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SPECTROPHOTOMETRIC DETERMINATION OF PROTEINS ASSOCIATED WITH VIRULENCE IN NIGERIAN STRAINS OF *AEROMONAS* SPECIES

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ABSTRACT
Twenty six Aeromonas isolates from fishes, poultry and humans in Zaria were quantified for total soluble proteins (enzymes) profiles in January, 2007 by spectrophotometric (Biuret) method. Isolates were grown on Brain Heart Infusion (BHI) broth, they were incubated at 37^{0}C and centrifuged at 1,000 g/dl using harous labofuge. The results indicated in poultry, virulent proteins were: A. hydrophila (3.58 g/dl) A. caviae (4.00 g/dl), A. salmonicida, (3.82 g/dl) and A. sobria (0.00 g/dl). In fish, the virulent proteins were: A. hydrophila (3.11 g/dl), A. sobria (4.63 g/dl), A. caviae (2.95 g/dl) and A. salmonicida (2.74 g). In humans, the virulent proteins were: A. hydrophila (4.07 g/dl), A. sobria (3.58 g/dl) and A. caviae (3.99 g/dl). These strains of Aeromonas species were known to produce pathogenic factors which could be involved in aeromoniasis.

Key words: Quantification, Soluble proteins, Aeromonas

INTRODUCTION

Aeromonads are heterogeneous groups of bacteria of pathogenic significance infecting humans, aquatic, (reptiles, frogs, fishes) terrestrial and arboreal animals (Villari *et al*., 2000). In humans the organism causes intestinal symptoms (diarrheic) and extra-intestinal symptoms such as meningitis, endocarditis and osteomyelitis (Zhang *et al*., 2002). Several soluble proteins could be involved in virulence and in *Aeromonas* pathogenicity. The include: aerolysins, hemolysins, enterotoxins, proteases, lipases, multidrug-resistance proteins, histone-like proteins, ribonucleases, tween 80 esterases and deoxyribonucleases, (Chacon *et al.,* 2003). Virulence factors of *Aeromonas* organisms are associated with structural components of the bacteria cell and exotoxins that are secreted during bacteria metabolism (Dean *et al.,* 1998). These genes were known to be associated with the *ast* gene wich codifies for a heat stable enterotoxin and the A*lt* gene that codifies for a heat labile enterotoxin (Chopra *et al.,* 2000).

To the best of our knowledge, there is no work done in Nigeria on *Aeromonas* proteins with the view of quantifying its virulence factors to enable better understanding of the molecular basis for enzymatic catalysis and the mechanism controlling the functions of these proteins. This research employs spectophotometric (Biuret) method to determine proteins associated with virulence in Nigerian strains of *Aeromonas* species. This will explain possible reasons for bacterial virulence and a better understanding of their pathogenic significance.

METHODOLOGY

Twenty six (26) strains of *Aeromonas* sourced from the bacterial zoonoses Laboratory of Ahmadu Bello University(ABU) Zaria were used for this study in January, 2007. Determination of total protein concentration in the *Aeromonas* organisms was carried out using Biuret method as described by Esievo and Saror (1992). The isolates were grown overnight in Brain Heart Infusion (BHI) broth. After incubation at 37^{0}C for 24hrs and later centrifuged at 10,000 g for 5 minutes using harous labofuge (Jenway® 640, UV/vis, USA). Aliquots (0.5ml) of supernatant was dispensed in 10 ml capacity pyrex test tubes (BDH Laboratories) and 0.2 ml of Biuret reagent was added to it. The mixture was agitated by shaking to apparent homogeneity and incubated at 37^{0}C for 30 minutes. Thereafter, the automated spectrophotometer (Jenway® 640, UV/vis, USA) was calibrated and the absorbance was measured at 570 nm.

Blank was set in parallel and was prepared by adding of distilled water0.5cm^3 and Biuret 2.0cm^3 reagent, without the experimental sample and was incubated at room temperature for 30 minutes under the same conditions described earlier, The blank was used to adjust (zero) the spectrophotometer before readings were taken. A control tube was prepared by adding BHI 0.5cm^3 (without organisms) and added to of biuret reagent (2.0cm^3) and the spectrophotometer reading taken at 570 nm. The difference in spectrophotometric readings of broth culture without organism was taken from the difference of colorimetric readings of broth culture with organisms. Protein values (mg/ml) were estimated using a standard curve earlier plotted from known concentration and absorbance of a standard protein Bovine Serum Albumin (BSA) (Figure 1). A chart was deduced from the values of protein concentration of the *Aeromonas* species (Figure 2).

