RESEARCH ARTICLE

BACTERIOLOGICAL ASSESSMENT OF UNUSED LITTERS OF POULTRIES IN UMUAHIA

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Abstract

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Bacteriological assessment of and associated antibacterial profile of poultries kept under different unused litter. Twenty four (24) unused litters) collected in Mc Cartney bottles were gently shook. stirred with sterile glass rod until the droppings mixed thoroughly and aliquot (0.1ml) was pure plated in nutrient agar and selective and differential media. The plates were inverted and incubated aerobically at 37°C for 24hrs after which the plates were examined for growth. The bacteria colonies that develop on all media plates were sub-cultured by streaking on a freshly prepared nutrient agar plates until pure colonies were obtained. Isolates obtained were characterized and identified on the basis of their microscopic and sugar fermentation characteristics, Gram staining, biochemical tests and morphological characteristics of the colonies through macroscopic features were also carried out. The bacterial isolates from feacal droppings were Klebsiella spp., Escherichia. coli, Streptococcuss spp., Citrobacter spp., Salmonella spp. and Aerobacter spp., while only Klebsiella spp., E. coli, Citrobacter spp. and Salmonella spp. were isolated from unused litters. The bacterial isolates have multidrug resistance (MDR) and showed high resistance to Ampicillin, Ceftriaxone, Augmentin, Tetracycline and Sulfamethoxasole. It was concluded from this study that the poultry droppings harboured pathogenic bacteria some of which were multiple antibiotics resistant which may be linked with indiscriminate use of antimicrobial agents, use of leftover antibiotics from a family member and improper discontinuation of antibiotics. There should be regulation on the use of antibiotics for growth promotion and disease prevention in poultry birds. Feed and water troughs should be cleaned daily and fresh feed and water should be supplied.

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Introduction:-

It is now established that the global consumption of chicken has witnessed tremendous upsurge in the last decade such that currently, chicken meat is reported to be the second most eaten meat worldwide (Kenedy and Wilbard, 2015). In Nigeria, poultry production is put at about 58.7% of the total livestock production (Akintunde and Adeoti, 2014) and so plays an important economic, nutritional and socio-cultural roles in the livelihood of many Nigerian citizens.

Poultry litter which is usually composed of a mixture of chicken manure, bedding materials, spilt feed and feather is today considered a primary source of odour and environmental nuisance (Duntop et al., 2016).

A number of bacterial diseases have been associated with poultry droppings and leading amongst them are those caused by Escherichia coli, Salmonella, Shigella, Bacillus cereus, Pasturella, Staphylococcus and Clostridia organisms. The isolation of microorganisms from poultry droppings will, therefore, improve knowledge on the types of bacteria which may be present in poultry droppings, possible diseases which may be contracted in the course of poultry production and possible preventive practices.

The aim of this work was to isolate and determine the susceptibility results of bacteria from unusedlitters from poultry in Umuahia, Abia State.

Materials and Methods:-

Study area
The work was done in Umuahia North and Umuahia South Local Government Area.

Study population
Three unused litters samples from each farm were collected.

Sample Collection
24 unused litters were collected randomly from poultry farms labeled appropriately with source, time and date of collection. They were transported to Microbiology Research Laboratory of Veterinary Microbiology Laboratory, Micheal Okpara University of Agriculture Umudike within one hour of collection for microbiological analysis.

Isolation of Microorganisms
Ten (10) fold serial dilution technique was employed in the inoculation of the litter samples. Each of the samples was diluted in the 10-fold serial dilution technique as described by Gurung et al. (2009). One (1) g of each litter sample was diluted in 9 ml of sterile physiological saline and diluted serially up to the sixth tube in the row. An inoculum of 0.1ml Diluents from tubes No. 3 and 4 were inoculated onto starch casein agar, tryptone soya agar and Sabouraud Dextrose Agar Plates. The diluents were spread plated on the agar plates using an alcohol flame sterilized glass rod. The inoculated plates were incubated at 35 $^{\circ}$C for 24 hrs. Aliquot (1.0ml) was pureplated in nutrient agar and some selective and differential media salmonella-shigella agar and manisol salt agar. The plates were inverted and incubated aerobically at 37˚C for 24hrs after which the plates were examined for growth. The bacteria colonies that develop on all media plates were sub-cultured by streaking on a freshly prepared nutrient agar plates until pure colonies were obtained different biochemical that were carried out for growth identification according to procedure elongated by Fawole, and Oso, (2004).

