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RESEARCH ARTICLE

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Antibiotic Resistance Pattern Of Bacterial Pathogens Isolated From Poultry Manure Used To Fertilize Fish Ponds In New Bussa, Nigeria

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Abstract

This study was carried out to isolate and identify antibiotic resistant bacteria from poultry manure usually used for pond fertilization. Poultry manure from 120 Chickens in National Institute for Freshwater Fisheries Research (NIFFR) integrated fish farms, New-Bussa, Nigeria was collected. Five bacterial pathogens; *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* and *Aeromonas hydrophila* were isolated. Antibiotic susceptibility testing carried out using the disk diffusion technique. Antibiotics used were; ofloxacin, amoxicillin, tetracycline, ampicillin, erythromycin, gentamicin, nalidixic acid and chloramphenicol. All the isolated organisms were 100% sensitive to ofloxacin. The multiple resistance pattern revealed that 100% were resistant to tetracycline, 84.34% resistant to ampicillin, 76.68% resistant to amoxicillin, 66% resistant to chloramphenicol, 66% resistant to gentamicin, 29% resistant to erythromycin, 28.34% resistant to nalidixic acid. The risk posed by untreated poultry manure used in fish pond fertilization and the public health implications of these results were discussed.

Keywords: Pathogenic bacteria, antibiotic resistance, sensitivity, poultry manure.

1. Introduction

Animal manure is used traditionally used to fertilize fish ponds. The pond water becomes fertile upon the application of manure, resulting in more food organisms, thus a high fish production [5]. Increased local fish production is desirable in Nigeria as Nigeria is reported to be the largest importer of frozen fish in the world, with a fish demand of between 106,200 to 128,052 metric tons per year [1]. However, the use of untreated livestock manure releases high concentrations of pathogenic microorganisms into the ponds constituting a high risk to fish and fish farmers. [18] reported that average counts of heterotrophic bacteria were significantly higher in the pond water on the application of livestock manure. The development of *Pseudomonas* and *Aeromonas* were also significantly higher (P<0.05). This has grave consequences as some of these bacteria have been reported to be resistant to conventional antibiotics [14]. Antibiotics and other antibacterial drugs are the major weapons against disease-causing bacteria. They act in a number of ways to kill bacteria or suppress their activity. Over time, however, bacteria can become resistant to antibiotics. Extensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have increased the occurrence of antibiotic resistance

bacteria [9] in the natural environment. Resistance genetic material transfer from environmental bacteria to commensal microflora may also cause bacterial pathogens to carry antibiotic resistance, complicating disease prevention and treatment [9,11].

In some developing countries like Nigeria, antibiotics are sold over the counter without a prescription which compounds the problem. In human medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients [21]. Other practices contributing towards resistance include the addition of antibiotics to feeds of livestock [12]. Antibiotic-resistant organisms in domestic animals such as poultry, beef and swine are well documented [18] and have been implicated as reservoirs for multidrug-resistant food borne pathogens. Also unsound practices in the pharmaceutical manufacturing industry such as production of counterfeit drugs can contribute towards the likelihood of creating antibiotic resistant strains [10]. Emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used, but occurrence of antibiotic resistance bacteria is also increasing in freshwater basins [3]. However, relatively very few studies on antibiotic resistant bacteria have been carried out on dumpsite soils, water sources, duck droppings in the environment and

hospital environment [8, 14, and 16] and even fewer studies have been undertaken in dynamic integrated aquaculture environment where manures are usually used to fertilize fish ponds.

Therefore, the present study was carried out to isolate and identify antibiotic resistant bacteria from poultry manure used to fertilize fish ponds in fisheries environment and also to determine their antibiotic susceptibility patterns.

2. Material and Methods

2.1 Sample collection: Faecal droppings of chicken were collected randomly from NIFFR integrated farms in New-Bussa, Niger-State of Nigeria. A total of ten samples were collected from 120 chickens each raised in the integrated farm. The faecal droppings were collected with sterile spatula into sterile peptone water. All samples were analyzed within 1 hour of collection.

2.2 Isolation and identification: Isolation and identification of bacteria were investigated according to the manual of [7].

