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Antibiotic Resistance Pattern of Bacterial Pathogens Isolated from Cow Dung Used to Fertilize Nigerian Fish Ponds

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Abstract

This study was carried out to isolate and identify antibiotic resistant bacteria from cow dung used for pond fertilization. Cow dung from over 200 cows in NIFFR integrated farms, New-Bussa, Nigeria were collected. Six bacterial pathogens; *Escherichia coli, E. coli O157:H7, Shigella dysentariae, Staphylococcus aureus, Salmonella typhi, and Aeromonas hydrophila* were isolated. Antibiotic susceptibility testing by the disk diffusion method was conducted using ofloxacin, amoxicillin, tetracycline, ampicillin, erythromycin, gentamicin, nalidixic acid and chloramphenicol. All the isolated organisms were 100% sensitive to ofloxacin. The multiple resistance patterns revealed that 100% were resistant to tetracycline, ampicillin (85.6%), amoxicillin (83.3%), chloramphenicol (66%), gentamicin (47.6%), erythromycin (44.4%) and nalidixic acid (18.3%). The Public Health risks posed by the cow dung manure include proliferation of ponds with these organisms that are pathogenic to fish and man, contamination of the environment and the possible retention of these organisms in the table size fish.

Keywords: antibiotic resistance, manure, pathogenic organism, sensitivity

Introduction

Integrated fish farming is widely acceptable in aquaculture and is being practiced by the National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Niger State, Nigeria. In this farming system, manure from livestock production is administered to fish ponds and the manure is directly consumed by fish. The release of nutrients supports the growth of fish with low input, with the fish receiving limited, if any, supplementary feed. The pond water becomes fertile upon the application of manure, resulting in more food organisms, thus a high fish production. However, organic manuring releases high concentrations of pathogenic microorganisms into the ponds constituting a high risk to fish and fish farmers. This is especially serious as an array of these bacteria has been reported to be resistant to antibiotics (Andreas *et al*., 2002; Olaitan *et al*., 2011). 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. Antibiotics are regularly used in treating sick and diseased cow in the integrated units. Over time, however, bacteria can become resistant to antibiotics. Other practices contributing towards resistance include the addition of antibiotics to livestock feeds (Mathew *et al.,* 2007).

In addition, unsavory practices in the pharmaceutical manufacturing industry such as production of counterfeit drugs can contribute towards the likelihood of creating antibiotic resistant strains (Larsson and Fick, 2009). 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 basin (Ash *et al.,* 2002). The use of antibiotics as growth promoters in animal husbandry has been linked to certain antimicrobial resistance patterns among human bacterial pathogens (Bager *et al.,* 1997; Wagener *et al.,* 1999) suggesting that there is a possible flow of antimicrobial resistance genes between animal and human pathogens. Potential transfer of resistant bacteria and resistance genes from aquaculture environments to humans may occur through direct consumption of antimicrobial-resistant bacteria present in fish and associated products. Few studies on antibiotic resistance bacteria have been carried out on dumpsites, water sources, duck droppings in the environment and hospital environment (Ikpeme *et al.,* 2011; Olaitan *et al.,* 2011; Omololu-Aso *et al.,* 2011) but fewer studies have been undertaken in dynamic integrated aquaculture environment where manure is used to fertilize fish ponds. Therefore, the objective of this study was to isolate and identify antibiotic resistant bacteria from cow dung used in fertilizing fish ponds. In addition, also examine the antibiotic susceptibility patterns of the isolated organisms.

Materials and methods

Sample collection

Samples of cow dung were randomly collected from NIFFR integrated farms in New-Bussa, Niger state, North

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central region of Nigeria. A total of 1000 samples were collected from over 200 cows raised in the integrated farm. The faecal samples were collected with sterile spatula into sterile peptone water and were analyzed within 1 hour of collection.

Isolation and identification

Isolation and identification of bacteria were investigated according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1997). Pure cultures suggestive of *E. coli* were sub-cultured on Sorbitol MacConkey (SMAC) agar and incubated at 44.5°C for 24 hours to check for colonies that were colorless to pale (non- fermenting), flat and smooth, circular or serrated at the edge typical of *E. coli* O157:H7.

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 (Bauer *et al.,* 1979). 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 (NCCLS, 2006): Tetracycline (30 µg); Ofloxacin (30 µg); Gentamicin (20 µg); Erythromycin (10µg); Ampicillin (10 µg); Chloramphenicol (30 µg); Nalidixic acid (30 µg) and Amoxicillin (30 µg). 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 was statistically analyzed using SPSS Version 12, level of significance 5%.

