

Isolation of *Aeromonashydrophila* in a case of wound infection in cattle in Nigeria

Awoyomi, O.J., Oyewusi, J.A, Talabi, A.O., Oyewusi, I.K., Biobaku, K.T, Mustapha, O.A., and Agbaje, M

College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria. jojuawoyomi@yahoo.com

**Abstract**

A three and a half year old N'dama cow weighing about 150kg was rescued from a steel jaw trap during grazing the previous day and presented at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Nigeria with a fresh, deep wound. The animal was treated but there were no satisfactory response to Penicillin/streptomycin intramuscular injection at 10,000IU/kg for seven days and also i/m 20% Oxytetracycline injection at 20mg/kg repeated after 48 hours. A loopful of pus samples aseptically taken from the wound were streaked on blood agar base enriched with 7% horse blood (Oxoid, UK) using the quadrant streaking method and incubated aerobically at 37°C for 24 - 48 hours. *Aeromonas hydrophila* was isolated which was resistant to the commonly used antibiotics on the farm. Following the results obtained from the sensitivity test, the animal was placed on Enrofloxacin for seven days, the swelling regressed, no pus was expressed from the wound site and no pain was elicited from the joint on application of pressure by the 18th day. *Aeromonas hydrophila* is pathogenic to man; hence care should be taken in handling of animal traps.

Key words: isolation, *Aeromonas hydrophila*, cattle, Nigeria

Introduction

Aeromonas hydrophila are Gram-negative bacteria, non-spore forming, oxidase-positive, rod shaped and facultative anaerobe of the family Aeromonadaceae. In animals, *Aeromonas specie* has been implicated as the distinct cause of diarrhoea in piglets and pigs (Efuntoye, 1995), abortion and reproductive problems in mares (Fontaine *et al.*, 1996), diarrhoea in foals (Browning *et al.*, 1991), septic arthritis in foals (Traub-Dargatz *et al.*, 1994), diarrhoea in horses (Hathcock *et al.*, 1999), septicemia in dogs (Andre-Fontaine *et al.*, 1995), mastitis in cattle (Bergman *et al.*, 1981), polyarthritis in calves (Love and Love 1984), seminal vesiculitis in bulls (Moro *et al.*, 1999), diarrhoea in sheep (Ilhan *et al.*, 2006) and

goats (Efuntoye 1995), gastroenteritis in deer (Pal *et al.*, 1989) and diarrhoea in rabbits (Efuntoye 1995).

Aeromonas hydrophila is commonly found in freshwater, sewage and soil (Martin-Carnahan and Joseph, 2005). Interest in *Aeromonas sp.* has increased in recent years since they cause gastroenteritis, cellulitis, peritonitis, meningitis and pneumonia in immune-competent humans and disseminated infections in immune-compromised humans or water- or soil associated traumatic wound infection (Semel and Trenholme, 1990; Von Graevenitz, 2007).

This paper describes a case of trap trauma where *Aeromonas hydrophila* alone was isolated on bacterial culture involving a cow in a herd of 78 mixed breed cattle kept

semi-intensively at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Nigeria.

Materials and Methods

Background

On the 9th of March 2011, a three and a half year old N'dama cow weighing about 150kg belonging to the University; was rescued from a steel jaw trap during grazing the previous day and presented at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Nigeria with a fresh, deep wound.

The Farm is situated at Latitude 7° 9' 39" N and Longitude 3° 20' 54" E, 76m above sea level in the rain forest vegetation zone. The area has a humid climate with mean annual rainfall and temperature of 1037 mm and 34.7°C respectively and average relative humidity of 82% (Dipeolu *et al.*, 2005).

Clinical examination and management

Rectal temperature, pulse and heart rates were within normal range (Blood *et al.*, 2007), a superficial wound of about 6cm in length was noticed and the assessment revealed a deep punctured wound just about the centre of the traumatic injury around the middle phalanx of the left fore-limb. The fetlock joint was inflamed and painful to touch. The animal could not bear weight on the affected limb.

The hair around the wound was clipped, thoroughly cleaned with methylated spirit and the following medications were administered: anti-tetanus vaccine; Charmyl[®] spray; i/m Penicillin/streptomycin injection at 10,000IU/kg and 10mg/kg; Cydic[®], an anti-inflammatory agent for seven days. There was no satisfactory response to the therapy and the affected joint relapsed into inflammation, with purulent exudate.

This prompted the following line of therapy: i/m 20% Oxytetracycline injection at 20mg/kg (repeated after 48 hours);

acetaminophen with daily dressing with chlorhexidine antiseptic and application of Gentian Violet/Oxytetracycline spray topically. Wound dressing continued with administration of Cydic[®], an anti-inflammatory agent for five days. These also did not produce a satisfactory response to the therapy and six days later, the punctured wound was discharging pus while the joint was still swollen and painful to touch.