2.3 Antibiotic Sensitivity Testing: Antibiotic resistance of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method [4]. The following eight clinical antibiotics, with their concentrations given in parentheses were used in the antibiograms as recommended by the National Committee for Clinical Laboratory Standards [13]: Tetracycline (30μg), Ofloxacin (30μg), Gentamicin (20μg), Erythromycin (10μg), Ampicillin (10μg), Chloramphenicol (30μg), Nalidixic acid (30μg) and Amoxicillin (30μg). After incubation, a clear circular zone of no growth in the immediate vicinity of a disk indicates susceptibility to that antimicrobial. Using reference tables the size of zones was related to the Minimum Inhibitory Concentration (MIC) and results recorded as whether the organism is susceptible (S) or resistant (R) to that antibiotic. Data were statistically analyzed using SPSS Version 12.

3. Results and Discussion

The occurrence of the 144 isolates from Poultry manure (Fig. 1) revealed that 60 (41.6%) were *Escherichia coli*, 24 (16.7%) were *Aeromonas hydrophila*, 24 (16.7%) were *Salmonella typhi* and 18 *(12.5%)* for both *Shigella dysenteriae* and *Staphylococcus aureus* respectively. The isolates were mostly enteric organisms.
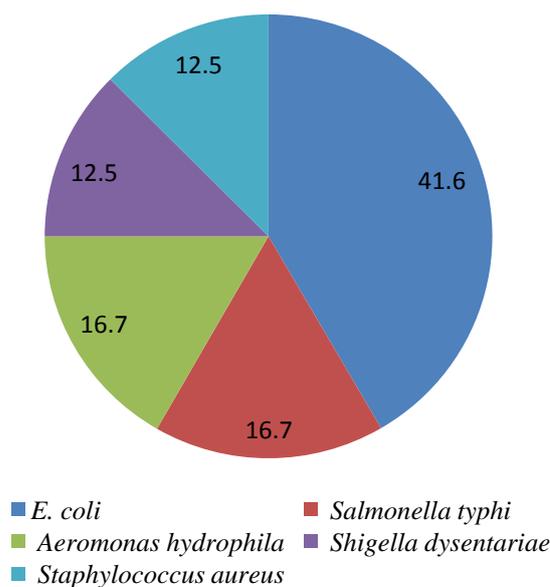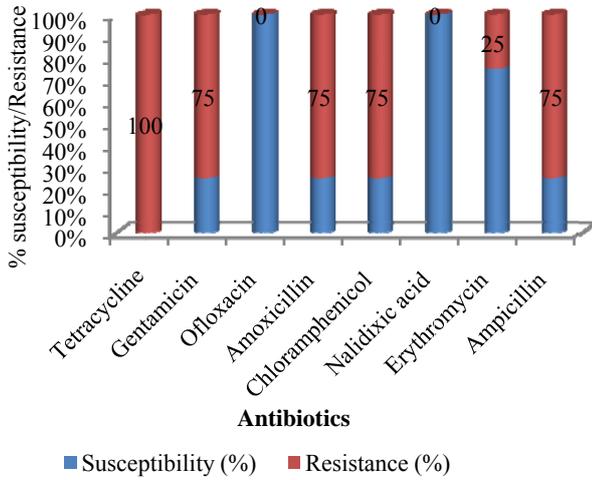

