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Research Paper

CORRELATIVE ANALYSIS OF *SALMONELLA* BACTERIAL ANIMAL FEED AND PIG INDUSTRY

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Animal feed account for more than 75% total cost of production in pig industry and feed spoilage is a common factor as well as disease infection. These negative factors were influenced on production process in pig industry. Hence animal feeds and faecal samples were collected from pig farms and analyzed for microbial prevalence. The results revealed that the pathogenic microorganisms that can be zoonotic in nature *E. coli* staphylococcus and bacillus species of microbes were most prevalent

Keywords: Animal feed, Microbial prevalence, Pig industry

INTRODUCTION

Salmonella is the leading cause of food borne illness Bacteria food borne disease are among the most serious health problems affecting public health and development worldwide. (Berends *et al.,* 1996). *Salmonella* therefore can be define as a gram negative, non spore forming, Catalase-positive, oxidase negative, facultative anaerobic which is the significant cause of mortality and in animals. *S. enterica, serovars typhimurium* being an emerging problem of animal origin particularly meat product from pigs. As pathogen they develop complex virulence mechanism to evade host defense mechanism although the organism do not cause sub clinical disease in pig. *Salmonella* are among the most common bacteria food borne pathogens worldwide and have emerged as the second most common cause of bacterial human food borne illness and a pathogen of public health concern (Brooks *et al.,* 2004). *Salmonellosis* result from the ingestion of variety of *Salmonella serovars* particularly *Salmonella typhimurium* and *Salmonella enteritidis. Salmonella* is characterized by septicemia, acute and chronicitis (Blaha, 2002). *Salmonella* organism are ubiqutous and gastrointestinal tract remain it ecological niche (Barber *et al.,* 2002). Although their primary habitat is the intestinal tract, the organism may be found in other part of the body. Contamination of parts of the body other than intestine may be contact with animals' spleen, urine and faeces during slaughtering.

Antibiogram is defined as a laboratory test use

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to determine the sensitivity pattern of a given microorganism to a range of antibiotics. The advantages' of antibiogram and the technology involved in running these tests are well known (Tauxe *et al.,* 1998). Isolation, identification and antibogram of pathogenic agent are normally carried out when a bacteria disease has produced a problem on farm, expressed as economic loss in form of mortality. The bacterial most frequently in involved were *E. coli, Salmonella*, *Haemophilus, Pasteurella* and occasionally gram positives. These microbes are isolated from specific post mortem lesions.

Pigs can be define as any of the animals in the genus Sus, within the Suidae family of even toed ungulates. Pigs include domestic pig, The domestic pig (Sus scrofa domesticus) is usually given the scientific Sus Scrofa, although some authors call it *S. domesticus*, reserving *S. scrofa* for wild boar. It was domesticated approximately 5,000to 6,000years ago. Their coats are coarse and canines form a sharp bristly (Stege *et al.,* 2000). They are born brownish colored and tend to turn more grayish colored with age. The upper canines form sharp distinctive tusk that curve upward and outward (Olsen *et al.,* 2001). Compared to other artiodactyles, their head is relatively of extinction and ecosystem change (Olsen *et al.,* 2001). They have been introduced long, pointed, and free of warts. Their head and body length range from 0.9to 1.8m and they can weigh between 35 and 50 kg. Feral pigs like other introduced mammals are major drivers of extinction and ecosystem change. They have been introduced into many parts of the world, and will damage crops and home gardens as well as potentially spreading disease. They uproot large areas of land, eliminating native vegetative and spreading weeds. This result in habitat alteration, a change in plant succession and composition and a decrease in native fauna dependent on the original habitat (Willeberg, 2000). Hence *Salmonella* species is not a friend to pigs and human therefore, the environment where they live and the water which they consume should be properly taken care off. If not properly taken off when consume by consume by human can lead to *salmonella* species in human.

OBJECTIVES OF STUDY

- To evaluate the isolation of bacteria from pigs and feeds and to identify the bacteria isolated.
- To assess isolates bacteria from environment and the water in which to identify the bacteria isolated.
- To determine the susceptibility pattern of the isolated bacteria to some commonly used antibiotic.

