between flocks) has an impact on the microbial diversity both in litter and in the intestines of young chickens, suggesting a bidirectional influence of microbiomes (Cressman et al., 2010). Much like the chicken cecum, poultry waste contains a significant number of resistance integrons, particularly in gram-positive bacteria.

Impact of antibiotic resistant microbes from poultry waste on environment

The impact of natural selection within the intestine of individual poultry hosts can be further scaled up to the inter-micro biome and inter-ecosystem level (Figure 1). Peterson et al., (2010) reported that the agricultural ecosystems interact with other ecosystems directly at local and regional levels, and more broadly through global movement of dusts and water as well as economic trade in feeds, animals, and animal waste. Although the industrial poultry house often is assumed to be biocontained and biosecure, multiple pathways connect it with surrounding ecosystems. These include ventilation practices required to keep crowded animals alive; movement of rodents, wild birds, and insects in and out of confinement facilities, and transfers of wastes. These conditions release viable microbes and resistance determinants into surrounding environments, water systems, and wild animal reservoirs.

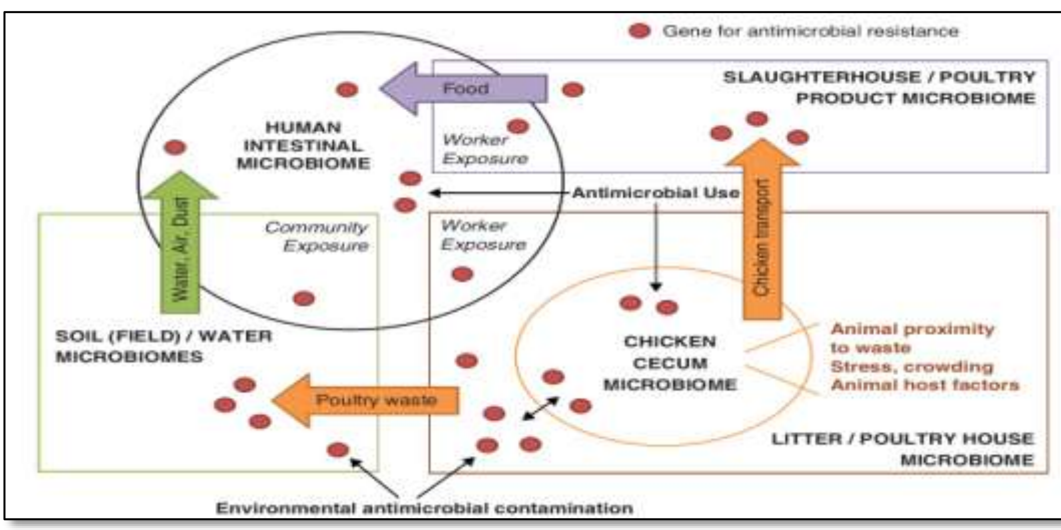

Figure 1 Transfer of antibiotic resistant gene from poultry to human and environment

Chee-Sanford et al., (2009) reported that antibiotic resistance genes are considered as a pollutant that can be transferred environmentally through horizontal gene transfer (HGT) without relying on their corresponding antibiotics or ARBs. The release of high concentrations of antibiotics and resistance genes in natural ecosystems can impact the structure and the activity of environmental microbial populations. It was also found that environmental microorganisms are the original source of resistance genes acquired by human pathogens through HGT, and that these changes were relevant for the future of human health (Martinez, 2009). Smalla et al., (2000) reported that pig manure promoted the mobilization of plasmids conferring antibiotic resistance. Thus, farmlands enriched with pig manure slurry are favorable to conjugal gene transfer. This has raised concern about the possible formation of an environmental reservoir of antibiotic resistance genes in farm land that could transfer resistance back to animals or humans via crops (Sengelov et al., 2003).

Bolan et al., (2010) reported that continued growth of India's poultry industry could be derailed by environmental and epidemiological facts. Livestock intensification has potential environmental externalities. Intensive poultry production implies birds in confined spaces with implications for handling waste and managing pests and diseases. These are aggravated when the intensive production systems cluster geographically, as is the case in southern India. A major problem facing intensive poultry production is the disposal of litter. Most of the litter produced by the poultry industry is applied to agricultural land. Poultry litter is a good organic source of nutrients for raising crops, such as maize but it can lead to environmental pollution when the litter is applied under agronomic, soil and climatic conditions that do not lead to the utilization of the manureborne nutrients. Poultry manure can also become a serious environmental pollutant and contributor to greenhouse gases. In addition, air quality has become a major environmental concern of the poultry industry. There are environmental and health issues linked to bioaerosols (e.g. microbes, endotoxins and mycotoxins suspended in air) generated at production, manure storage facilities and during land spreading of poultry litter.

