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Full Length Research Paper

Isolation, antibiogram and haemolytic activity of *Aeromonas hydrophila* from goat

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Aeromonas hydrophila **are ubiquitous in the soil and water. Though most species of the genus** ***Aeromonas*** **are apathogenic and may only exhibit very low pathogenicity in terms of human infections, some (especially** ***A. hydrophila*****) are important human pathogens in that they cause a number of gastrointestinal and extra-gastrointestinal infections in man.** ***A. hydrophila*** **in particular cause acute diarrheal disease that is similar to cholera in humans. Septicaemia, bacteraemia and meningitis are other clinical conditions associated with** ***A. hydrophila*** **in man. Domestic animals (for example, goat) that feed on waste matters and dirty water in the environment are prone to an infection with** ***A. hydrophila*****, and these animals can transfer the pathogen to humans either indirectly or by direct contact with infected goats. In this study, rectal swabs (n=50) were obtained from locally breed goats in Abakaliki metropolis, Ebonyi State, Nigeria. The rectal swabs were analyzed microbiologically for the presence of** ***A. hydrophila*** **using alkaline peptone water (pH 6.8), blood agar, MacConkey agar medium and thiosulphate-citrate bile salt (TCBS) agar, and all suspected isolates were tested for susceptibility to chloramphenicol, ampicillin, ciprofloxacin, gentamicin and ceftazidime, then were confirmed using standard microbiological identification techniques. Overall, 24 isolates of** ***A. hydrophila*** **(48%) was isolated from the 50 rectal swabs analyzed. Only 16 isolates showed haemolytic appearance on blood agar. However, they were all resistant to the tested antibiotics except for ceftazidime that inhibited 23 out of the 24** ***A. hydrophila*** **isolates analyzed. The presence of resistant** ***A. hydrophila*** **in goats portends a public health issue to humans that come in contact with these animals directly or indirectly. Thus it is vital for the general public to observe strict personal hygiene after coming in contact with goats, and animal farmers should control their goats for them not to wander away to feed on garbage and waste water in the environment.**

Key words: *Aeromonas hydrophila*, antimicrobial susceptibility, haemolysis, Nigeria.

INTRODUCTION

Aeromonas hydrophila is facultative anaerobic, oxidase-positive and Gram-negative rods (Brooks et al., 2004). It is predominantly found in fresh water, and can also be found in cold and warm blooded animals (Janda and Abbott, 1996). *A. hydrophila* are notorious in causing gastroenteritis (that is, diarrheal disease that resembled cholera) in humans and it has also been implicated in other diseases such as bacteraemia and wound infections (Belgin et al., 2011; Cheesbrough, 2002). *Aeromonas* species produces a broad range of extracellular enzymes such as enterotoxins and hemolysins which aids its disease mechanism (Trower et al., 2000). Once inside the gastrointestinal tract (GIT) of its host, the organism attaches to the host cells through the production of flagella, pili and adhesions (Gavin et al.,

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Table 1. Distribution of the isolated *Aeromonas hydrophila*.

Age (years)	No. of goats studied	No. of *Aeromonas hydrophila* isolated
< 2	10	4 (8%)
2-4	20	8 (16%)
5-6	20	12 (24%)
Total	50	24 (48%)

Table 2. Results of haemolysis on blood agar.

Microorganism	No. positive	No. negative
A. hydrophila	16 (66.7%)	8 (33.3%)

2005). Some of the clinical manifestations of the disease caused by *A. hydrophila* include cellulitis, gastrointestinal perforations and diarrhea (Mukhopadhyay et al., 2008; Qui et al., 2003). *A. hydrophila* occur worldwide, and they have been distributed through contaminated freshwater when released in the dung of cold-blooded and warm blooded animals (Isoken et al., 2012). It has been noted that *A. hydrophila* is commonly isolated from drinking water contaminated by animal dungs (El-Taweel and Shaban, 2001). *A. hydrophila* is usually present in most water sources used for drinking and other purposes.

Studies have shown that *A. hydrophila* is usually more susceptible to chlorine and monochloramine additives in wastewater treatment plants than coliforms (Chauret et al., 2001). Thus, the prevention of spread of *A. hydrophila* through water distribution system is vital, and it can be achieved via the effective treatment and maintenance procedures in wastewater treatment plant systems (Chan et al., 2000). Because *A. hydrophila* is usually susceptible to tetracyclines, aminoglycosides and cephalosporins (Brooks et al., 2004), this research was aimed to isolate and determine the antibiotic profile and haemolytic abilities of *A. hydrophila* recognized from goats in Abakaliki metropolis, Nigeria.

MATERIALS AND METHODS

Animal study

The 50 Nigerian local breed goats aged between 1-6 years old were recruited from local farms in Abakaliki metropolis, Ebonyi State of Nigeria. All healthy and unpresented with any form of diarrheal disease goats were recruited for sample collection. The ages of the goats were recorded, and ethical approval was obtained from the animal section of the State's Ministry of Health, and consent was also obtained from the farm owners whose goats were recruited.

Sample collection

Rectal swabs (n=50) were aseptically collected from each of the goats and transported in alkaline peptone water medium to the Microbiology Laboratory of Applied Microbiology Department, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria.

The rectal swabs collected were processed within 2 h of collection using MacConkey agar medium (Oxoid, UK) and thiosulphate-citrate bile salt agar (Oxoid, UK) for *A. hydrophila* isolation.