Figure 1: Frequency of Occurrence of Isoated Organisms In Poultry Manure used to Fertilize Fish Ponds
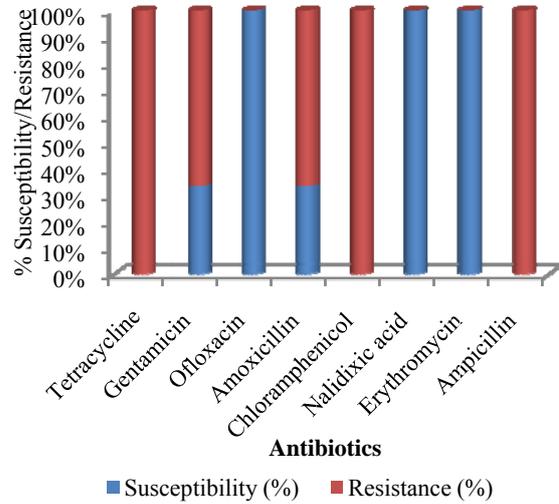
Salmonella typhi had 100% susceptibility to ofloxacin and nalixdixic acid; erythromycin (75%). However the same isolates had 100% resistance to tetracycline and 75% to resistance to ampicillin, amoxicillin, gentamicin and chloramphenicol respectively (Fig. 2). *Escherichia coli* recorded 100% susceptibility to ofloxacin and nalidixic acid and 80% susceptibility to erythromycin. However, it had 100% resistance to amoxicillin and tetracycline; 80% resistance to chloramphenicol, gentamicin and ampicillin respectively (Fig. 3). Fig. 4 shows *Shigella dysenteriae* with 100% susceptibility to ofloxacin, erythromycin and nalidixic acid; with 100% resistance to tetracycline, chloramphenicol and ampicillin respectively. It also had 66.7% resistance to amoxicillin and gentamicin. *Aeromonas hydrophila* was 100% susceptible to ofloxacin; with 100% resistance to tetracycline, amoxicillin, ampicillin, and erythromycin respectively. It however, had 75% resistance to gentamicin, nalidixic acid, and chloramphenicol respectively (Fig.5). *Staphylococcus aureus* recorded 100% susceptibility to chloramphenicol, erythromycin, and ofloxacin respectively; gentamicin (66.7%). However, it had 100% resistance to tetracycline, 66.7% to amoxicillin, ampicillin and nalidixic acid respectively. Overall, there was a 100% resistance to tetracycline by all the isolates, 84.34% resistant to ampicillin, 76.68% to amoxicillin, 66% to gentamicin, 66% to

chloramphenicol, 29% to erythromycin, 28.34% to nalidixic acid. There was 0% resistance to ofloxacin by all the isolates (Fig. 7).
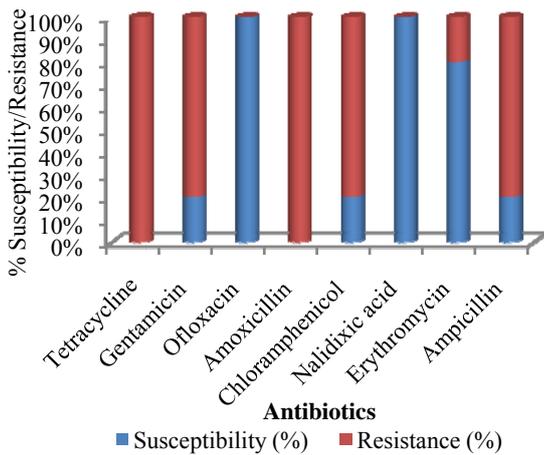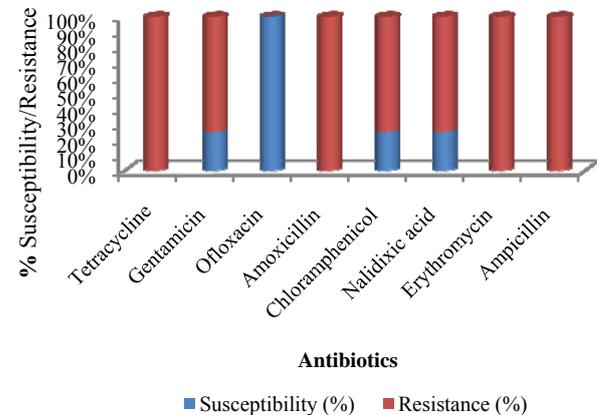

Figure 2: Antibiogram of *Salmonella typhi* isolated from Poultry manure used to fertilize Fish Ponds

**Figure 3**:Antibiogram of Escherichia coli isolated from Poultry Manure used to Fertilize Fish Ponds

The results of this study show that the antibiotics tetracycline recorded 100% resistance from all the bacterial isolates; >75% resistance for ampicillin and amoxicillin; >50% resistance gentamicin and chloramphenicol; <30% resistance for erythromycin and nalidixic acid. All the bacteria isolated had 0% resistance to ofloxacin. This trend is quite worrisome as these antibiotics represent first line antibiotics in the treatment of many bacterial infections [15]. Resistance of a single bacterial isolate to more than one antimicrobial drug is commonly reported. Multiple antimicrobial drug resistance profiles have been reported [8, 14]. This type of testing is simple,

cost-effective, and suitable for surveillance and it has been used for enteric bacteria from human and animal sources [20].

