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Isolation of Aerobic Bacteria from Poultry litter and Antibiotic profile, Kassala state- Sudan (2020)

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Abstract

This study was conducted to verify the presence of aerobic bacteria in both types of poultry (Broiler and Laying) and sensitivity of these bacteria to antibiotics.

The study was conducted in Kassala city, eastern Sudan. Where (60) samples were taken at random from (4) poultry farms (a farm with (2) broiler batteries, a farm with a broiler barn, one closed system deep litter, a farm with a layer battery, and an open system farm with a layer barn). The samples included (30) samples cloacal swabs and (30) samples fresh manure. Where (10) samples were taken from each barn except from the open system (20) samples were taken.

The samples were sent to Kassala Veterinary Research Laboratory, where the samples were cultured on Blood agar, and Macconkey agar, stained by Gram stain, and the bacteria were chemically analyzed by Oxidase test and Catalase test. Then the antibiotic sensitivity test was done on Nutrient agar. Where a special tablet was used for Gram-negative bacteria (AS, BA, CF, TZP, CH, CP, CR, TE, OF, GM, AK, LE.) And a special tablet was used for Gram-positive bacteria (AS, BA, PR, TE, CF, CP, LE, LZ, CX, RF, LM, GM).

All isolated bacteria were sensitive to ciprofloxacin, some bacteria showed partial resistance to some antibiotics, and some bacteria were resistant to Tetracycline (E. coli &Streptococci), The Escherichia coli also showed resistance to piperacillin.

This study concluded that poultry can been a source of bacteria, and one of causes of antibiotics resistant.

Therefore, don't uses of antibiotics except in necessary case, and interest of biosecurity in the farms.

Keywords: Bacteria, Antibiotics, Resistant, Poultry, Sensitivity

1. Introduction

Poultry manure can contain a variety of pathogens. Some are host-adapted and, therefore, not a health risk for humans. Others can produce infection in humans and are thus termed zoonotic. The more common zoonotic pathogens in manure include Escherichia coli 0157:H7, Campylobacter, Salmonella (EPA, 2013). These deposited wastes may serve as a reservoir for the multiplication of several pathogenic microorganisms that can cause severe disease outbreaks (Mathan periasamy et. al 2013). Therefore, to ensure the absence of pathogens in the fresh chicken litter, poultry compost, or the physically heat-treated chicken litter, additional approaches such as physical, chemical, and biological treatments, should be considered as another

means for pathogen control (Kelleher *et al* 2013). The antibiotics used in poultry farming may sometimes be the same, or belong to the same class, as those used in human medicine, Witte. W. (1998). Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years (Dragana Ljubojevic 2016). The fate of antibiotic residues in the manure of treated animals is of considerable concern because of the potential development of antibiotic resistant bacteria in the environment and because of the effect of these residues on manure treatment systems, Osman A. Arikan Lawrence et.al. The office international des Epizooties (OIE) published international standards on Antimicrobial Resistance in 2001. Further, the European Union (EU) has issued a strategy to reduce the prevalence of antibiotic resistant bacteria by surveillance of the evaluation of resistant bacteria and antibiotic consumption and by conducting research into the development of alternative preventive methods (Rev. Sci. off. int. epiz. 2001).

1.2 Problem statement:

Poultry litter is a potential reservoir and transmission vehicle for pathogens and zoonotic pathogen. Many of these bacteria are resistant to antibiotics, these bacteria might reenter the flock to produce diseases, contaminate broiler carcasses, transfer to cattle as it used as cattle feed, transfer to human through vegetables as it used as organic fertilizer. In the Sudan this poultry product usually used as fresh without treatment with any of the known methods. In addition, this product is considered as an important source of farm income.

1.3. Objectives of the study:

1.3.1 General objective:

To assess resistant bacteria in the poultry litter to certain antibiotics in kassala state farms.