Sterile stick cotton swab was used to obtain a sample of the pus from the wound for bacterial culture and antibiotic sensitivity test since the two commonly used antibiotics were not effective. Samples were also taken on April 5th, 6th and 7th, 2011.

Microbiological tests

Bacteriological examination of pus samples: All reagents, kits and media used in this study were of analytical grade, obtained from Oxoid. Media used included: Nutrient Agar (NA) and Blood Agar Base (BA)(Oxoid, UK). All media were prepared according to the manufacturer's specification and sterilized at 121°C 1 bar for 15 minutes. Biochemical characterization was carried out using Oxoid Microbact[®] kits while bacteriological examination was done according to the methods described by Quinn *et al.*, (1994) and CLSI, (2003). A loopful of pus samples were streaked on blood agar base enriched with 7% horse blood (Oxoid, UK) using the quadrant streaking method. Blood agar plate was incubated aerobically at 37°C for 24 - 48 hours. The plates were examined for gross colony morphology, pigmentation and haemolytic characteristics after 24 - 48 hours. Discrete, pure colonies of isolates were selected and subcultured on nutrient agar (Oxoid, UK) and incubated aerobically at 37°C for 24 hours. After incubation on nutrient agar, bacterial isolates were identified on the basis of their

Gram reaction, microscopic and macroscopic characteristics as well as catalase and oxidase reactions. Biochemical tests were carried out using Microbact® 24E (*Enterobacteriaceae* and miscellaneous Gram-negative bacilli) Identification kits.

Although general purpose media which are acceptable were used for culture in this report, availability of selective media like Difco *Aeromonas* medium (BD, Becton, Dickinson and Company Sparks, USA) and Oxoid *Aeromonas* ampicillin medium (Oxoid, CM 833, SR136) would have been more preferable. Also where available, speciations of the genus are better done by the 16S rDNA-RFLP method (Figueras *et al.*, 2000).

Antimicrobial resistance pattern test: Antimicrobial susceptibility test was conducted on the pure isolates. The isolates were tested using the Kirby-Bauer disk diffusion method (Quinn *et al.*, 1994; CLSI, 2003) on 16 antimicrobials. The inhibition zone was recorded as the diameter of the zone surrounding the individual disk in which bacterial growth was absent. Based on this, the isolates were classified as resistant or susceptible, according to the guidelines of the CLSI (2003).

Results

Pure isolates from the pus culture yielded Gram-negative rods that were catalase positive, aerobic and grew on blood agar. On sheep blood agar, colonies were convex, smooth with entire edge after incubation for 24 hours. The isolates were identified as *Aeromonas hydrophila* on the basis of Gram reaction, macroscopic and microscopic characterization as well as various metabolic characteristics using Microbact® 24E (*Enterobacteriaceae* and miscellaneous Gram-negative bacilli) Identification kits (Table 1). The organisms

were identified with the aid of Microbact Bacteria Identification software, 2003 (Oxoid, UK).

The pure isolates tested were resistant to Gentamycin (GN, 10 µg), Colistin (CT, 10 µg), Erythromycin (E, 15 µg), Amoxycillin (AML, 10 µg), Streptomycin (S, 10 µg), Chloramphenicol (C, 30 µg), Ceftriaxone (CRO, 30 µg) Cefuroxime, Penicillin, Sulphamethazole and Tetracycline. However, isolates were susceptible to Ciprofloxacin (CIP, 10 µg), Pefloxacin (PEF, 5 µg), Ofloxacin (OFX, 5 µg), Enrofloxacin (5 µg) and Furazolidone (Table 2).

Treatment and clinical outcome: The only organism that was cultured from the wound swab was *Aeromonas hydrophila*. When the animal was placed on Enrofloxacin for seven days, the swelling regressed around the fetlock, no pus was expressed from the wound site and the animal was able to bear weight on the limb. No pain was elicited from the joint on application of pressure by the day 18 post treatment.

Discussion

Aeromonas hydrophila is increasingly recognized as an emerging opportunistic pathogen of clinical relevance (Tichoniuk *et al.*, 2010). This bacterium is motile by polar flagella and very toxic to many organisms because of its structure. When it enters the body of its victim, it travels through the bloodstream to the first available organ. It produces Aerolysin Cytotoxic Enterotoxin (ACT), a toxin that can cause tissue damage (Ormen and Ostensviki, 2001). The risk of infection due to traumatic wound depends on several factors which include the extent of potential contamination, the contaminating dose of bacterial cells, and their virulence. Also the physiological and physical nature of the wound (i.e. area of necrosis, poor blood as oxygen supply) will also affect the risk of

Table 1. Some phenotypic tests and results of isolates

| Phenotypic tests | Results |
|------------------|---------|
| Oxidase | + |
| Catalase | + |
| Nitrate | + |
| Lysozyme | + |
| Oxidation | - |
| Glucose | + |
| Mannitol | - |
| Xylose | - |
| D-galactosidase | - |
| Indole | - |
| Urease | + |
| V-P | - |
| Citrate | - |
| TDA | + |
| Gelatin | + |
| Maltose | - |
| Inositol | - |
| Sorbitol | - |
| Rhamnose | + |
| Sucrose | - |
| Lactose | - |
| Arabinose | - |
| Adonitol | - |
| Raffinose | - |
| Salicin | - |
| Arginine | + |

V-P = Voges-Proskauer

TDA = Tryptophan Deaminase

infection (Pommerville, 2010).