Figure 4: Antibiogram of *Shigella dysenteriae* isolated from Poultry Manure used to fertilize Fish Ponds

Figure 5: Antibiogram of *Aeromonas hydrophila* isolated from Poultry Maure used to fertilize Fish Ponds

Results from this study show *Escherichia coli* had a 100% resistance to tetracycline, amoxicillin and ampicillin, this is similar to the report of [2] who reported 100% resistance of their *E. coli* isolates to amoxicillin and ampicillin. This study also observed 100% susceptibility of *Escherichia coli* to ofloxacin and nalidixic acid respectively. This is also similar to the report of [14] who recorded 100% and 85.71% susceptibility to ofloxacin and nalidixic acid respectively. The high level of resistance of *Shigella dysenteriae* to tetracycline, chloramphenicol,

ampicillin and gentamicin reported in this study was similar to the report of [8] who reported resistance to tetracycline, chloramphenicol, ampicillin and gentamicin. The susceptibility recorded by *S. aureus* in this study for chloramphenicol and erythromycin were 100% respectively and is however slightly
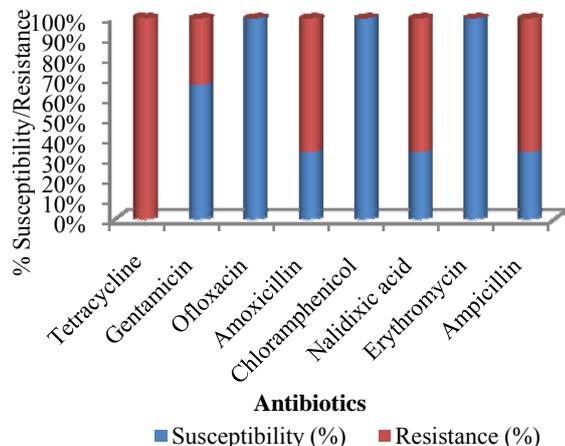
Figure 6: Antibiogram of *Staphylococcus aureus* isolated from Poultry Manure used to fertilize Fish Ponds
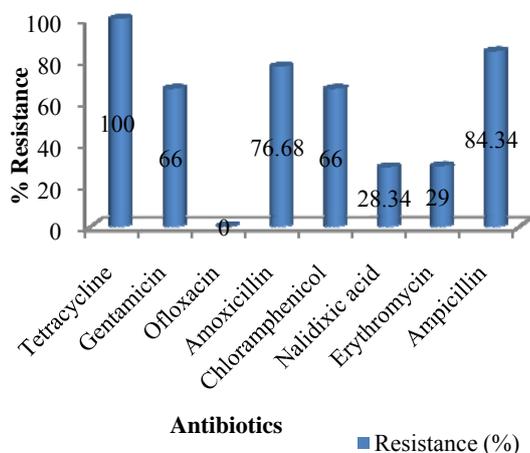
Figure 7: Antibiotic Multiple Resistance Pattern of Bacteria isolated from Poultry Manure used for Fertilizing Fish Ponds

higher than the susceptibility reported by [16] for both chloramphenicol and erythromycin which were 95.12% and 73.98% respectively. However the susceptibility to gentamicin reported was 70.73% which was slightly higher than those reported in this study.

The patterns of resistance to the antimicrobial agents may be due to indiscriminate, widespread and lengthy use of tetracycline, chloramphenicol and gentamicin in poultry. Tetracycline is a commonly used first-line antibiotic in the poultry and is often

used before the antimicrobial agent resistance of a pathogen has been determined [17]. The highest levels of susceptibility to all bacterial isolates found in this study were to ofloxacin and nalidixic acid (Fig. 6) is in line with the findings of [6, 19].

4. Conclusions

The results of this study indicate that poultry manure is a potential carrier of pathogenic bacteria which is capable of transmitting zoonotic diseases to humans due to contact with the manure, pond water and the fish from the pond. These antibiotic resistant organisms may be further transferred to consumers in improperly processed fish. The antibiotic sensitivity testing show that all the organisms were 100% sensitive to ofloxacin whereas the isolates were most resistant to antibiotics like, tetracycline, penicillin, amoxicillin which are the common antibiotics in treating bacterial infections in both man and animals. This study further revealed that fish, fertilized fish ponds and the environment are easily contaminated by poultry manure intended for fish production; therefore it is important to use untreated animal manure with caution. The implication of this on the choice of antibiotics in relation to zoonotic infections in our environment should be noted and effort should be made to stop indiscriminate use of untreated animal manure for fish pond fertilization.

6. References

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