Results and discussion

One hundred and thirty-two (132) bacteria were isolated from 1000 cow dung samples. These were: *Escherichia coli*: 66 (50%); *Aeromonas hydrophila* 30 (22.7%); *Salmonella typhi* 18 (13.6%); *Staphylococcus aureus* 12 (9.1%) and *Shigella dysenteriae* 6 (4.6%) (Fig. 1). Twelve 12 (18.2%) of the sixty-six (66) *E. coli* isolates from cow dung were non-fermenters typical of *E. coli* O157:H7 which represent 9.1% of total isolates from cow dung.

Overall, there was a 100% resistance to tetracycline by all the isolates, ampicillin (85.6%), amoxicillin (83.3%), gentamicin (47.6%), chloramphenicol (66%), erythromycin (44.4%) and nalidixic acid (18.3%). Multiple antibiotic drug resistance profiles have been reported in enteric bacteria from human and animal sources (Ikpeme *et al.,* 2011; Olaitan *et al*., 2011; Troy *et al*., 2002). Resistance of a single bacterial isolate to more than one antimicrobial drug is commonly reported. There was no resistance to ofloxacin by all bacteria isolated. *Salmonella typhi* recorded 100% susceptibility to ofloxacin and nalidixic acid; 66.7% to erythromycin and 100% resistance to tetracycline; 66.7% resistance to gentamicin and ampicillin respectively (Fig. 2). These antibiotics are very common and are readily available as over-the-counter drugs to consumers in Nigeria (Omojasola and Omojasola, 2001) and may not be very useful for therapeutic purposes. The susceptibility pattern of *E. coli* (Fig. 3) showed the organism was susceptible to ofloxacin and nalidixic acid (100%), chloramphenicol (77.8%) and erythromycin (66.7%). However, it had 100% resistance to amoxicillin, tetracycline and 66.7% resistance to ampicillin. *E. coli O157:H7* recorded 100% susceptibility to ofloxacin, amoxicillin and nalidixic acid with 100% resistance to tetracycline, gentamicin, erythromycin and ampicillin respectively and 50% resistance to chloramphenicol (Fig. 4). Multiple antibiotic drug resistance in *E. coli* to ofloxacin and nalidixic acid has also been reported (Aibinu *et al.* 2004; Olaitan *et al.* 2011). This agrees with earlier reports that *E. coli* O157 isolates had high prevalence of resistance to tetracycline, sulfamethoxazole, cephalothin, and ampicillin (Carl *et al*., 2002; Zhao *et al*., 2001). Cattle is thought to be primary reservoirs of *E. coli* O157 and it is highly infectious as the infective dose for humans is reported to be 10 cfu, the lowest of the common foodborne pathogens. In addition, *E. coli* O157; H7 has high survival rates in as fecal counts as high as 4.2×103 cfu/mL have been reported in 100 day old manure (Kress and Gifford, 1984).

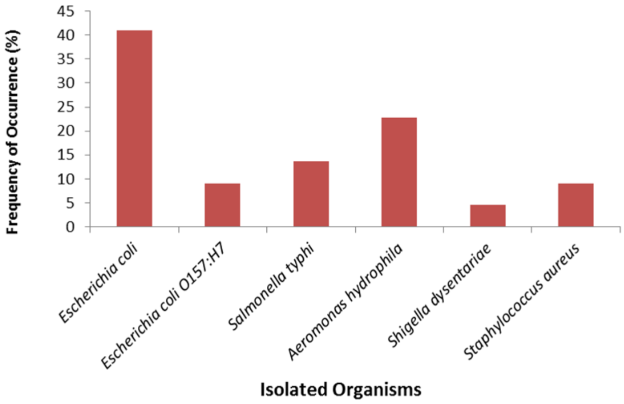

Fig. 1. Frequency of Occurrence of Isolated Organisms In Cow Dung used to fertilize Fish Ponds in New Bussa, Nigeria

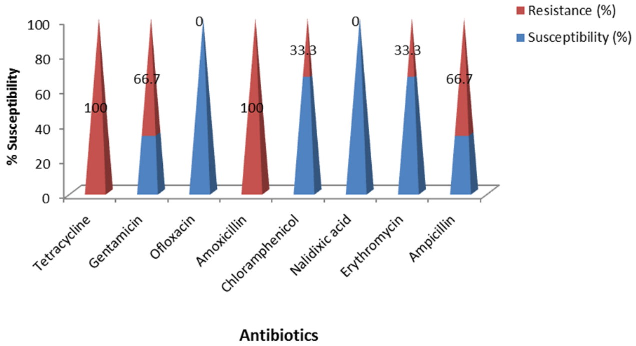

Fig. 2. Antibiogram of *Salmonella typhi* isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

Tab. 1. Antibiotic Resistance profile of pathogenic bacteria isolates from Cow Dung used to fertilize fish ponds in New Bussa Nigeria