Specific objectives:

1. To determine aerobic bacteria in fresh poultry litter collected from poultry farms in Kassala state.
2. To determine the antibiotic susceptibility of isolates to commonly available antibiotics.
3. To determine pattern of resistance of isolates.

2. Material and methods:

2.1 Study area

The samples were collected randomly from five barns in Kassala state (Alswagi aljnobia) from 26 May to 26 September. Kassala town located between longitudes 37 E and 35 W and latitudes 14.15 and 17.15 N.

2.2 Study population

Layer, broiler farms (open, closed system).

2.3 Sample size

60 (30 feces and 30 cloacal swabs), from each barn took ten samples (five feces and five cloacal swabs); only one barn took twenty samples (ten feces and ten cloacal swabs), because it is only open system that present during the study.

2.4 Samples

The samples (Litter samples and cloacal swabs) were collected according standard microbiology procedures, Microbiology manual by james cappuccino, Natalie sherman (2017) & (Steubing, P.M. (1993)) in brief, about 2 gram of samples were collected in sterile containers using sterile spatula; and use sterile swabs; and immediately transported to Kassala veterinary research laboratory. In battery cage the fecal samples took from manure belts. The samples were collected in six times; in each one ten samples.

2.5 Media and Reagents

The media are prepared before the day of collection of samples according to direction on container; and prepared for ten samples only in each time. The blood agar prepared by suspend 4.25gm in 100ml distilled water ; and put in autoclave at 25ibs pressure for 15minutes and after that put in room temperature ; then were added 10ml from blood sheep; mixed well and pour into five sterile petri dish(about 22ml/plate) and put in room temperature to remove the moisture and finally enter into the incubator at 37c for 24houres .At time of culture in sterile condition the petri dishes are divided by marker into two sides (s for swabs and m for manure), sterilized all the equipment's and then were took the loop and insert into the waste and spread on the media also spread the swab and then labeled by name and date , incubate at 37c for night .

In next day were read the growth of colony of bacteria. Then subculture in Macconkey agar and were stained by gram stain.

Macconkey is an indicator, a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric, and differentiated based on lactose fermentation. The crystal violet and bile salts inhibit the growth of Gram-positive organisms. The media were prepared by added 4gm from media in 100ml distilled water; put in autoclave at 15ibs pressure (121c) for 15 minutes then cool to 45-50c and pour into five sterile petri plates(21ml/plate), incubated at 37c for night, took from colony of blood agar and spread in Macconkey agar after divided the plates into two (s and m) as in case of blood agar. After culture incubated at 37c for 24houres then read the result. Lactose fermenting bacteria produce colonies that are pink in color, but colonies of non-lactose fermenting bacteria appear colorless.

Gram stain used to distinguish and classify bacteria species into two groups (Gram-positive and Gram-negative).

2.6 Staining procedure

Put a drop distil water in sterile slid and took by the sterile loop part from colony on blood agar and done thin smear, fixed the smear by flam; flood slide with crystal violet for 1minute then washed, flood the slide with iodide for 30seconds then washed; flood the slide by alcohol and rapidly washed, finally flood the slide by safranin (counterstaining) for 1 minute. After dry put drop from oil and detected under microscope (lens 100).

Gram-positive bacteria appeared in purple color, but Gram-negative as red color.

2.7 Identification of Bacteria

2.7.1 Catalase test: it helps in converting H_2O_2 to H_2O and O_2, the reagent that it used is hydrogen peroxide (3%).
Put a drop from the reagent in sterile slide and took part of colony from Macconkey agar by loop and spread on reagent, the positive result produce bubbles (due to O_2 production) but negative result indicated by lack of bubbles.

2.7.2 Oxidase test:
Oxidase test used to determine the presence of bacteria cytochrome oxidase enzyme using the oxidation of substrate "tetramethyl-p-phenylenediaminedihydrochromide" to indophenols a dark purple colored end product. A positive test (presence of oxidase) indicated by the development of a dark purple color. No color development indicates a negative test and absence of the enzyme.