MATERIALS AND METHODS

Material and Equipments Used

Test tubes, weighing balance ,aluminum foil, spatula, filter paper (Whattman No 1), normal saline, autoclave, Petri-dishes, glass slide, and cover slide, pipette, conical flask, reagents for biochemical analysis, measuring cylinders, Forceps, hand gloves and disinfectants(formalin, ethanol), Antibiotic disks, bijoux bottles were used in this study. All glassware were washed very well in water using detergent, disinfectant and brushes and sterilized in hot air oven at 160 ^{o}C for 30 min to achieve maximum sterilization. The incubator, hot air oven and other equipment used in this study were all thoroughly cleaned, disinfected and sterilized.

Sampled Collection

Animal feeds and pig's faecal samples were collected from a piggery farm in Sapelle. Two types of feeds were collected via: Treated feeds (treated) with long lasting antibiotics and heat and the non-treated feed. The faecal samples were collected from pigs that were feed with the two kinds of feeds (Treated and untreated feed). The samples were collected in piggery farm using a sterile universal container and it was transported in salinities F medium to the laboratory for microbiological investigation.

Samples collected	Site collected
Treated feeds	Site A
Untreated feed	Site B
Fecal sample Pigs dropping	Site C, Site D

Media Used

Deoxycolate citrate agar, Salinite F, and peptone water were used in this study. They were prepared following the manufacturing's guide as shown in the appendix section of this work.

Sample Processing

A portion of each samples obtained was placed (socked) in normal saline, in other to get a stock microbial solution (i.e. A stock solution of each of the sample was measured by weighing 10 g of each sample to 90ml of sterile normal saline, in each case giving a 10% stock solution). This solution was then used for microbiological studies as shown below.

Microbiological Analysis

Serial Dilution and Culture

1. Two row of twelve's test tubes, six for untreated and six treated feed

   Were setups on a test tube rack?

2. With the use of sterile pipette a total of 9milliliter of distilled water was introduced into each of the test tube.
3. One milliliter of the stock solution was taken with pipette (1 ml dropped pipette) and dispensed into the first tube and mixed well.
4. Then one milliliter was transfer from the first test tube into the second, and again on the third and in this manner to the last test tube(tube 6) and
5. 1 ml was discarded from the test tube last test tube, to get a 1:10 dilution all through.
6. Molten nutrient agar was poured unto each of the Petri dishes containing the different dilutions and allowed cool and solidify
7. The plates were then inverted and incubated at 35-37 ^{o}C for 24 h.
8. Plates were examined for discrete colonies that were counted and count noted.
9. The count was multiplied by the dilution factor to get the total count in colony forming units per milliliter.

Identification of Isolates

Each of the samples were inoculated (using a sterile wire loop) into Deoxycolate citrate agar and incubated at 37 ^{o}C overnight. Culture plates were examined in preliminary identification of isolate was done using their colonial morphologies and biochemical tests.

Antibiogram Test (Determination of Resistance Pattern of Isolate to Some Antibiotics)

Each of the bacteria isolates identified from the step above was subjected to antimicrobial susceptibility screening in other to determine their resistance pattern to some antibiotics.

Standard antibiotics multi-disks (containing the following Offorasin, Streptomycin, Gentimycin, Chlomramphenicol, Nalidixic acid, Erythromycin, peflacine, Ciprofloxacin, Norfloxacin, Lincocin, Ampiclox, Rfampin) were used and disc diffusion method for determining antimicrobial susceptibility were used to carry this work as follows.

Peptone water was prepared and dispensed into bijoux bottles and labeled according to the isolates being investigated. Each isolates was then inoculated into peptone water to give 10^5 cfu/ml. The bottles were shaken vigorously and each of them was poured then poured separately onto nutrient agar plates for each sample. The plates were then rocked from side to side so that so that the sample will spread evenly on the plate. With the help of sterile forceps; the standard antibiotic discs were then impregnated on each of the plates. The plates were then inverted and incubated at 35-37 ^oC for 24 h incubation. The plates were examine for resistance patterns of the isolate. Resistances were determining by the absence of zones of inhibition around antibiotics disks, while zones of inhibition marked Susceptibility. The profile and sensitivity profile was recorded.