A major concern regarding *Aeromonas* infections is that they may mimic streptococcal or staphylococcal soft-tissue infections, because the organisms are potentially highly pathogenic and are resistant to penicillin, ampicillin, tetracycline, amoxicillin, carbenicillin and cephadrine, but sensitive to gentamycin, chloramphenicol and neomycin, consequently, the standard empirical antibiotic therapies for wound infections are ineffective against *Aeromonas* infection (Valley et al., 2004).

Previous studies on the susceptibility of clinical isolates of *Aeromonas* to

antimicrobials in Taiwan concluded that these pathogens are susceptible to certain cephalosporins (e.g. maxalactam, ceftazidime, and cefepime), aminoglycosides (e.g. amikacin), aztreonam, imipenem, and quinolones and are therefore reasonable choices for empirical treatment (Ko WC et al., 1996). Also in 2009, a report in France recommended that cephalosporin or a quinolone be prescribed along with an aminoglycoside in cases of severe *Aeromonas* infection (Lamy et al., 2009). Hence, the observation of emergence of resistance of *Aeromonas* to aminoglycosides (gentamycin), cephalosporins (Ceftriaxone, Cefuroxime) and other commonly used synergistic drug combinations (penicillin, streptomycin), broad spectrum drugs (tetracycline) in this study and other previous reports is of significance to clinicians in their choice of drugs for the empirical treatment of *Aeromonas* infection. However, further studies are needed to understand the mechanisms by which these organisms acquire resistance traits.

The high resistance of *Aeromonas* observed in this report could be due to easy accessibility of most antibiotics (tetracycline, streptomycin, penicillin, etc) as over-the-counter drugs, sub-optimal usage by unqualified persons and the licensing of some as growth promoters which ultimately result in abuse thereby encouraging emergence of resistance.

The pathogenicity of *Aeromonas* infections appears to be due to the action of several extracellular toxins that result in a very short incubation period and rapid progression of infection. Consequently, delays in the administration of appropriate antibiotics to individuals infected with *Aeromonas* species increase the risk of serious sequelae, especially in those who

are immune-compromised. From this case report there is need to embark on immediate initiation of combination of antimicrobial treatment until susceptibility result becomes known in case of trap wound. Finally, there should be a reassessment of prescriptions as soon as sensitivity results are available.

Furthermore, in case of trapping injury it is expedient to alleviate pain in the animals. Many of the animals caught in traps especially steel, jaw leg hold traps suffer excruciating pain – because the trap tears the flesh, cut tendons and ligament and can also break bones. Injuries from these traps can be so severe that may even warrant amputation of the injured limbs. At times the injury may seem to be superficial, but with a hidden tunneling wound. Therefore apart from the analgesic drugs, anti-tetanus vaccine should also be administered when livestock or horse is involved.

Trapping has many adverse effects on non-target species. The adverse effects include

stress and pain, weight loss due to temporary limping, anxiety, physical exertion due to struggling (Marks *et al.*, 2004) and changes in hormones, enzymes and electrolyte pH. Trapped animals have increased levels of serum cortisol (Warburton *et al.*, 1999), leading to increased anxiety finally resulting to increased body temperature and increased heart rate (Kreeger *et al.*, 1990) and long term muscle damage (Duncan *et al.*, 1994).

Apart from the effects it has on the animals, the owners and livestock attendants are also adversely affected. The effects on these people involved include economic losses due to death, treatment or untimely culling of the affected animal, injury sustained while trying to set the animal free and psychological trauma of having to look for the missing animal. It is therefore expedient to control/restrict trapping to a given area, especially where livestock are kept in an extensive system.

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Table 2: Susceptibilities of *Aeromonas hydrophila* isolated from the pus to antibiotics tests

| S/N | Antibiotic | Result |
|-----|-----------------|-----------|
| 1 | Amoxicillin | Resistant |
| 2 | Ceftriaxone | Resistant |
| 3 | Cefuroxime | Resistant |
| 4 | Chloramphenicol | Resistant |
| 5 | Ciprofloxacin | Sensitive |
| 6 | Colistin | Resistant |
| 7 | Erythromycin | Resistant |
| 8 | Enrofloxacin | Sensitive |
| 9 | Furazolidone | Sensitive |
| 10 | Gentamycin | Resistant |
| 11 | Ofloxacin | Sensitive |
| 12 | Pefloxacin | Sensitive |
| 13 | Penicillin | Resistant |
| 14 | Streptomycin | Resistant |
| 15 | Sulphamethazole | Resistant |
| 16 | Tetracycline | Resistant |

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