2.8 Sensitivity test:

In sensitivity test use nutrient agar, it prepared by add 2.8gm in 100ml distilled water and heated to boiling to dissolved the medium completely. Sterilized by autoclaving at 15Ibs pressure (121c) for 15 minutes. Then pure in sterile petri dishes (20 ml/plate), put in room temperature and after that incubated for 24 hours at 37c. At next day after under flam took a part of bacterial colony from Macconkey agar and spread on plate that contain nutrient agar, then put the antibiotic disc and incubated for night, finally we read the result according to bacterial lysis.

3. Result:

Barn	Age by day	Rearing system	Type of production	Sample No	Spp. of bacteria
A	27	Battery cage	Broiler (ross)	10	5 *E.coli* (4 swab and 1 manure sample) 3 *Salmonella* (1 swab and 2 manure sample) 2 *Staphylococci* (manure sample)
B	25	Deep litter (close system)	Broiler (cobb)	10	9 *E.coli* (5 swab and 4 manure sample) 1 *Staphylococci* (manure sample)
C	30	Battery cage	Broiler (cobb)	10	6 *E.coli* (2 swab and 4 manure sample) 2 *Salmonella* (swab sample) 1 *Staphylococci* (swab sample) 1 *Streptococci* (manure sample)
D	308	Battery cage	Layer (hi sex)	10	6 *E.coli* (4 swab and 2 manure sample) 2 *Salmonella* (1 swab and 1 manure sample) 2 *Staphylococci* (swab sample)
E	260	Deep litter (open system)	Layer (hi sex)	20	8 *E.coli* (7 swab and 1 manure sample) 5 *Salmonella* (3swab and 2 manure sample) 2 *Staphylococci* (manure sample) 1 *Streptococci* (manure sample)

The bacteria are more loading in broiler specially in battery cage and also in manure samples than in swabs samples.

Sensitivity test:
Antibiotics resistance can emerge in animals and can transfer to humans, therefore we concentrated in antibiotics that uses in humans.

Antimicrobial resistance profile of bacteria:

- **Gram negative bacteria:**

No	Antimicrobial Agent	Bacteria spp	
		Salmonella	*E.coli*
1	Ampicillin (20mcg)	++	++
2	Co-Ttimoxazole (25mcg)	+	++
3	Cefotaxime (30mcg)	++	++
4	Piperacillin (100\10mcg)	+	_
5	Chloramphenicol (30mcg)	++++	+++
6	Ciprofloxacin (5mcg)	+++	+++
7	Ciftriaxone (30mcg)	++	+
8	Tetracycline (30mcg)	+	_
9	Ofloxacin (5mcg)	+	+
10	Gentamicin (10mcg)	++	++
11	Amikacin(30mcg)	+	+
12	Levofloxacin (5mcg)	++	++

- **Gram positive bacteria:**

No	Antimicrobial Agent	Bacteria spp	
		Staphylococci	*Streptococci*
1	Ampicillin (20mcg)	++	+
2	Co-Trimoxazole (25mcg)	+	+
3	Cephalexin (30mcg)	++	++
4	Tetracycline (30mcg)	+	-
5	Cefotaxime(30mcg)	++	++
6	Ciprofloxacin (5mcg)	+++	+++
7	Levofloxacin (5mcg)	++	++
8	Linazolid (30mcg)	++	+
9	Cloxacillin (5mcg)	+	+
10	Roxithromycin (15mcg)	++	++
11	Lincomycin (2mcg)	+	++
12	Gentamicin (10mcg)	+	++

4. Discussion:

Several bacteria species are the major causes infection in poultry. Most infections are linked to outbreak, live animal contact, poor hygiene and environmental exposure. Wet litter in poultry houses increase bacterial level.
All samples tested gave a positive result, the deep litter is containing more number of bacteria specially in case of broiler chicken. Therefore, to reduced bacterial number in

manure that use in agriculture, chemical, physical and biological treated manure is very important to reduced risk of contamination.