RESULT'S

Table 1 shows the various samples collected. The mean bacterial count of treated feeds sample A is 1.0 x 10^4, sample B is 1.2 x 10^4, sample C is 1.2 x 19^4 and sample D is 1.5 x 10^4 while the mean bacterial count (cfl/ml) of untreated feeds of sample A is 2.3 x 10^4, sample B is 2.8 x 10^4, sample C is 3.1 x 10^4 and sample D is 2.7 x 10^4. It there fore shows that the microbial load in untreated feeds is more than the microbial load in treated feed.

Out of the samples collected from piggery form, i.e., treated feeds and the pig's facial samples. Among the samples collected it was found that *Salmonella* was not present or isolated from the feeds, but *Salmonella* was only isolated from the pigs fences. This is an indication that *Salmonella* present in the fences may not necessarily be from the feeds, but from other source which could be from dirty water in which pig play or drink.

Table 1 shows the mean total viable count of bacteria (cfu/ml) isolated from treated and untreated feeds.

Morphology: *Salmonella* are gram-negative rods. With the exception of s. pillorum-galllinarem, all *Salmonella* are actively motile. They are non-spring and with the exception of s. Typhi and non-capsulate. Biochemical test was carried out, to know their gram reaction.

Gram	–	(-ve)
Motility	–	(+ve)
Indole	–	(+ve)
Catalase	–	(-)

Table 1: Mean Total Viable Count of Bacterial (CFU/ML)

Treated Feeds	Mean Bacterial Count (CFU/ml)	Untreated Feeds	Mean Bacterial Count (CFU/ml)
A	1.0×10^4	A	2.3×10^4
B	1.2×10^4	B	2.8×10^4
C	1.2×19^4	C	3.1×10^4
D	1.5×10^4	D	2.7×10^4

Oxidase	–	(-)
Citrate	–	(+)
Glucose	–	(+g)
Lactotse	–	(+(A))
Sucrose	–	(+A)
Manitol	–	(+)

This is fully explained in Table 3 of this work.

Each of the bacterial identified were subjected to antimicrobial susceptibility screening (antibiogram) in order to determine their resistance pattern to some antibiotics. It was observed that some were resistance while some were sensitive to antibiotics disk as shown in Table 2.

Table 2: Antibiogram Pattern – Antibiotics Sensitivity Pattern

OFX Tarvid	S
CEP Ceporex	R
CN Gentamycin	S
AU Augumentin	R
NA Nalxidic Acid	R
CPX Ciprofloxacin	S
S Streptomycin	S
PEF Petlacine	R
SXF Septrin	S
PN Ampicillin	R
RD Rifampin	R
FLX Floxapen	R
E Erythromycin	R
CH Chloramphenicol	S
APX Ampiclox	R
NB Norfloxcin	S
LC Lincocin	R

Note: S = Sensitive; R= Resistant.

This following antibiotics such as Tarvid (OFX), Gentamycin (CN), Ciprofloxacin (CPX), Streptonmycin (S), septrin (SXT), Chloramphenicol (CH), and Norfloxcin (NB), are sensitivity and therefore can be use for treatment while other antibiotics such as ceporex (CEP), Augumentin (AU) Nalxidic Acid (NA), Petlacine (PEF), Amplicillin (PN), Rifampin (RD), Floxapen (FLX), Erythromycin (E), Ampiclox (APX) and Lincocin (LC0 are resistant to these antibiotics.

Table 3 shows that samples collected from treated feeds with a mean bacterial count (cfu/ml) of $10x10^4$ and isolated bacterial species found are *Bacillus* spp and *Staphylococcus*. While untreated feeds have a mean bacterial count (cfu/ml) of $18x10^4$ and the bacterial species isolated are Bacillus spp. *E. coli* and *Staphylococcus* aureus and that of pig dropping bacterial isolated are *Bacillus* species, *E.coli* and *Klebsiella* spp. This also show that untreated feed have a high microbial load than the treated feed.

Table 3: Colony Counts (Viable Count) and Isolated Bacterial

Sample	Mean Bacterial Count (cfu/ml)	Bacterial Species isolated
Treated feeds	10×10^4	*Bacillus* spp and *Staphylococcus aureus*
Untreated feeds	18×10^4	*Bacillus* spp, *E. coli* and *Klebsiella* spp.
Pig droppings		*Bacillus* spp, *E. coli* and *Klebsiella* spp.