Effect of veterinary manure in plants

Heuer et al.,(2011) reported that wastes from poultry farm used as a veterinary manure is an important reservoir of antibiotics and ARBs. Usage of veterinary waste could significantly result in the selection of ARBs and an increase in the prevalence of resistance genes in manure-fertilized soil.

Baquero et al.,(2008) showed that antibiotics from veterinary manure might act at very low concentrations, as signalling agents (a kind of hormones) in microbial environments. Common receptors have been identified in plants for a number of antibiotics and disinfectants affecting chloroplast replication (fluoroquinolones), transcription–translation (tetracyclines, macrolides, lincosamides, aminoglycosides, pleuromutilins), folate biosynthesis (sulfonamides, and probably trimetoprim), fatty acid synthesis (triclosan), and sterol biosynthesis (azoles, statins).

PROBIOTIC FOODS

Most commonly used probiotic product is yoghurt. In spite of this, cheese, milk either fermented or unfermented, smoothies, juice, nutrition bars, cereals and infant formula feed all are examples of probiotic food (Ranadheera et al., 2010). Along with food, probiotics can also be available in the form of liquid, powder, gel, granules, paste, capsules, sachets, drugs and as dietary supplements. All these forms contain a large number of bacteria which remains in a stable condition. These forms of products are more convenient as we can deliver a large number of bacteria from manufacturer to customer in stable condition. There are various products made by different companies using different strains of probiotics. These products have various clinical or therapeutic applications. The use of probiotics in food or other products depends on many factors like stability of product, humidity, pH, age of customer, quantity or number of bacteria used, etc. These products are helpful for all age groups like children, infants, old age. The aim of using these microbes as probiotics is mainly to increase the beneficial flora of the host.

In the United States, dairy products such as yoghurt and fermented milk are the food products that contain the probiotics exclusions. LAB are associated with the fermented milk (Shah, 2015). The most commonly used bacteria in dairy products

(containing probiotics) are members of Lactobacillus and Bifidobacterium genus (Backhed, 2012). Probiotics commonly are not the colonizers of the GI tract for long term, whereas they can stick provisionally to the epithelial layer. They remain metabolically active, although they divide very slowly in the intestine. Milk and milk products contain probiotic bacteria, which improves the beneficial microbiota in the intestine (Isolauri, 2001). Probiotics are a group of microorganisms that are involved directly in increasing the resistance of bacteria against intestinal pathogens and thus are involved in the prevention of the diseases. Probiotic bacteria involved in the production of a variety of compounds, which shows an inhibitory effect to the growth of pathogenic microorganisms, inhibitory compounds include bacteriocins, reuterin and organic acids such as acetic acids and lactic acids. Food is a common medium for probiotic microorganisms delivery in consumer body. Probiotic microorganisms that are given by food systems have to fulfill some conditions like they have to first survive during the transfer through the upper gastrointestinal tract, and then survive in the gut to produce beneficial effects to the host. Fermented foods can have probiotics, prebiotics or both, and they are associated with good health. Yogurt is a well-known probiotic used today. It contains a very good nutritional value and provides health benefits to the host. L. delbrueckii subsp. Thermophilus bacteria, Streptococcus salivariussubsp, and L. bulgaricus. The various factors that affect the growth of probiotic microorganisms are H2O2, dissolved oxygen, pH, buffering capacity, lactic acid/acetic acid concentration etc.

CONCLUSION

Morphologically nine different bacterial colonies were isolated from 10-6 and 10-7 dilutions of poultry waste. In the nine isolates, multiple antibiotic drug resistance strains were selected for biochemical tests and 16S rRNA identification. In biochemical test, the selected five multiple antibiotic resistant bacteria were identified as Pseudomonas sp., Acinetobactor sp., Enterococcus sp. and Bacillus sp. to the generic level according to Bergey's Manual.Among the four probiotic bacteria, Weissellacibaria KTSMBNL 28 exhibited high probiotic characteristics than Weissellacibaria KTSMBNL 27, Weissellacibaria KTSMBNL 29 and Weissellacibaria KTSMBNL 30. Hence Weissellacibaria KTSMBNL 28 was used for the studies against antibiotic resistant bacteria from poultry waste. In conclusion, the present study demonstrates that the W.cibaria KTSMBNL 28 strain can be used to treat antibiotic resistance in poultry waste. Due to the significant problem of advancing antimicrobial resistance, the global scientific community has attempted to find alternative solutions and one of the most promising attempts is use of probiotics. Probiotics generally recognized as safe can be studied and used as an alternative and/or adjunctive therapy to conventional antibiotic therapy in treating various infectious diseases. A complete study serves as an important stepping stone for the use of probiotics in the restoration of the environment from antibiotic resistant bacteria.

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