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Isolation and Identification of the most Common Bacteria Isolated from Intestine of Broiler Chickens in Egypt

Eman M. Desouky[1], Heba N. Deif*[2] and Jakeen K. Eljakee[2]
[1]Master student at Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.
[2]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.
*Corresponding Author, Heba N. Deif, E-Mail: naim_heba@yahoo.com

ABSTRACT

Bacteria are seriously affecting broiler chickens and the poultry industry in many countries, as well as in Egypt. Both pathogenic and nonpathogenic bacterial species have been reported in broiler chickens. The present study aimed to isolate and identify the most common bacteria recovered from apparently healthy broiler chickens by conventional methods and the VITEK system. Samples were taken from 30 broiler chickens of different ages (3 – 5 weeks) from different retail markets in Cairo during the period from January to August 2019. Bacteriological examination of the samples showed both Gram-positive and Gram-negative bacteria as well. *Lysinibacillus sphaericus* (n=7), *Escherichia coli* (n = 5), *Proteus* species (n = 4), *Bacillus cereus* (n = 3), with isolation rates of 23.3 %, 16.6 %, 13.3 % and 10 %, were identified. In addition to its pathogenicity and hazardous action, some strains of *Lysinibacillus sphaericus* have a larvicide effect on some species of mosquitoes. So, further studies and investigations will be conducted to test *Lysinibacillus sphaericus* and *Bacillus cereus* isolates for their biological control activity and/or their potency to remove, control, or reduce petroleum hydrocarbon soil contaminants in different localities. It will be used as an alternative control agent for numerous synthetic commercial formulations.

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INTRODUCTION

The intestines of both healthy and diseased chickens serve as a reservoir for both pathogenic and nonpathogenic bacteria **(Clavijo and Florez, 2018).** The gastrointestinal compartments of chickens are densely populated with complex microbial communities dominated by Bacteria **(Wei *et al*., 2013).**

Enterobacteriaceae is a family of rod-shaped, aerobic, facultative anaerobic bacteria. *E. coli* belongs to this family, found in humans and other animals commonly in their large intestines **(Campbell *et al*., 2002).** It can be commensal or pathogenic and cause diseases to its host **(Salyers and Whitt, 2002).** Although it is found in lower numbers than other major commensals, *E. coli* is considered the most common internal bacteria causing diseases may be due to carrying several virulence factors (Stecher and Hardt, 2008).

Fires, affecting many countries worldwide, are found to cause losses of soil nutrients and infertility. *Lysinibacillus sphaericus*, a Gram-positive, spore-forming Bacilli, isolated from soil and water **(Martínez and Dussán, 2017)** and reported to have a very important role in improving the soil quality which helps the replantation processes. Consequently, *L. sphaericus* could be used as a good nutrient enhancer for plant growth in fire-impacted soils **(Aguirre-Monroy *et al*., 2019).**

Bacillus cereus is a novel emerging pathogen contaminated extensively in animal feed and food chains, posing a huge economic loss for the animal industry and a high risk for human health **(Haque *et al*., 2021).** It is distributed in the environment but can also colonize human and invertebrate intestines **(Jensen *et al*., 2003).** Biochemical testing of *B. cereus* shows its ability to generate acid from glucose but not from mannitol, xylose, and arabinose; oxidase negative, motility positive, catalase-positive, citrate

utilization positive, casein hydrolysis positive, nitrate reduction positive, Voges-Proskauer (VP) reaction positive, l-tyrosine reduction positive, and growth in 0.001% lysozyme positive **(Adams and Moss, 2005**). *Bacillus cereus* may cause two different and distinct forms of food-borne diseases: the emetic (vomiting) and diarrheal types (**Forghani *et al*., 2014**).

Various species of *Proteus*, which mainly exist as saprophytes, are known to cause septic infections in humans (**Wilson and Miles, 1975**) and animals **(Pine *et al*., 1973; O'Driscoll, 1977)** under certain conditions. Here, we describe the isolation and identification of bacilli from apparently healthy broiler chickens.

MATERIIALS AND METHODS

Samples Collection:

Between January and August 2019, 30 intestinal samples were collected from broiler chickens purchased from the retail market in Cairo. The birds were sacrificed and their intestines were collected for the bacteriological examination which was carried out in the Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. Under complete aseptic conditions, each intestinal sample was placed in a sterile Petri dish and opened using sterile forceps. Intestinal contents were collected using sterile cotton swabs, then were inoculated on a selective medium.