In poultry there are many antibiotics used for preventive, or for treatment and as growth promoter.

Both FDA and WHO rank antibiotics relative to their importance in human medicine the ranking is critical important.

The contact of birds with litter rich in bacteria remnants from a previous flock, beginning with the arrival in the house, facilities the early development of the intestinal flora, colonization of intestinal mucous by the great and diversified number of bacteria in normal in several animal species including chickens (Lee,1985).

Ingestion of litter microbiota can confer some protection to chicken against the colonization of some pathogens, particularly Salmonella spp.

E. coli everywhere in the environment and it usually plays a positive role in normal digestion. Antibiotics reduced the prevalence of E. coli but now that antibiotics are used less; the bacteria have made a comeback. Some genes that make the bacteria able to cause disease and known as avian pathogenic E. coli (APEC). E. coli may form biofilms in the poultry houses, which are hard to eliminate.

E. coli isolated in this study is showed resistance to Cefotaxim, Tetracyclin, Amikacin. But is susceptible to Ciprofloxacin.

E. coli isolated in Ghana, all isolated showed some degree of resistance to Ceftriaxone (1.34%), Cefotaxim (0.67%), Gentamycin (2.01%), Tetracyclin (2.0%) and Ampicillin (3.36%), (Yao Gm,2015).

In this study E. coli is suceptible to Gentamycin and Ampicillin.

Salmonella isolated in this study is susceptible to Ciprofloxacin, Chloramphenicol, Gentamycin, Amoxicillin. And some resistance to Trimoxazol, Ceftoaxime, Tetracycline and Amikacin.

Staphylococcus is susceptible to ciprofloxacine and most cephalosporine group, Amoxycillin, but it shows resistance to Tetracycline, Gentamycin, Cloxacillin, Trimoxazole and Lincomycin.

Susceptibility of chicken to intestinal Salmonella spp. colonization is higher during the first days of life, being decreased afterwards as normal intestinal microbiota grows (Baily,1988).

Streptococcus, is susceptible to ciprofloxacin, Lincomycin, Cephalosporin, Gentamycin, Levofloxacin, but it resistance to Cloxacilline, Trimoxazole, Amoxicillin and Tetracycline.

Cooperation between the processing plant and live production is important to reduce human footboards in final product.

The egg-gathering practices at the breeder farm must ensure clean eggs for the hatchery, the hatchery must be clean regularly and disinfect properly.

Maintaining microbial balance in gut microbiome can affect bird's health.

Regardless of the detected bacteria, the variation in the prevalence estimates might be due to difference in husbandry and management system, type of feed, geographical location, and the concentration of the farms in each location, Sahin, *et al* (2015), Humphery, et.al (2007). Which can have an impact on the dissemination and transmission of pathogens. Additionally, in poultry this variation might also be due to seasonal effects and difference in the hatchery sources, feed composition, vaccination programs, and flock disease status, (Alali, *et al*. 2010).

Acidification of litter with pH that can go under 4 promotes a decrease in the concentration of viable bacteria in litter and improves the environmental conditions inside the house (Ivanov, 2001).

Aluminum sulfate lowers the pH of the litter, Burgess *et al*. (1998) observed that it's addition in dose of 10% of weight of the litter causes a decrease in pH from 7.47 to 4.43 in litter composed of rice shells.

5. Conclusion:

This study has provided some important information's about the aerobic bacteria of poultry litter and sensitivity of the bacteria to the antibiotics.

The poultry consider as source of bacteria, and the litter has been applied as organic fertilizer. Therefore, don't uses of antibiotics except in necessary case, and interest of biosecurity in the farms.

6. Recommendation:

The study recommends to treatment of litter before used as fertilizer, biosecurity is very important, restricted used of antibiotic in poultry, don't used for prevention or as growth Promoter, but used only in case of treatment of specific diseases and replaced by probiotic, prebiotic, essential oils and organic acid.

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