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ISOLATION AND IDENTIFICATION OF MULTIDRUG-RESISTANT BACTERIA FROM PIG FEEDS AND FAECES

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

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A total of 15 bacteria were isolated from pig feeds and feace from two pig farms in Osun state. The isolated bacteria were identified by biochemical tests to include *3 strains of Pseudomonas aeruginosa, 2 strains of Corynrbacterium xerosis, 2 strains of Escherichia coli, 2 strains of Kiebsiella aerogenes, 2 stains of Providencia rettgeri and a strain of Micrococcus roseus, Staphylococcus epidermis, Citrobacter Freunlii and Proteus vulgaris.* These organisms were subjected to antibiotics susceptibility tests and, 86.70% of the organisms showed multi-drug resistance. *E. coli and Pseudomonas* showed resistance to clinically important antibiotics. 73.30 % of all the bacteria isolates were susceptible to *Gentamycin* while between 40 to 50 % of the isolates were susceptible to all the antibiotic drugs used for the study. Persistence or unguided use of antibiotic drugs in livestock keeping could results in resistance of organisms to drugs thus reducing the efficacy of these life-saving drugs. Proximate analysis of pig feed samples indicates that brewery wastes (pig feed) had the highest amount of protein (24.95 % ± 0.06), crude fat (5.69 % ± 0.11), crude fibre (9.71 % ± 0.50), total ash content (7.87 % ± 0.21) and carbohydrate (42.85 % ± 3.51). However, there was no significant difference in the proximate compositions of all the pig feeds investigated.

INTRODUCTION

Society is facing a crisis of antibiotic resistance. Distinguished bodies and agencies which include World Health Organization, the Centers for Disease Control and Prevention are raising the alarm over antibiotic resistance. Antibiotics are among the most potent life-saving interventions in all of medicine. The reductions in death afforded by effective antibiotics for bacterial infections of all types, ranging from simple skin infections to infections of the bloodstream, lung, abdomen, and brain, are enormous (Spellberg, 2010; Spellberg *et al.*, 2011).

Within a few years of their availability, antibiotics had reduced the rate of death from infections in the United States by nearly 80 percent and similar results were reported from other countries (Spellberg, 2010). The availability of effective antibiotics is necessary to enable modern medical advances that range from intensive care unit medicine to aggressive surgeries,

cancer chemotherapy, care for premature neonates, and organ transplantation.

Loss of antibiotic efficacy threatens to return society to a time when one in ten patients with a skin infection died and one in three patients with pneumonia died (greater than 10-fold higher death rates compared to the antibiotic era (Spellberg, 2010; Spellberg *et al*., 2008b; Spellberg *et al.*, 2009). Without effective antibiotics, medicine would be paralyzed by an inability to treat infectious diseases (Spellberg, 2010; Spellberg *et al*., 2011; Spellberg *et al*., 2013).

Feeding sub-therapeutic concentrations of antibiotics to livestock animals in order to improve their growth is an age-long practice in livestock keeping (Elliott, 2015). The mechanism with which this works remains unclear until recent evidence from mice that suggested that efficacy of antibiotics in livestock keeping may be due to alterations in the intestinal microbiota, resulting in decreased extraction of calories from food by the bacteria, leaving more available to the host to absorb (Cho *et al*., 2012). This established mechanism in mice studies remains speculative in livestock. Nevertheless, there is evidence that feeding antibiotics to livestock can sometimes cause a growth-promoting effect.

In Western Europe, efforts have been undertaken over the past 10 to 20 years to curb antimicrobial growth promotion and prophylactic antibiotic use in livestock (Marshall and Levy, 2011; O' Neill, 2015). Such efforts have been largely impossible in the United States and other countries because of politics. Even as many countries have continued to experience the growing crisis of antibiotic resistance over the last decades, the amount of antibiotics purchased for use in livestock has increased tremendously (Animal Health Institute, 2008; FDA, 2015).

Although few studies have evaluated antibiotic-resistant bacteria in Nigeria (Okeke *et al*., 1999, Aibinu *et al*., 2004 and Umolu *et al*., 2006), most available data are specific to strains that are pathogenic either to human or animals. There is a paucity of information on the bacterial isolates in the Nigerian piggery farms. This study, is, therefore, aimed at determining the susceptibility of some commonly used antibiotics of drugs in livestock to different bacterial isolates in the commonly used pig feeds in Nigeria.

METHODOLOGY

SAMPLES COLLECTION

Pig feeds and faeces were collected from two different locations in Osun state (Osogbo and Ede towns) and labeled as A and B in sterile bottles and transported to the laboratory within 24 hours of collection. All apparatus used were sterilized before usage. Different agars were prepared according to the standard methods.

