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Short Communication

QUANTIFICATION OF TOTAL SOLUBLE PROTEIN CONCENTRATION IN *AEROMONAS* SPECIES BY SPECTROPHOTOMETRIC METHODS

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INTRODUCTION

***Aeromonas* isolates from fishes, poultry and humans in Zaria were quantified for total soluble proteins (enzymes) profiling using spectrophotometric (Biuret) method. The results indicated that in poultry, virulent proteins were: *A. hydrophila* (3.58 g) *A. caviae* (4.00 g), *A. salmonicida*, (3.82 g) and *A. sobria* (0.00 g). In fish, the virulent proteins were: *A. hydrophila* (3.11 g), *A. sobria* (4.63 g), *A. caviae* (2.95 g) and *A. salmonicida* (2.74 g). In humans, the virulent proteins were: *A. hydrophila* (4.07 g), *A. sobria* (3.58 g) and *A. caviae* (3.99 g). Aeromonads are heterogeneous groups of bacteria of pathogenic significance. They infect humans, aquatic (reptiles, frogs, fishes), terrestrial and arboreal animals (Villari *et al.*, 2000). In humans the organism is known to cause intestinal symptoms (diarrhoea) and extra-intestinal symptoms, such as meningitis, endocarditis and osteomyelitis (Zhang *et al.*, 2002). Several soluble proteins which are responsible for virulence have been associated with *Aeromonas* pathogenicity. These includes: aerolysins, hemolysins, enterotoxins, proteases, lipases, multidrug-resistance proteins, histone-like proteins, ribonucleases, tween 80 esterases and deoxyribonucleases (Chacon *et al.*, 2003). These 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). A study by Chacon *et al.* (2003) has revealed a significant statistical association between *Ast* and *Alt* genes. These genes were known to be associated with the *Ast* gene which codifies for a heat stable enterotoxin and the *Alt* gene that codifies for a heat labile enterotoxin. To the best of our knowledge, there is little work done in Nigeria on soluble *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 was conducted in order to quantify the proteins (enzymes) by spectrophotometric (Biuret) method so as to identify virulent markers in *Aeromonas* species. This will explain possible reasons for bacterial virulence and a better understanding of their pathogenic significance.**

KEY WORDS: Quantification, Soluble proteins, *Aeromonas*, Biuret method

MATERIALS AND METHODS

Twenty-six strains of *Aeromonas* was sourced from the bacterial zoonoses Laboratory of Ahmadu Bello University (ABU) Zaria was used for this study. Determination of total protein concentration in the *Aeromonas* organisms was carried out using the Biuret method as described by Esievo and Saror (1992). The isolates were grown overnight in Brain Heart Infusion (BHI) broth. After incubation at 37°C for 24hrs, it was centrifuged at 10,000 g for 5 minutes using Harous Labofuge (Jenway® 640, UV/vis, USA). Aliquots (0.5ml) of supernatant were 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 27°C for 30 minutes. Thereafter, was calibrated and the absorbance was measured

at 570 nm using a spectrophotometer (Jenway® 640, UV/vis, USA).

A blank was set in parallel and was prepared by adding 0.5ml of distilled water and 2.0 ml of Biuret 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 0.5 ml of BHI (without organisms) to 2.0 ml of Biuret reagents and the absorbance reading taken at 570 nm. The difference in absorbance readings of broth culture without organism is taken from the difference of absorbance readings of broth culture with organisms. The readings of protein values (mg/ml) were estimated using a standard curve earlier plotted from the known concentration and absorbance of a standard protein Bovine Serum Albumin (BSA). A chart was deduced from the values of protein concentration of the *Aeromonas* species.

RESULTS AND DISCUSSION

Table I contained the various *Aeromonas* species isolated from fishes, poultry and humans that were subjected to protein analysis. The mean value of protein concentration per gram of cells was determined for the 26 isolates of *Aeromonas* species using spectrophotometric analysis with slope indicating y=0.047x (Fig. 1). The chart in Fig. 2 was extrapolated and it showed the total amount of protein and the output of protein expression of the *Aeromonas* species involved in pathogenicity. The results of the chat showing extracted and quantified *Aeromonas* proteins revealed high protein concentration of 4.63 g from *A. sobria* isolated from the fish and 4.063 g from *A. hydrophila* from humans. No protein was recorded for *A. sobria* from poultry. A lower protein concentration was recorded for *A. salmonicida* from fish and non from humans.

TABLE I: Various Isolates of *Aeromonas* Species Subjected to Spectrophotometric Profiling

S/N	Sample	Original
1	C138	*A.hydrophila*
2	T3	*A. salmonicida*
3	ZF87	*A. caviae*
4	HTH67	*A. caviae*
5	HTH44	*A. caviae*
6	MS37	*A. sobria*
7	BC29	*A. salmonicida*
8	HTH41	*A. hydrophila*
9	CG18	*A. hydrophila*
10	T30	*A. caviae*
11	T22	*A. hydrophila*
12	MS37	*A. sobria*
13	HS7	*A. hydrophila*
14	RF59	*A. salmonicida*
15	ZF16	*A. hydrophila*
16	BC11	*A. salmonicida*
17	SM23	*A. sobria*
18	SM12	*A. hydrophila*
19	CM45	*A. hydrophila*
20	HS2	*A. caviae*
21	HTH25	*A. hydrophila*
22	CL18	*A. hydrophila*
23	HTH64	*A. hydrophila*
24	ZF53	*A. hydrophila*
25	HS37	*A. hydrophila*
26	ZF26	*A. hydrophila*