Isolation and Culture of *Enterobacteriaceae:*

Each sample was grown on MacConkey agar medium at 37°C for 24 h. Colonies appearing after 24 h were re-streaked to obtain a pure culture and then subjected to preliminary identification using microscopy, biochemical tests. Colonies representing each bacterial species were identified and characterized by using standard bacteriological methods according to the methods described by **Barrow and Felthan (2004).** The biochemical reagents and tests used included: Oxidase test, Triple sugar iron agar, PPA (Phenyl pyruvic acid), Urease, Simmons citrate, Indole, Methyl Red and Motility test was performed by referring to Bergey's Manual **(Holt *et al.*, 1994)** and the Manual for the Identification of Medical Bacteria **(Barrow and Felthan, 2004)**

Isolation and culture of Gram-positive bacilli:

Intestinal samples were cultured in buffered peptone-water (Oxoid). Aerobic spore-forming isolates were selected by heat (**Nicholson and Setlow, 1990**). Subsequent plating of buffered peptone-water was done aerobically on Difco nutrient agar. *Unless otherwise stated, bacillus* isolates were routinely grown aerobically at 37°C for 48h on Difco nutrient agar. Identification of the pure isolates was made based on staining, colony morphology, cultural, physiological and biochemical characteristics of pure isolates by using standard bacteriological and biochemical procedures as described by **Quinn *et al*., (2002).** The catalase activity of bacterial isolates was detected by resuspension of a colony in a 3% solution of hydrogen peroxide (Sigma). Hemolysis was determined on Columbia 5% sheep blood agar plates (bioMérieux), streaked with colonies from fresh Difco nutrient agar plates. Readings were taken after incubation at 37°C for 24 h.

VITEK 2 COMPACT:

The biochemical profile of test isolates was determined with the VITEK system- bioMérieux following the manufacturer's instructions. After overnight incubation at 37°C, colonies showing different morphologies were picked up from each selective plate and tested separately with VITEK for identification.

RESULTS

Isolation of Gram-negative bacteria:

The results illustrate that *Escherichia coli* (n=5) and *Proteus* species (n=4) were identified from the examined samples.

Isolation of spore-forming bacteria:

Spore-forming bacteria were selected by the heat of chicken fecal material. Treated samples were subsequently plated and incubated aerobically. Microscopic examination of the isolates showed a diverse collection of rod-shaped bacteria producing endospores of different sizes and shapes, including those that caused swelling of the mother cell (Fig. 1). A bacteriological examination showed the isolation of 7 *Lysinibacillus sphaericus* isolates and 3 *Bacillus cereus* isolates. All the isolates were catalase-positive, a characteristic that differentiates *Bacillus* from the anaerobic spore-forming *Clostridium* spp. All *Bacillus* isolates presented some level of hemolysis on 5% sheep blood agar.

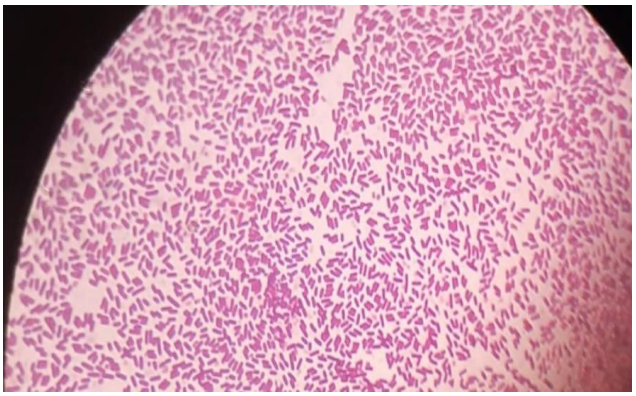

Fig. 1. Morphology of fecal bacilli isolated from broilers.

DISCUSSION

A broiler is any chicken that is bred and raised specifically for meat production. The chicken gastrointestinal (GI) tract harbors complex communities of bacteria **(Ranjitkar *et al*., 2016)**.