	Antibiotic Susceptibility (Zone of inhibition in mm)							
Isolates	TET	GEN	OFL	AMX	CHL	NAL	ERY	AMP
Salmonella typhi	R(18)	R(16)	S(25)	R(13)	S(21)	S(26)	S(27)	R(11)
E. coli	R(17)	R(11)	S(28)	R(12)	S(23)	S(28)	S(29)	R(10)
E. coli O157:H7	R(17)	R(10)	S(27)	R(13)	S(20)	S(26)	R(10)	R(11)
Shigella dysentariae	R(16)	R(11)	S(21)	R(10)	R(13)	S(20)	S(21)	R(10)
Aeromonas hydrophila	R(14)	R(10)	S(20)	R(9)	R(11)	R(16)	R(16)	R(8)
Staphylococcus aureus	R(17)	S(25)	S(26)	R(10)	S(23)	S(28)	S(24)	R(10)

R = Resistant, S = Susceptible, TET= Tetracycline, GEN = Gentamicin, OFL = Ofloxacin; AMX = Amoxicillin; CHL= Chloramphenicol; NAL= Nalidixic acid; ERT= Erythromycin, AMP = Ampicillin

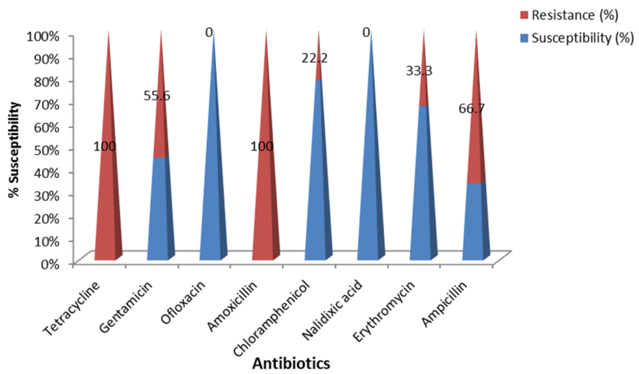

Fig. 3. Antibiogram of *E. coli* isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

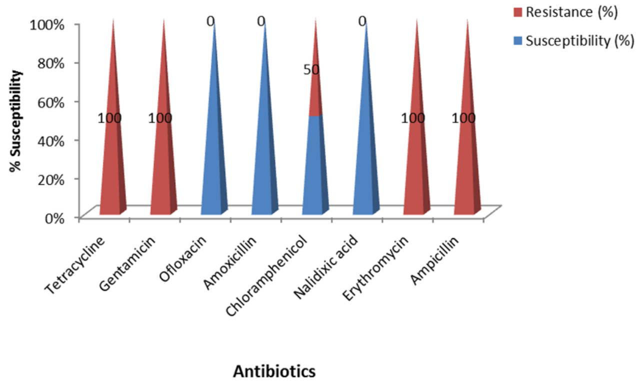

Fig. 4. Antibiogram of *E. coli* O157:H7 isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

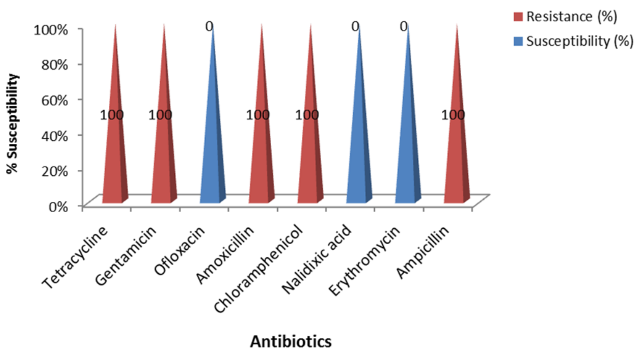

Fig. 5. Antibiogram of *Shigella dysenteriae* isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

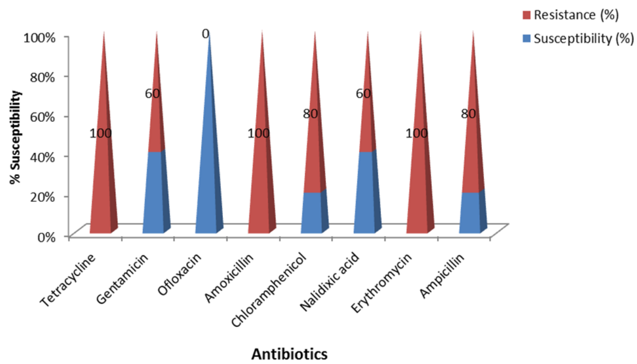

Fig. 6. Antibiogram of *Aeromonas hydrophila* isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

