

## New applications in treatment and control of Enterobacteriaceae-causing diseases in poultry

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**Abstract:** Enterobacteriaceae family is subdivided into eight genera including Escherichiae, Edwardsiellae, Salmonellae, Citrobacterae, Klebsiellae, Proteae, Yersinae, and Erwinae. Pathogens members are causing health problems in both humans and animals. Pathogens members are considered the most important avian intestinal pathogens and induced many disease conditions and losses in poultry including chickens, turkey, ducks, and pigeons. Enterobacteriaceae was reported as negatively impacts feed conversion, body weight gain, mortality, low egg production, increase dead in shell and low hatchability, condemnations at processing, cost of medication and control. Furthermore, antibiotic resistance bacteria occurs in poultry farms and surrounding environment can be spread to humans. Biochemical or serologic laboratory examinations do not help in differentiating primary from secondary infections but it must come in combination with bird clinic to determine the importance of bacterial isolation for a bird species and a specific disease cause. Poultry diseases control based on hatching eggs and hatchery sanitation as well as farm biosecurity, disinfectants, good management practices, vaccination, accurate diagnosis and treatment (antimicrobial therapy). Corrective drugs are indicated and used very carefully according to manufacture (dosage and directions of application) to get good results and avoid drug resistant bacterial infections. Combinations of antibiotics with probiotics or prebiotics are useful to prevent and control bacterial infections in poultry. Also competitive exclusion proved to be valuable in control of enteric bacterial pathogens. Used disinfectants must be of high safety, of minimal residual toxicity and effectiveness for a variety of infectious agents. It is recommended to apply the recent points in control of enteric pathogens of economic and public health importance including: genetic selection is one of the choices to improve and obtain chicken resistant lines, Phytobiotics (Plant spices and extracts) is used in organic poultry production as some have growth promoting effects and others have antimicrobial properties against bacterial pathogens. Additionally, application of nanotechnology in disinfection, medication and vaccination.

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### Introduction

Enterobacteriaceae is a family of rod-shaped, aerobic, facultative anaerobic bacteria. The Enterobacteriaceae family is subdivided into 8 genera including Escherichiae, Edwardsiellae, Salmonellae, Citrobacterae, Klebsiellae, Proteae, Yersinae, and Erwinae (*Nagy and Fekete et al., 2005*). Many genera and strains either non-pathogenic (commensally: intestinal bacteria can be part of the normal flora) or pathogens (those are not routinely found in the GI tract) are colonize the small and large intestine of human and animals including birds.

The Economic impact of Enterobacteriaceae bacterial infections was reported as negatively impacts feed conversion, body weight gain, mortality, low egg production, increase dead in shell and low hatchability as well as condemnations at processing. More than 45% of negative economic effects beginning in the earliest subclinical disease stages in man (*Kaper et al., 2004*).

Poultry diseases prevention based on: 1. Management of hatching Eggs and hatchery

management to obtain healthy one-day-old chicks. Many pathogenic bacteria species [ *Salmonella Enteritidis* (*S. Enteritidis*), *Escherichia coli* (*E.coli*), *Cetrbacter frundi* (*C. frundi*), *Protues vulgaris* (*P. vulgaris*), *Campylobacter jejuni* (*C. jejuni*)], *Pseudomonas aeruginosa* (*P.aeruginosa*) and *Klebsiella pneumonia* had been isolated from hatchery and hatchery wastes (*Amer et al., 2013 and 2017*). 2. On farm level disinfectants, good management practices, vaccination and treatment (antimicrobial therapy).

Control of salmonellosis in man has a positive economic impact on poultry industry from many aspects: costs of medical expenses, sick leaves and loss of productivity. Other members of Enterobacteriaceae as *Edwardsiella* and *Yersinia* have the same impact on human health.

The hazard use of such antibiotics for treatment of most common Gram-negative bacteria which are responsible for infections, such as *E. coli*, *Salmonella* sp., *Shigella* spp., *Campylobacter* sp. and *Yersinia* sp. had growing threat of multidrug resistance (*Horská et*

*al., 2013*). Therefore, *in vitro* antibiotic sensitivity test by disc diffusion method (*CLSI 2013*) is recommended for selection of more effective antibiotic.