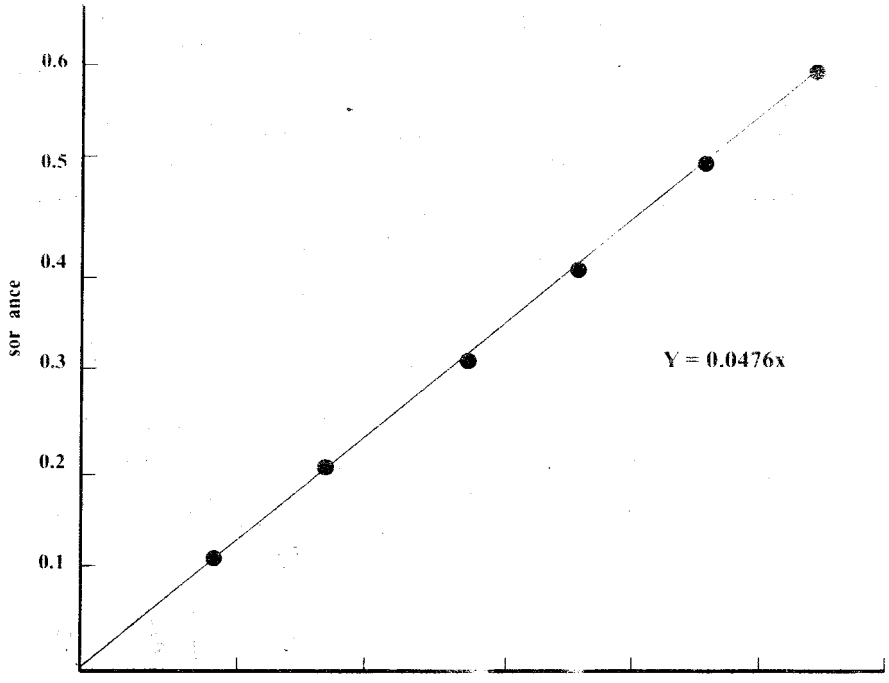

Fig. 1: Standard Curve for protein analysis of *Aeromonas* species

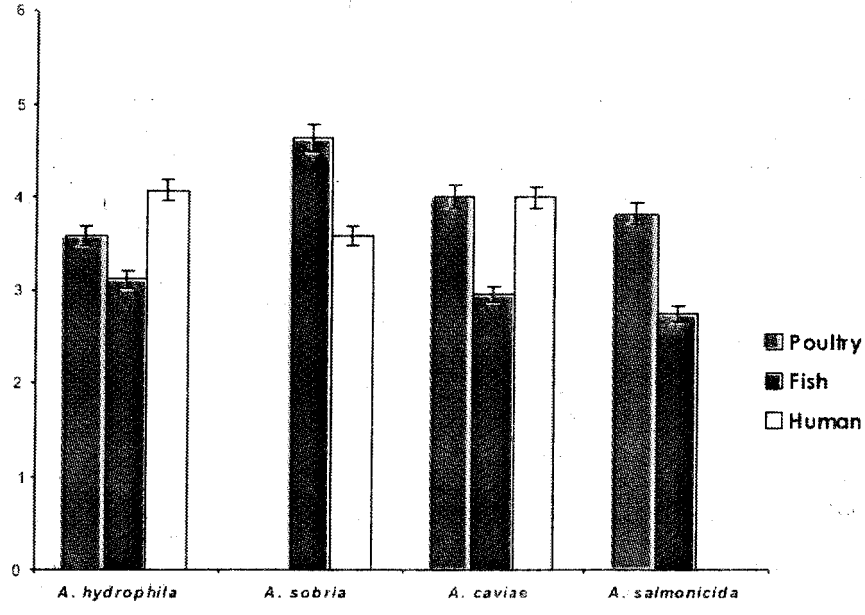

Figure 2: Chart showing average proteins of species of *Aeromonas* isolated fishes, poultry and man.

It could be deduced that the highest protein concentration came from *Aeromonas sobria* from the fish or poultry; this may be an indication for a particular fish or poultry disease or other pathological lesions caused by *A. sobria* as reported by Cipriano and Bullock (2001). More so, quantity of proteins is a reflection of synthesis of the 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 concentration in *A. salmonicida* isolated from the fish may be associated with lower virulence and may also be an indication for the lower outbreaks of fish diseases in some of our cultured ponds (Okpokwasili and Ogbulie, 2001). Furthermore, 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 concentration in *A. hydrophila* isolated from man may be responsible for the source of common gastrointestinal ailments and diarrhea associated problems in humans (Bechet and Blondeau, 2003). This further attests to the likelihood of the proteins playing a crucial role in the pathogenicity of aeromonads. Appropriate measures needs to be put in place to control and destroy these organisms. Lower concentrations of the proteins may be associated with lower pathogenicity recorded in some areas of aeromoniasis outbreak. This could be useful tool in ascertaining variations between strains of the same species.

CONCLUSION AND RECOMMENDATIONS

From this study, we were able to demonstrate soluble proteins responsible for virulence in *Aeromonas* species by spectrophotometric (Biuret method). This may form a basis for classification and identification of some strains within the genus *Aeromonas*. 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 understand the pathogenic role of proteins as virulence markers in aeromoniasis. This will help in the control of infections due to *Aeromonas* species.

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