Bacteriological examination showed that *Lysinibacillus sphaericus* (n=7), *Escherichia coli* (n=5), *Proteus* species (n=4), and *Bacillus cereus* (n=3), were identified with 23.3 %, 16.6 %, 13.3 % and 10 %, respectively. **Hossain *et al*., (2008)** identified 22.86% *E. coli* isolates from broilers and 38.71% isolates from layers. From internal organs of broiler **Roy *et al*., (2012)** isolated *Escherichia coli* from 26 (52%) samples; similarly, *Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp., and *Pasteurella* spp. were isolated from 15 (30%), 10 (20%), 9 (18%) and 4 (8%) samples, respectively. Of the 235 strains out of 981 fecal swabs obtained by **Dandachi *et al*., (2018)** from poultry farms, 217 were identified as *E. coli* (92%), eight as *Klebsiella pneumoniae* (3%), three as *Proteus mirabilis* (1%) and three as *Enterobacter cloacae* (1%). Out of 140 chicken dropping samples collected by **Bushen *et al*., (2021)**, 61 (43.6%) showed bacterial growth. Of these, *E. coli* accounts for 39.0%, followed by *K. pneumoniae* (22.0%), *P. mirabilis* (19.3%), and *Salmonella* species (17.7%).

Recent studies have shown that spores of a laboratory strain of *Bacillus* decreased different aspects of colonization of young chicks by the avian colibacillosis agent *Escherichia coli* O78:K80, *Salmonella enterica* serotype Enteritidis, and *Clostridium perfringens* **(Barbosa *et al*., 2005).** *Bacillus* spores are being used as human and animal probiotics despite studies now indicating extensive mislabeling of constituent *Bacillus* strains **(Green *et al*., 1999, Hoa *et al*., 2000).** Several of these strains have also been multidrug-resistant and harbor toxin genes **(Hoa *et al*., 2000; Duc *et al*., 2004).**

Therefore, it is becoming increasingly clear that a more rigorous selection process is required for *Bacillus* probiotic candidates. Our understanding of competitive exclusion and probiotic properties relates mainly to lactic acid bacteria; however, with relatively very little known regarding *Bacillus* spp. **(Barbosa *et al*., 2005).** Nevertheless, it is anticipated that the competitive exclusion of pathogens by *Bacillus* probiotics will result from one or more modes of action, including immune exclusion, competition for adhesion sites, and production of antimicrobial agents, such as bacteriocins **(Patterson and Burkholder, 2003).**

Thus, in light of these arguments, we aimed in this study to isolate and identify the most common bacilli bacteria recovered from the gastrointestinal tract of apparently healthy broiler chickens by conventional methods and the VITEK system. The work described in this article identified 10 aerobic spore-forming bacilli (7 *Lysinibacillus sphaericus* and 3 *Bacillus cereus*).

Three *Bacillus cereus* isolates were detected from the examined samples. It is a facultatively anaerobic, toxin-producing Gram-positive bacterium that can be found in soil vegetation and even food. The pathogenicity of *B. cereus*, whether intestinal or nonintestinal, is intimately associated with the production of tissue-destructive exoenzymes. Among these secreted toxins are four hemolysins, three distinct phospholipases, an emesis-inducing toxin, and proteases **(Hölzel *et al*., 2018; Nguyen and Tallent, 2019).** The Centers for Disease Control (CDC) website states that, there were 619 confirmed outbreaks of *Bacillus*-related poisoning from 1998 through 2015, involving 7385 illnesses **(Thein *et al*., 2010; May *et al*., 2016).**

In the present study, 7 *Lysinibacillus sphaericus* isolates were identified. It is a Gram-positive, mesophilic, rod-shaped bacterium commonly found in soil. The reclassification from *Bacillus sphaericus* to *Lysinibacillus sphaericus* is based on the fact that the *Lysinibacillus* genus, in contrast to the type species of the genus *Bacillus*, contains peptidoglycan with lysine, aspartic acid, alanine and glutamic acid **(**Iftikhar ***et al*., 2007).** It can form resistant endospores tolerant to high temperatures, chemicals, and ultraviolet light and can remain viable for long periods. It is of particular interest to the World Health Organization due to the larvicide effect of some strains against two mosquito genera, *Culex* and *Anopheles* **(Colin, 2012)** more effective than *Bacillus thuringiensis*, frequently used as a biological pest control.

Recent studies have shown that a laboratory strain of *Bacillus* could suppress poultry colonization by different avian pathogens **(Barbosa *et al*., 2005).**

CONCLUSION

Most *Bacillus cereus* strains produce toxins accused of causing food-borne illnesses. However, the antifungal compounds produced by some *Bacillus cereus* strains have been used as useful biological control agents in the suppression of fungi and crop disease. So, future studies should focus on the antibacterial activities of aerobic spore-forming bacilli.

Declaration of Conflicting Interests

The authors revealed that there was no potential conflicts of interest.

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