Disinfectants are used to free poultry environment from pathogenic organisms are one of the important lines of control of disease; disinfectant is low cost per unit of disinfecting value, solubility used water, of high safety, ready availability, stability in air, no or minimal residual toxicity, effectiveness for a variety of infectious agents, various disinfectants and methods should be consulted (*Block, 1991*).

Biosecurity including all of the measures that can be taken to prevent or provides safety from transmissible infectious diseases from entering or surviving and infecting poultry flock (*Bermudez, 2008*). These measures are very important to avoid that hatching eggs, day-old chicks, feed, water, the poultry house, rodents and residual environmental contamination become the source of infection in fresh uninfected stock.

#### **Control of Enterobacteriaceae**

Controlling all environmental, managerial and microbial factors are imperative to control bacterial infections in poultry as well as food borne infections in both poultry and man.

#### **Treatment:**

**Antibiotic:** The use of antimicrobials, combined with improvements in sanitation, nutrition and immunization, has lead to a dramatic decrease in deaths and a major gain (*AMDUCA 1994, WHO 2002*).

#### **Alaminopencilin group:**

- a. Ampicilin
- b. Amoxicillin

#### **Aminoglycosides group:**

Aminoglycosides are a bactericidal, affects the Gram negative bacteria. Its members are streptomycin, gentamycin, turbamycin, kanamycin and framcetin:

- a. Streptomycin
- b. Neomycin
- c. Gentamycin
- d. Kanamycin

#### **Aminocyclitone group:**

- a. Spectromycin

#### **Tetracycline group:**

It is a bacteriostatic and has a wide-ranging effect, including oxytetracycline, chlorotetracycline and doxycycline.

- a. Oxytetracycline
- b. Chlortetracycline
- c. Doxycycline

#### **Quinolones group:**

- a. Nalidixic acid
- b. Flumequine
- c. Floxacins

**Polypeptides Group:** Including poly-moxin B or poly-moxin E (Colistin). Colistin

#### **Chloramphenicol group:**

Including Chloramphenicol and Thiamphenacol.

#### **Drug Interaction:**

Drugs can be used in single or in combinations.

a. Synergism: The effect of combined two drugs of similar action is greater than the sum of both.

b. Antagonism: A combination of two drugs or the presence of a substance hindering the effect of each other.

#### **Bacterial resistant:**

Wide increase of resistance to all antibiotic classes under surveillance for the most common Gram-negative bacteria infections. The growing threat of multidrug resistance which is being observed more frequently in some Gram-negative bacteria such as *E. coli*, *Salmonella* sp., *Shigella* spp., *Campylobacter* sp. and *Yersinia* sp. Some bacterial species are naturally resistant to antibacterial compounds (primary resistance), by continuous exposure to antimicrobials or sub dose, microorganisms developed acquired resistance (secondary) (*Fuchs and Wannmacher, 1999*).

#### **Antibiotics sensitivity test (CLSI 2013):**

Disk diffusion method ( *in vitro* sensitivity test ) is the mostly used for testing to detect and compares the antibiotic's ability to prevent bacterial growth, in order to assure its activity at a certain concentration. The result by measuring the area of inhibited bacterial growth with a ruler and interoperated according zone sizes on a standardized chart to give a result of sensitive, resistant, or intermediate.

#### **Probiotics, Prebiotics, and Synbiotics:**

a. Probiotics, prebiotics, and synbiotics can be used to modify the gut environment to prevent introbacterial pathogens colonization, invasion, multiplication, and shedding. Under modern production systems, chicks acquire gut flora from the incubator.

**a. Probiotics** are products containing a single viable strain of beneficial bacteria or multiple bacterial strains that colonize the crop, small intestine, ceca, and cloaca (*Fuller, 1989*). Beneficial microorganisms in probiotics ( in feed or water) may inhibit enteric pathogens by competing for attachment sites on surface of enterocytes, competing for nutrients, and producing antibacterial compounds (volatile fatty acids, low pH, and bacterocins) Moreover, probiotic may negatively affect pathogen metabolism by increasing or decreasing enzyme activity and stimulating immunity by increasing antibody levels and macrophage activity (*Fuller 1989, Patterson and Burkholder 2003*).