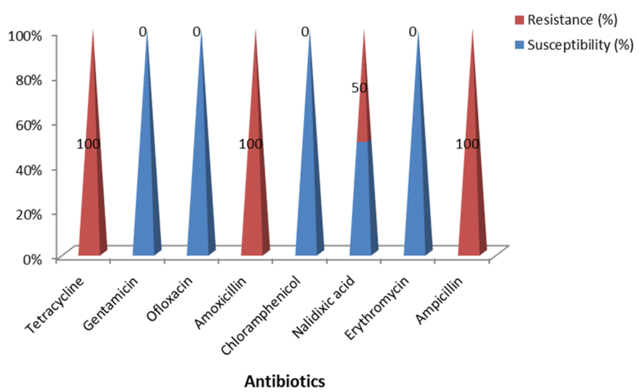

Fig. 7. Antibiogram of *Staphylococcus aureus* isolated from cow dung used to fertilize fish ponds in New Bussa Nigeria

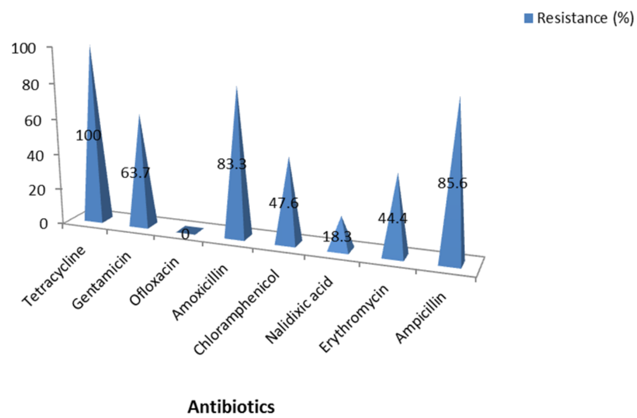

Fig. 8. Percentage Multiple Antibiotics Resistance

Antibiogram results of the various isolates as presented in Tab. 1 show multidrug resistance by all isolates. Ofloxacin was the only antibiotic with no resistance by the isolated bacterial pathogens. However, the isolates had 100% resistance to tetracycline, ampicillin (85.6%), amoxicillin (83.3%), chloramphenicol (66%), gentamicin (47.6%), erythromycin (44.4%) and nalidixic acid (18.3%).

There was 100% susceptibility of *Shigella dysenteriae* to ofloxacin, erythromycin and nalidixic acid respectively with 100% resistance to tetracycline, chloramphenicol and ampicillin respectively (Fig. 5) The high level of resistance of *Shigella dysenteriae* to tetracycline, chloramphenicol, ampicillin and gentamicin recorded in this study is similar to that of Ikpeme *et al.* (2011) who also reported resistance to tetracycline, chloramphenicol, ampicillin and gentamicin. *Aeromonas hydrophila* was 100% susceptible to ofloxacin while it had 100% resistance to tetracycline, amoxicillin and erythromycin respectively. It also had 80% resistance to chloramphenicol and ampicillin respectively while it had 60% resistance to gentamicin and nalidixic acid respectively (Fig. 6). Moreover, *Staphylococcus aureus* was 100% susceptible to gentamicin, ofloxacin, chloramphenicol and erythromycin respectively while it was 50% susceptible to nalidixic. It had 100% resistance to tetracycline, amoxicillin ampicillin respectively (Fig. 7). The susceptibility recorded by *S. aureus* in this study for chloramphenicol and erythromycin were 100% respectively and is however slightly higher than the susceptibility reported by Omololu-Aso *et al*. (2011) for both chloramphenicol and erythromycin which were 95.12% and 73.98% respectively. Also, the susceptibility to gentamicin reported was 70.73% which was slightly lower compared to 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 treatment of cow infections. Tetracycline is a commonly used first-line antibiotic in the animal husbandry and is often used before the antimicrobial agent resistance of a pathogen has been determined (Prescott *et al.*, 2000). The highest levels of susceptibility to all bacterial isolates found in this study were to ofloxacin and followed by nalidixic acid (Fig. 8) (Engberg *et al.*, 2001; Sayah *et al.,* 2004). The results suggest that livestock is a reservoir of resistant bacteria for environmental contamination, in agreement with the report of Sayah *et al.* (2004). Cow dung manure serves a potential carrier of pathogenic bacteria which are capable of transmitting zoonotic diseases to humans as a result of contact with the manure, when this untreated manure is used to fertilize fish ponds, it may lead to increase in bacterial infections in the fish and serves as a potential source of food borne infections for the fish consumers.

Conclusions

From this study, pathogenic bacteria were isolated from cow dung manure. The antibiotic sensitivity testing shows that all the organisms were 100% sensitive to ofloxacin. The isolates were most resistant to tetracycline, ampicillin and amoxicillin. Therefore potential role of manure fertilized ponds as source of antibiotic resistance in the environment should further be studied. It is recommended that manure intended for pond fertilization should be treated before use. In addition, the implication of this high level of antibiotic resistance on the choice of antibiotics in relation to zoonotic infections should be noted and efforts should be made to stop indiscriminate use of antibiotics.

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