BACTERIA ISOLATION

One gram of each sample was suspended into 9 ml of sterile distilled water and serially diluted up to 10^6. MacConkey Agar was prepared into two 250 ml conical flasks. Aliquot of 1 ml each of serially diluted sample solution was plated in triplicates and incubated at 35 ^{0}C for 24 hours. Distinct colonies of each sample were selected and purified by sub-culturing on fresh MacConkey plates. The pure cultures were then stored on MacConkey slants at 4 ^{0}C in the refrigerator and identification of isolated bacteria was done.

BIOCHEMICAL CHARACTERISATION

Gram's reaction, biochemical tests, catalase test, motility, indole and urase tests and other tests were performed according to the official methods. Proximate analysis of the pig feeds was performed according to the official methods of analysis by A.OA.C Int'l. (2000) while susceptibility test was

determined on Nutrient Agar plates using the disc diffusion method (Scott, 1989).

ANTIBIOTICS SUSCEPTIBILITY TESTING

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion method (Cheesbrough, 2000), on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006). Susceptibility was tested against 10 µg each of *augumentin gentamycin, perfloxacin, tarivid, streptomycin, septrin, chloramphenicol, sparfloxacin, ciprofloxacin and ampicillin* (Oxoid, England).

RESULTS

Table1: Average Bacterial Count of Isolate from Pig Feeds and Faeces

Samples	Bacterial count CFU/g (± SD)	
	Site A	Site B
Compounded Feed	$2.67 \times 10^5 \pm 1.67$	$1.99 \times 10^6 \pm 5.22$
PKC	$1.75 \times 10^6 \pm 1.02$	$1.68 \times 10^6 \pm 0.11$
BDG	$1.88 \times 10^6 \pm 0.08$	$1.98 \times 10^6 \pm 0.09$
Corn bran	$1.57 \times 10^5 \pm 0.55$	$1.38 \times 10^6 \pm 0.06$
Faeces	$1.77 \times 10^6 \pm 3.71$	$1.46 \times 10^6 \pm 2.49$

Table 2*:* Result of Biochemical Characterization of Isolates from Pig Feeds and Feaces

Isolate code	Bacterial Isolates
FEDA1	*Cornebacterium xerosis*
FEDA2	*Pseudomonas aeruginosa*
FEDA3	*Klebsiella aerogenes*
FEDA4	*Citrobacter freundii*
FEDB1	*Escherichia coli*
FEDB2	*Provindencia rettgeri*
FEDB3	*Corybacterium xerosis*
FACA1	*Pseudomonas aeruginosa*
FACA2	*Staphylococcus epidermidis*
FACA3	*Klebsiella aerogenes*
FACA4	*Proteus vulgaris*
FACB 1	*Pseudomonas aerugnosa*
FACB 2	*Escherichia coli*
FACB 3	*Pronindencia rettgeri*

Table 3: Proximate compositions of Pig Feeds and Composite Feed (mg/g)

Composition	Corn bran	Brewery waste	PKC	Compounded feed
Crude Protein	10.67 ± 1.02	24.95 ± 2.06	18.94 ± 1.04	22.69 ±2.01
Crude fat	6. 49 ± 0.45	5.69 ± 0.34	3.12 ± 0.12	3.88 ± 0.52
Crude fibre	7.67 ± 0.47	9.71 ± 1.03	6.16 ± 0.67	3.63 ± 0.34
Total Ash	3.78 ± 0.46	7.87 ± 0.42	5.88 ± 0.35	6.22 ±0.36

Moisture content	11.77 ± 0.34	8.67 ± 0.38	12.12 ± 1.25	9.16 ±0.52
Carbohydrate	58.70 ±3.17	42.85 ±1.56	59.67 ±2.15	54.43 ±1.18