RESULTS

Figure 1 shows the standard curve for *Aeromonas* proteins subjected to analysis. The slope was taken from y = 0.0476x. The curve is constructed when the protein concentration was plotted against absorbance. Extracted and quantified *Aeromonas* protein by Biuret method revealed high protein concentration of 4.63 g/dl from *A. sobria* from fish and of 4.063 g/dl of *A. hydrophila* from humans. No protein concentrations were recorded for *A. sobria* from poultry. Others had relatively lower *Aeromonas* protein concentration (Figure 2).

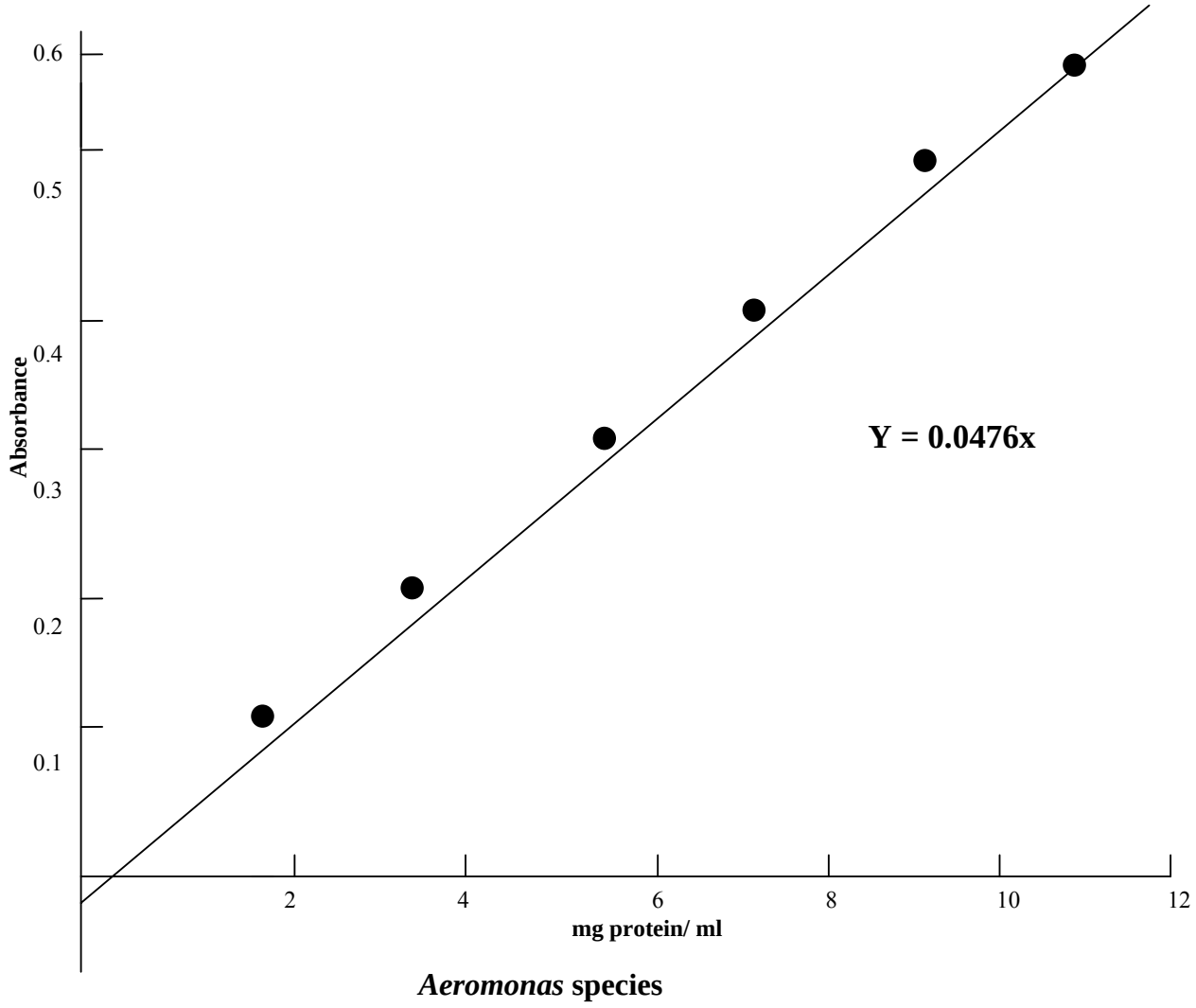

Figure 1: Standard Curve for Protein estimation of *Aeromonas* species

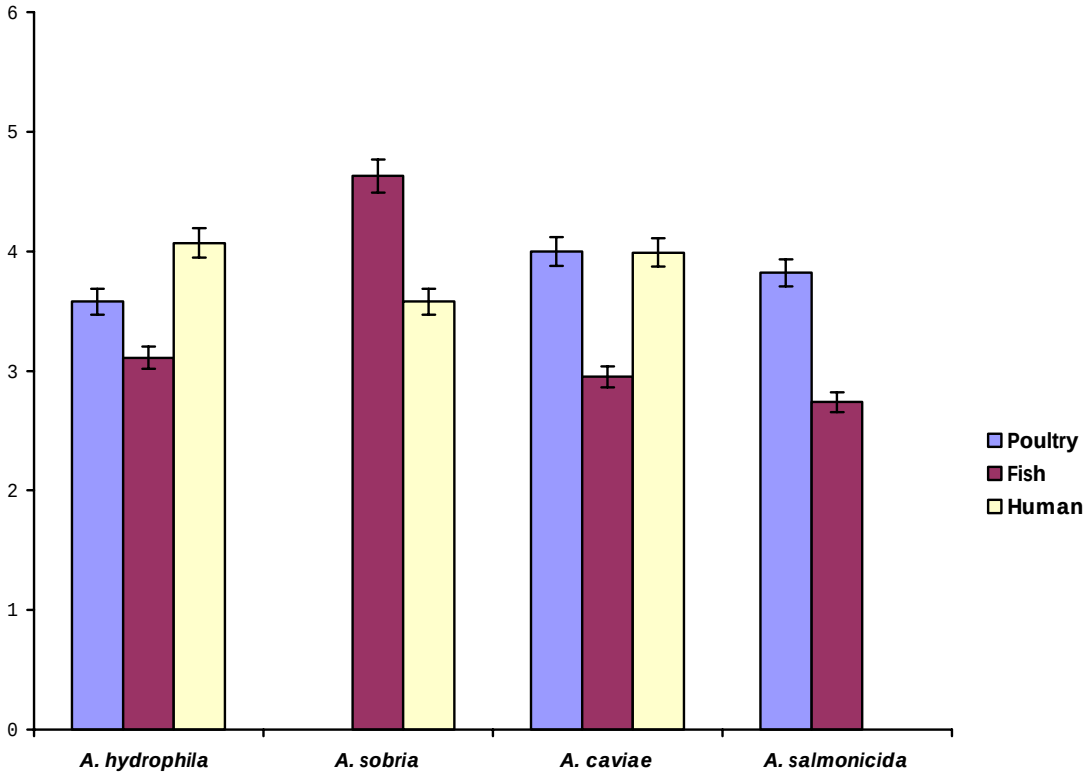

Fig 2 Chart showing average protein concentration of species of *Aeromonas* isolated from fishes, poultry and man.

DISCUSSION

The average amount of protein concentration per gram of cells was determined for the 26 isolates of *Aeromonas* species using spectrophotometric analysis. The chart showed the total amount of protein. It could be deduced (Fig 2) that the highest protein concentration was in *Aeromonas sobria* from fish or poultry, this may be indicated for a particular fish or poultry disease or other pathological lesions caused by *A. sobria* as reporteded by Cipriano and Bullock (2001). Moreso, protein quantity is a reflection of synthesis of proteins and these proteins are expressed by the DNA which could entail that there may be mutation leading to renewed synthesis of new proteins to cope with the adverse effects of the environment. The lower protein in *A. salmonicida* in fish may be associated with lower virulence and may be indicated for the lower outbreaks of fish diseases in some of our cultured ponds (Okpokwasili and Ogbulie, 2001). Moreso, common fish carriers of *A.salmonicida* such as *Salmo salar* are scarce in our environmental water which could serve as definitive host that may aid dissemination of *Aeromonas salmonicida* (furunculosis).

The presence of moderately high amount of protein in *A.hydrophila* from man may be responsible for the source of common gastrointestinal ailments and diarrhea associated problems in humans (Bechet and Blondeau, 2003). Thus further attesting to the likelihood of the proteins playing a crucial role in pathogenicity. Appropriate measures need to be put in place to control and destroy these organisms. Lower concentrations of the proteins may be associated in lower pathogenicity recorded in some areas.

CONCLUSION AND RECOMMENDATIONS

From this study we were able to demonstrate soluble proteins responsible for virulence in *Aeromonas* species by spectrophotometric (Biuret method). Further studies may be carried out to determine types of proteins and evaluate the virulence of these proteins in mice, guinea pigs, or other animal species in order to produce effective *Aeromonas* vaccine for use in Nigeria.

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