DISCUSSION

This study is based on bacterial isolates from animals feeds and pigs faecal samples and their resistant pattern to some commonly used antibiotics. In this study, animal feeds were subjected to contamination from diverse sources, including environmental pollution and activities of

Table 4: Isolates That are Sensitive and those that are Resistant to the Antibiotics

Feed samples	Isolate	Antibiotic Sensitive to Profile	Antibiotics Resistant to Profit
Treated feeds	*E.coli*	GN, CH, PN, APX, AM, CP, and S.	Taw SXT
	Staph aureus	CFT, T, GW, PN S and CP.	CH, SXT, AM and APX
	Bacillus SPP	CFT, GN and T	PN, S, CP, APX, AM and SXT.
Untreated FEEDS	*E. coli*	CP, APX, CFT AM S and GN.	T and SXT.
	Staph aureus	GN, CP, CFT, TS and PN.	CH, AM, STX, and PN
	Bacillus SPP	S, GN, CP, APX, CH, T, and SXT	AM and PN
Pigs faecal samples	*Salmonella* spp isolated	CN, OFX, and S	NA, AU, NA, AU, and PN,
		SXT, GN, CN, and S	LC, APX, RD, PEF and CPX.
		CPX, CH, NB, and S.	E, LC, CN, APPX, RD, and FLX.

Note: STX – Septrin; S – Streptomycin; AM – Amoxicilline; PN – Ampicillin; T – Tetracyclin; APX – Ampiclox; CH – Chlomyamphenicol; CFT – Cefliazone; OFX – Ofloxacin; CN – Gentamycin; NB – Norfloxacin; E – Erythromycin; RD – Rifampin; FLX – Floxapen; LC – Liconcin; CPX – Ciprofloxacin; PEF – Peflacin.

insects and microbes according to (Van Barneverld 1999). In this study, the isolation of *Staphylocollus aureus*, *Escherichia coli* and *Bacillus* spp from animal feeds obtained from piggery farm in Delta. In this was not present in the antibiotic treated feeds. This finding could be attributed to the effect of the antibiotics and heat treatment given to this set of feeds. Pigs may acquire antimicrobial resistance when feeding on the treated feeds. From the antibiotic sensitivity test done on the various isolates, the result showed that isolates obtained from treated and untreated feeds samples gave similar antibiotic profile. However, *Bacillus* spp isolated from treated feed samples were highly resistant to a number of antibiotics tested when compared to *Bacillus* spp from of the *Bacillus* spp acquiring resistant to the antibiotics used for treating the feeds. It similar observation had been reported by (Jeffrey *et al.*, 1998).

Several incidents have been reported in which human illness was traced to contaminated animal feed. In 1985, outbreak of infection association between the use antimicrobial agents in animal feeds and an increased risk that humans will contract infection by resistant bacterial strains such as *Salmonella* spp, *E. Coli*, and other enteric isolates. Research reports in recent time have documented the use of antibacterial drugs to combat various diseases of pigs such as mastitis. The use of these antibacterial drugs has led to selection of antibiotics resistant strains of bacterial pathogens including *Salmonella* species. In this regard, it is find that it will be of serious health implication for humans who may acquire these bacteria from consumption of improperly looked pig meat or though direct contact with infected animals and their feeds.

CONCLUSION AND RECOMMENDATION

In conclusion, therefore that animal's feeds (Swine feeds) contain pathogenic micro-organisms that can cause diseases to animals and even to humans if proper care is not taken in handling the feeds. I therefore recommend that

proper microbiological analysis must be carried out to ensure that no pathogenic bacterial is present in the feeds before taken to the markets. The feed must be put in well demarketed place to prevent contaminants.

The following step below can also help to improve sanitary condition of the farm environment.

- Properly maintaining the waste and preventing it of getting to the feeds.
- Ensure all the brushing surrounding; the piggery farm is cleared so that insect would not infect the animals.
- Using effective microorganisms (microflora) on the animals to reduce the population of pathogenic microorganisms in the intestinal treats of the animals.
- A legislative law should be put in place to guide against the treatment of feeds with drugs that are out of used.

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