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2015) on Mueller Hinton agar (Hardy Diagnostics, USA).

Statistical Analysis
The data from the study was subjected to statistical analysis by IBM SPSS version 25 using Analysis of variance (ANOVA). The group mean zone of inhibition of the various extracts on the isolated organisms were compared to the control (ciprofloxacin), all analyses were carried out.

Results:-

After the various analysis, the following micro-organisms were isolated; E. Coli, Klebsilla spp, Salmonella spp, streptococcus spp, Proteus aerobacter, Citrobacter and Pseudomans spp. Klebsilla (41.7%) was seen as the highest occurring bacteria followed by E.coli (33.3%), Salmonella (20.8%) and Citrobacter (4.2%), in the unused litter.

Table 1:- Bacteria isolated from unused litter.

| FARM	Bacteria Isolate			
	Klebseilia (%)	**E. Coli** (%)	**Salm**(%)	**Citrobacter**(%)
1	3 (30)	-	-	-
2	1 (10)	2 (25)	-	-
3	1 (10)	-	2 (40)	-
4	2 (20)	-	-	1 (100)
5	-	3 (37.5)	-	-
6	-	-	3 (60)	-
7	3 (30)	-	-	-
8	-	3 (37.5)	-	-
TOTAL (%)	10(41.7)	8(33)	5(20.8)	1(4.2)

KEY:

E.: Escheriachia.