**b. Prebiotics** are nondigestible food ingredients (fructo-oligosaccharides and galacto-oligosaccharides)

that affect the host by stimulating growth or activity of a limited number of beneficial bacteria in the intestinal tract and promote increased numbers of beneficial bacteria with reduction in numbers of undesirable bacteria (Fuller, 1989, Park et al., 2013). Prebiotics resist enzymatic degradation and absorption in the upper gastrointestinal tract until reaching the cecum oligosaccharides, where they are hydrolyzed and fermented by bacteria, such as Bifidobacteria and Lactobacillus (Park et al., 2013).

c. **Synbiotics** are products that contain selective mixture both probiotics and prebiotics to favors the efficacy of each other (Schrezenmeir and De Vrese, 2001).

#### **Probiotics and Competitive Exclusion:**

Alternative methods for prevention and treatment of *E.coli* and *salmonella* infection to declining use of antibiotics including prebiotics, probiotics, enzymes, digestive acidifiers, vitamins and immune enhancers. probiotics and competitive exclusion compete with pathogenic bacteria for nutrients and attachment sites on the intestinal mucosa, and produce antimicrobial compounds, such as bacteriocins and volatile fatty acids. Beneficial intestinal bacteria has an immunostimulant effect on intestinal mucosa by inducing production of cytokine by subepithelial lymphocytes, activating macrophages, and stimulating production of immunoglobulin A (IgA) as immune barrier. Colonization with *E. coli* begins immediately after hatching making early administration of probiotics is essential. Administration of Lactobacillus provided chicks protection against challenge with Avian pathogenic *E. coli*. *Pediococcus pentosaceus* and *Lactobacillus johnsonii* (*L. johnsonii*) had been reported to completely eliminate *E. coli* for at least 24 hours (Mono et al., 2006).

#### **Disinfectants:**

A disinfectant is a substance that destroying pathogenic microorganisms or rendering them inactive; to sanitize is to reduce microbial populations and keep them from multiplying. An ideal disinfectant is low cost per unit of disinfecting value, ready solubility in hard water, relative safety for humans and animals, ready availability, non-destructibility to utensils and fabrics, stability when exposed to air, absence of objectionable or lingering odor, no residual toxicity, effectiveness. For large variety infectious agents, and no deleterious accumulation of any portion of the disinfectant in meat or eggs (Block, 1991).

#### **Salmonella control and eradication (Kabir 2010):**

Hygienic measures for controlling rodent and insect together with combinations of antibiotics with probiotics or prebiotic may be useful to prevent bacterial contamination in broilers. vaccination against salmonella can reduce egg contamination in laying hens.

#### **Detection and elimination of chronic carriers:**

Control of the disease in poultry breeder farms depends on periodical detection and elimination of chronic carrier in breeders by the pullorum test using stained *S. pullorum* antigen in rapid whole agglutination blood test Preferred for chickens and non stained antigen in tube agglutination test used for turkeys.

#### **Vaccination of poultry against Salmonella:**

There is an increasing usage of *Salmonella* vaccination in poultry especially against serovars of public health. The aim of vaccination for Salmonella infections in poultry is to reduce or prevent the intestinal colonization resulting in reduced fecal shedding and egg shell contamination, to prevent systemic infection resulting in a diminished localization in the reproductive tissues and to protect man from food borne infection (Saif et al., 2003).

#### **Control of salmonella in feed:**

Limiting *Salmonella* multiplication in contaminated finished ration requires keeping ingredients and finished ration dry (Holder, 1993). Feed delivery trucks should be covered to avoid wet or contamination. Ration and ingredient storage bins should be protected from rain, rodents, and wild birds (Davies, 1994).

#### **Sanitizing hatching eggs:**

*Salmonella*, *E. coli*, *citrobacter* and *proteus* had been isolated as egg shell contaminant. *Salmonella* and *E. coli* are transmitted through shell contamination, therefore sanitizing hatching eggs can decrease their prevalence, reducing a source of cross-contamination during incubation and hatching (Scott and Swetnam, 1993). Reducing numbers of Enterobacteriaceae can improve hatchability of dirty or floor eggs (Rashid et al., 2011).