Alkaline peptone water (pH 8.6) was used as the enrichment medium. Blood agar containing 15 mg/l ampicillin was used as the plating medium for the detection of haemolytic activity. All cultured plates were incubated at 37°C for 24-48 h. The suspected colonies of *A. hydrophila* were tested for oxidase production and identified based on standard microbiological identification techniques (Cheesbrough, 2002; Vandepitte et al., 2003).

Antibiogram

Antimicrobial susceptibility was evaluated as per the Clinical Laboratory Standard Institute, CLSI (formally National Committee for Clinical Laboratory Standards, NCCLS) criteria using the disk diffusion technique [14]. Ampicillin (10 µg), gentamicin (10 µg), tetracycline (10 µg), chloramphenicol (10 µg), and ceftazidime (30 µg) were used to test the antibiotic susceptibility of the *A. hydrophila*. All antibiotics were procured from Oxoid Limited (Oxoid, UK).

RESULTS

Table 1 shows the frequency of isolation of *A. hydrophila* from the 50 rectal swabs included in this study. Overall, a total of 24 (48%) *A. hydrophila* was isolated. Goats aged less than 2 years old showed 4 (8%) *A. hydrophila*, while 8 (16%) and 12 (24%) were isolated from goats aged 2-4 and 5-6 years old, respectively.

The results of the haemolytic test were shown in Table 2. Only 16 isolates of *A. hydrophila* appeared on blood agar as haemolysis. All the *A. hydrophila* isolates were oxidase positive.

The susceptibility test result shows that the test organism, *A. hydrophila* were resistant to virtually all the tested antibiotics (Table 3). Notably, *A. hydrophila* were completely resistant to ampicillin (100%) and tetracycline (100%). Chloramphenicol and gentamicin were also least effective in inhibiting *A. hydrophila*. Yet ceftazidime was very effective in inhibiting *A. hydrophila*.

Table 3. Results of the antimicrobial susceptibility studies.

Antibiotics (µg)	Resistant n (%)	Susceptible n (%)
Ampicillin (10)	24 (100)	0 (0)
Gentamicin (10)	23 (95.8)	1 (4.2)
Tetracycline (10)	24 (100)	0 (0)
Chloramphenicol (10)	23 (95.8)	1 (4.2)
Ceftazidime (30)	1 (4.2)	23 (95.8)

DISCUSSION

Goat is a well known domestic animal in most African communities, and in Nigeria, the animal is slaughtered for meat and rarely are they used as pets in this part of the world. In many Nigerian homes, goat is reared for both subsistence and commercial purposes. Most farms in Nigeria that rear the animal rely on foliage and other solid foods as the main source of nutrition for their goats. However, it is possible that some may incorporate antibiotics in their water or food as a way of taking care of some bacterial infections in the animal or enhancing growth. This practice which is not backed by law could result in the development of resistant strains of bacteria in the animals and these could go on to infect humans through consumption or contact with these animals. In this study, we investigated the frequency, haemolytic ability and antibiogram of *A. hydrophila* from Nigerian local breed goats in Abakaliki metropolis, Ebonyi State, Nigeria. The goats included in this study were found to have a higher rate of *A. hydrophila* carriage (48%), and this could be attributable to the contamination of their drinking water with the organism. In Abakaliki, goats may be allowed to walk freely in search of food and water, and this scenario raises the tendency for them to become infected with *A. hydrophila* – known to be very prevalent in water. Though the reason for the high prevalence of *A. hydrophila* from goat in our study may not be obviously known, environmental factors may play a significant role in the infection rate of the organism in these animals. Studies on the frequency of *A. hydrophila* in goats are scarce in Nigeria; however, similar findings have been reported in other domestic animals, pets, water and even from foods (Belgin et al., 2001; Trower et al., 2000; El-Taweel and Shaban, 2001). *A. hydrophila* has been associated and implicated in a number of human enteric diseases, and their emergence in the community as important human pathogens (especially amongst animals as reported in this study) is becoming a public health issue which must not be ignored (Gavin et al., 2005; Mukhopadhyay et al., 2008; Isoken et al., 2012). Their presence in food should also raise suspicion about feacal-matter contamination of water and food materials from animal origin (Belgin et al., 2011). Out of the 24 isolates of *A. hydrophila*, only 16 showed haemolytic activity on blood agar. The *A. hydrophila* isolated in this study was resistant to ampicillin, chloramphenicol, tetracycline and ciprofloxacin. However, ceftazidime, a third-generation cephalosporin, was very effective against the *A. hydrophila* with only one isolate showing resistance to the antibiotic. Farm owners that rear goat either for pastoral or commercial purposes should ensure that the water used for rearing their animals are from a reliable source since *A. hydrophila* are ubiquitous in water. As shown in this study, goats harbor *A. hydrophila*, and they stand as potential source via which the pathogen can reach humans and cause gastroenteritis. It is therefore advisable for humans to ensure proper personal hygiene especially regular washing of their hands after coming in contact with these animals so as to keep the microbes at bay.

Conclusion

The presence of *A. hydrophila* in goats may pose an important public health problem in human populations due to the close proximity of these animals with human beings. These animals also serve as source of protein or meat and food to humans, and they could serve as route via which resistant strains of *A. hydrophila* are transmitted to humans. Thus, it is very important as a public health interest to expand this study beyond this scope, and incorporate other molecular tools that will assist in getting an updated epidemiological data on *A. hydrophila* in this environment not just in goats but in other animals that could be a potential reservoir of the microbe.

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