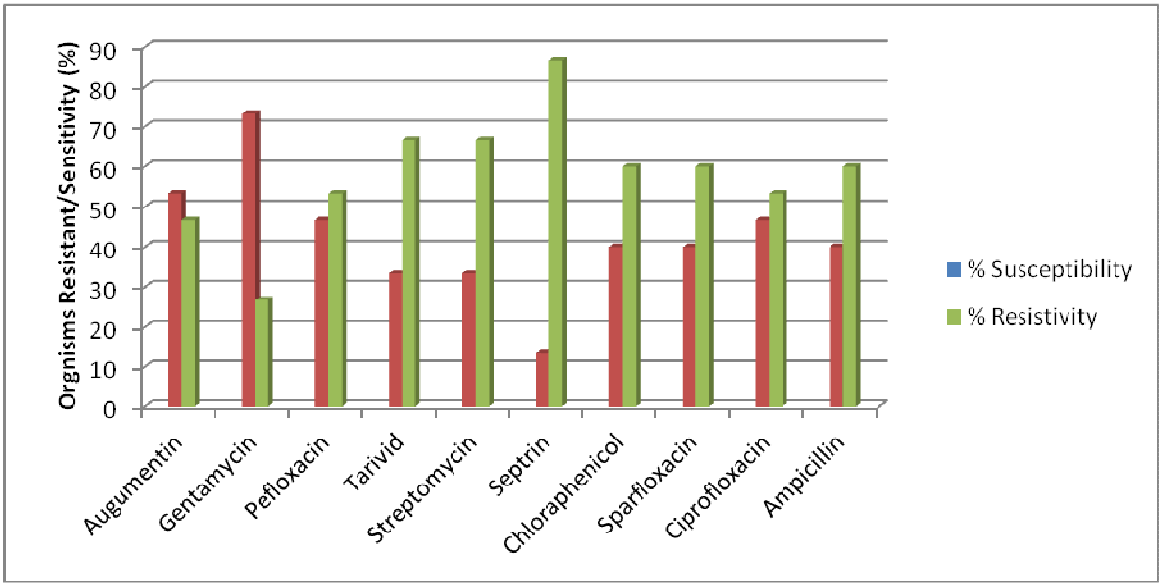

Figure 1: Organisms Susceptibility Profile To Multiple Drugs (%)

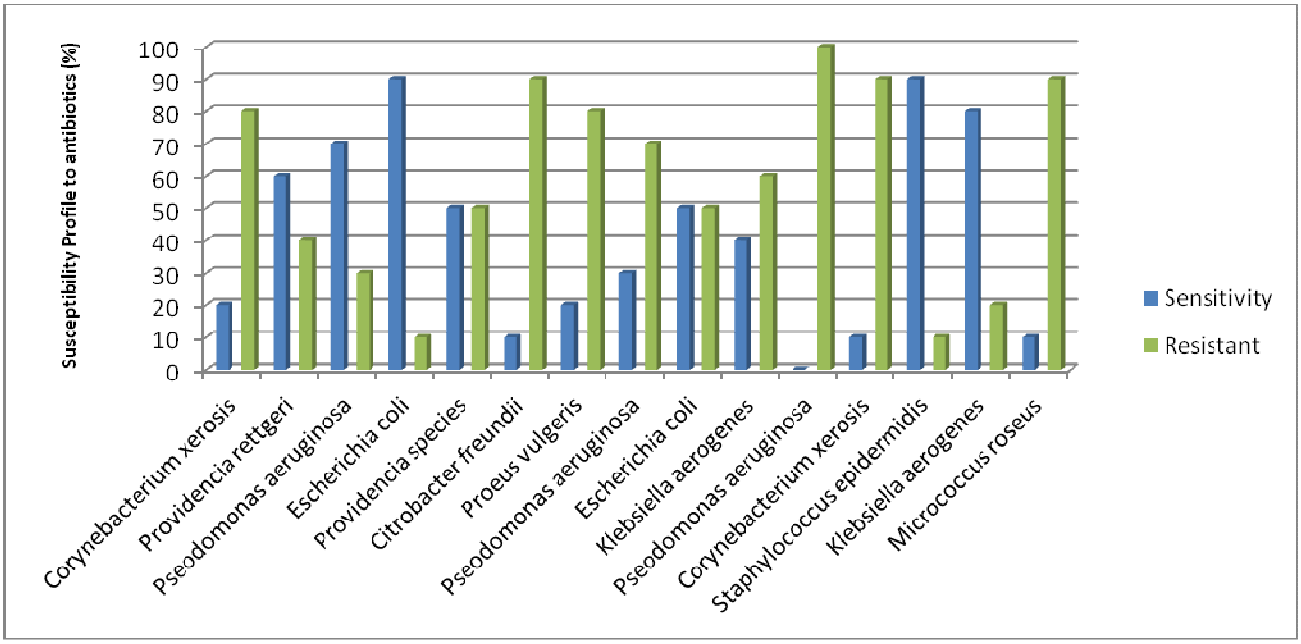

Figure 2: Bacterial Percentage Susceptibility Profile to Antibiotics

DISCUSSIONS

A total of 15 bacteria were isolated from the samples (Pig feeds and faeces) and identified by different biochemical tests. The identified bacteria were: 2 strains of *Crybacteriaxerosis*, 3 strains of *Pseudomonas aeruginosa*, 2 strains of *Escherichia coli*, 2 strains of *Klebsiell-aaerogenes*, 2 strains of *Providencia rettgeri*, and a strain of *Micrococcus reseus*,

Staphylococcus epidermis, *Citrobacterfreundii* and *Proteus vulgaris* respectively (Fig 1). Table 1 shows the total bacteria counts in feed A and feeds B ranged between 2.67 x 10^5 CFU/g (± 1.67) to 1.99 x 10^6 CFU/g (±50.22). Similarly, the bacteria count in faeces A and faeces B ranged between 1.77x 10^6 CFU/g (±3.71) to 1.46 x 10^6 CFU/g (±34.90) respectively.