#### **Hatchery contamination and control measures:**

The major source of contamination within the hatchery is the poor sanitary condition of the hatching eggs, use of dirty eggs, hatchery personnel, visitors and vehicles. Embryos may become infected with bacteria and moulds during incubation and hatched chicks are susceptible to infection with various microorganisms (e.g. *Salmonella* spp., *E. coli*, *Pseudomonas* spp., *Proteus* spp. and *Aspergillus fumigatus*) (Mayes and Takeballi 1983).

#### **Control of Colibacillosis:**

**Hygienic measures:** *E. coli* on the egg shell surface can be reduced or eliminated with sanitizers (Shane and Faust, 1996) in correlation with incubation and hatching process. Elimination of diseases and dead birds, Survival birds must supplied with high protein diets, increased vitamins A and selenium (Larsen et al., 1997).

#### **Vaccination against E.coli:**

#### **Live and Inactivated Vaccines:**

A live vaccine prepared from nonpathogenic *E. coli* strain (BT-7) was effective in chickens older than 14 days of age and provide protection against both homologous and heterologous strains (*Frommer et al., 1994*). Effective inactivated *E. coli* vaccines against serotypes including O2:K1 and O78:K80 have been produced and provide protection against the homologous serogroups not against heterologous serogroups (*Trampel and Griffith, 1997, Saif et al., 2003*).

#### **Biosecurity measures in poultry:**

Biosecurity in poultry production is the application and practices against infectious biologic agents to non-infectious levels and to limit their spread in the farm level and poultry product handling (processing and Marketing) to be save for human. Biosecurity practices can eradicate or reduce pathogens. Inadequate biosecurity can resulting epidemics of highly pathogenic or exotic disease in the industry, condemnation of flocks, infection by a low-virulent organism can reducing production, increase costs to clean, sanitize and disinfect as well as spread pathogens to human (*Nascimento et al., 2003*).

#### **Factors to obtain maximum health and productivity (*Sambo et al., 2015*):**

a. The most important step is to obtain New flocks (chicks, pullets, breeding males) from a clean known source (breeder flocks and hatchery). Birds should be purchased only from *Salmonella pullorum* (*S. pullorum*) clean sources. Chicks must originate from *S. pullorum* clean stock.

b. Managing poultry flocks for maximum health and productivity needs a well instructed facility, will trained manager and work team to keep the birds as healthy as possible and able to respond quickly to any disease condition.

#### **Recent Applications.**

##### **Phytobiotics (Botanical alternatives to antibiotics):**

Phytobiotics as botanical alternatives to antibiotics is new approach started to take place in organic poultry production to fulfill a number of consumer concerns regarding the use of antibiotics including residue contamination of poultry products and antibiotic resistant bacterial pathogens. Plant extracts and spices had been evaluated, some have growth promoting effects others have antimicrobial properties (*Diaz-Sanchez et al., 2015*).

##### **Genetic resistance to *Salmonella* and *E. Coli*:**

Demonstration of heritability of poultry lines for resistance to disease including *Salmonella* and *E. coli* to demonstrate the genetic component proved differences for disease resistance between chicken genetic lines. Combined with the need in molecular genetics for controlled disease challenges. Many genes are reported to be associated with genetic control of mice to infection with *S. typhimurium*. Chicken

homologs of major loci controlling natural resistance were examined. Therefore; The strategy of comparative genomics was effectively used to identify genes controlling chicken resistance to Salmonellosis (*Beaumont et al., 1999*).

#### **Nanotechnology:**

Nanoscience and Nanotechnology are new approaches to research phenomena at atomic, molecular and macromolecular scales. A nanomaterial has a diameter ranging between 1 and 500 nm, made from different materials, like copper, zinc, titanium, magnesium, gold, alginate and silver. The use of silver nanoparticles is one of the most promising strategies to combat biofilm infections related to indwelling medical devices (*Gong et al., 2007*).

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