Salm: Salmonella

%: Percentage

Susceptibility results of Isolate from unused litter from 8 farms

Figure 1: Shows bacterial susceptibility of isolates from unused litter and the susceptibility profile of the bacteria isolated form unused litter showed that Klebsiella was more susceptible to APX and least susceptible to S, E. coli was more susceptible to S and least susceptible to AMP and P, Salmonella was more susceptible to CN and least susceptible to AMP and CT while Citrobacter was more susceptible to APX, TE and CPX and least susceptible to AMP, P, E, S, AG and CT. The mean average sensitivity of Klebsella was highest in CT (45.8%), followed by AP (41.6%), Te (39.5). Ag (33.3%), E (31.2%), P (20.8%), AMP and C (18.7%) and S (6.2%), while E. coli was highest in Te (25%) followed S (20.8%). E (14.5%) CN (14.5%), CT (12.5%), CTX (6.2%), APX (41%) and was not sensitive to AMP and P, Salmonella was highest in CN (20.8%) Te(14.6%), APX, E and AG(10.4%), CPX(6.2%), S and P(4.1%) and was not sensitive to AMP, CT. Citrobacter was highest in APX, TE, CN, CPX(12.5%) respectively but was not sensitive at P, AMP, E, AG and CT as seen in Table 4.3
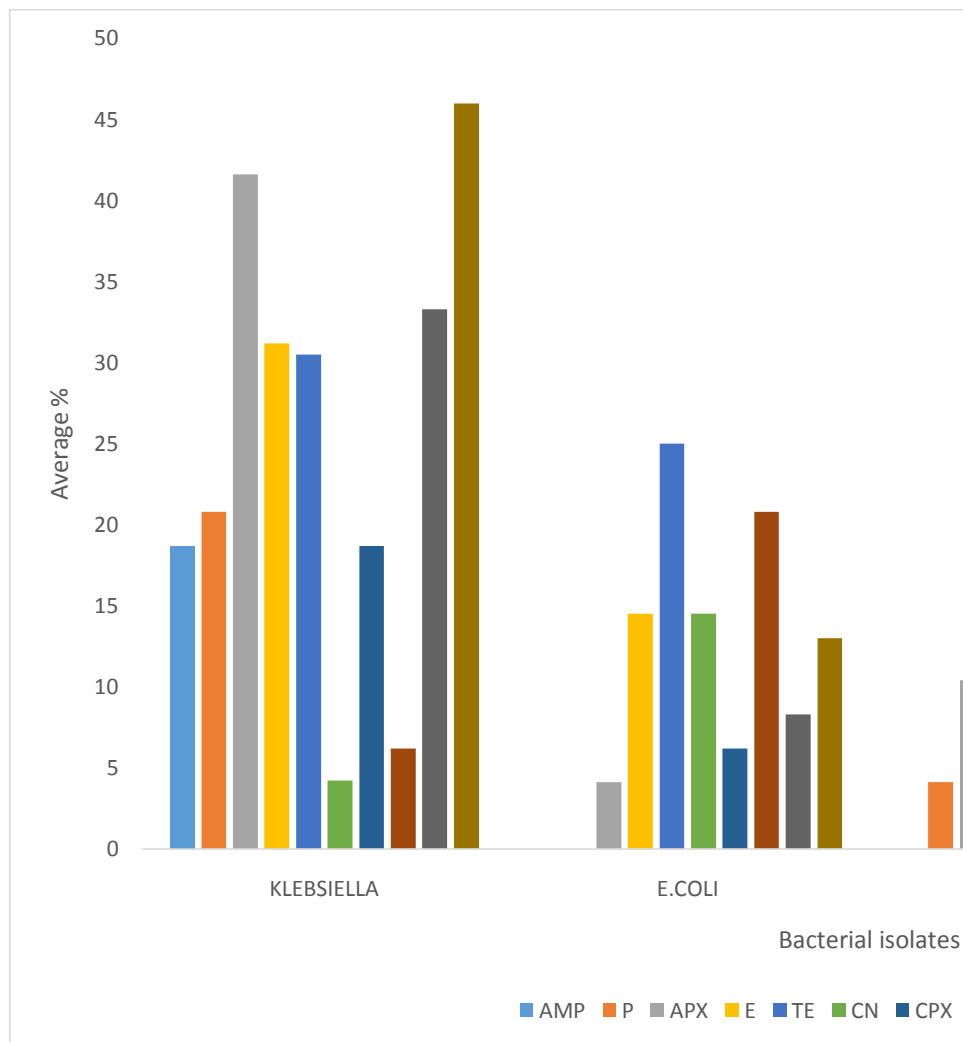
Discussion:-

The levels of total bacteria in the samples were much high as those detected by Martin and McCann (1998) but in line with reports of Omoya and Ajayi, (2016). E.coli (43.8%) accounted for the majority of the total aerobic plate counts, whereas citrobacter, Aerobacter, Pseudomans (7.3%) were only minor components of the total bacteria cultured from poultry litter. The bacterial pathogen isolated were mostly Gram-negative bacterial with E. coli having the highest prevalence both in unused litters and feacal material. The result is line with earlier reports (Afunwa, et al., 2020; Bushen et al., 2021). In this study, more than half of isolates showed MDR. Such report has been reported from Bangladesh (Nahar et al., 2014), Vietnam (Vdouba et al., 2019) and China (Yassin et al., 2017). Emergence of MDR bacteria especially enterobacteriaceae, has increased in recent years. The occurrence of MDR may be linked with indiscriminate use of antimicrobial agents such as wrong induction, wrong duration and improper route of administration, use of leftover antibiotics from a family member and improper discontinuation of antibiotics (Nahar et al., 2014; Yassin et al., 2017).

Conclusion:-

The most resistant isolates were E. coli and Pseudomanas which were found to be resistant to the entire antibiotics. These have very important implications on human health, as antibiotic resistant bacteria infections and difficult to treat and often require extensive antibiotics and long therapy can increase cost of treatment and even mortality.

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