The result of susceptibility pattern of the isolates in fig.2 revealed that *Pseudomonas aeruginosa* strain obtained in pig faeces sample from Osogbo showed 100% resistant to all the ten antibiotics used for the study while the strains *Micrococcus roseus, Corynebacterium xerosus, and Citrobacterer freundii* shows 90% multiple antibiotics resistant. Similarly, the strains of *Corynebacterium xerosis* from feeds and *Proteus vulgaris* showed 80% drug resistant to all the antibiotics. Others strain like *Pseudomonas aeruginosa* from feeds (70%), *Klebsiellaaerogenes* (60%), *Providencia species* (50%), *Escherichia coli* (50%), and *Providencia rottgeri* (40%) Respectively Table 1, showed resistant at a veried percentage to all the antibiotics. Conversely, *Staphylococcus epidermidis* (90%), *E. coli* from feed sample (90%), *Klebsiell-aaerogenes* from feeds (80%), *Pseudomonas aeruginosa* (70%) were sensitive to some of the antibiotics.

The performance of all the organisms to individual antibiotics shows that 86.66% of the organisms were resistant to *Septrin* (30ug), 66.67% of the test organisms were resistant to *Streptomycin* (30ug), and *Tarivid* (10ug). Also, all the organisms exhibited 60% resistant to *Ampicillin* (10ug), *Sparfloxacin* (10ug), *Chloramphenicol* (30ug), 53% of the organism were resistant to *Pefloxacin* (30ug) and *Ciprofloxacin* (30ug) respectively (Fig 1). More so, 73.33% of all the organism were sensitive to *Gentamycin* (30ug) and 53.33% were sensitive to *Augumentin* (30ug), while 40% were sensitive to *Ampicilin* (10ug), *Sparfloxacin* (10ug) and *Chloranphenicol* (30ug) respectively.

The multidrug resistance by some of the organisms could be attributed to inadequate usage and inconsistent use of antibiotics to farm animals. Since most of the organisms were enteric bacteria, resistance must have been transferred to other pathogens through interaction in the intestines. This is in conformity with WHO, (2011) that resistant by pathogens is due to cross and co-resistance via horizontal gene transfer of resistance genetic elements between the bacteria. This assertion was also supported by Glad *et al.*, (2013) that antibiotics usage increases resistance among pigs Table 1- shows the result of proximate analysis of brewery dried grain (BDG), corn brand PKC and composed feed. The results of proximate composition of corn bran are: crude proteins (10.67% ± 0.02), crude fats (6.49% ± 0.01), crude fibre (7.67% ± 0.02), total ash (3.78% ± 0.09), moisture (11.77% ± 0.09) and carbohydrates (58.70% ± 0.44) respectively. The proximate composition in brewery wastes are: crude protein (24.95% ± 0.06) crude fats (5.69% ± 0.01), crude fibre (9.71% ± 0.05), total ash (7.87 ± 0.02) moisture (8.6% ± 0.01) and carbohydrates 42.85% ± 0.35) respectively. Also, the results of proximate composition in PKC are: crude protein (18.94% ± 0.04, crude fats (3.12% ± 0.01), crude fibre (6.16% ± 0.01), total ash (5.88% ± 0.01), moisture content (6.23% ± 0.01), and carbohydrates (59.67% ± 0.01).

Lastly, the results of proximate composition in composed feeds (composite) are: crude protein (22.69% ± 0.01), crude fats (3.88% ± 0.02), crude fibre (3.62% ± 0.02), total ash (6.22% ± 0.01), moisture content (9.16% ± 0.01) and carbohydrates (54.43% ± 0.02) respectively. Statistical analysis revealed that there is no significant difference $p > 0.05$ in the percentage composition of the pig feeds. The outcome of proximate composition in all the four

samples was in line with the values reported by Maisanari, (1986), Olomu, (1995), and Kwarl *et al*., (1999). The variability in the proximate composition may be due to the difference in crop variety and methods of processing.

CONCLUSIONS

The isolates obtained from the samples revealed that there was a high population of septrin resistant bacteria in samples from all locations. The septrin resistant bacteria might be the result of the use of antibiotics in the animal feeds. The isolates from sample location exhibited multiple resistant to antibiotics. This implied the antibiotics are less effective on the bacteria isolates. The proximate analysis of the feed samples indicated that the feeds have different nutritive value depending on their compositions.

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