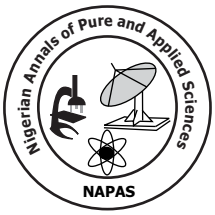

Effects of B. bacteriovorus (ATCC™ 1534) Injection on Some Serum Chemistry Parameters in Rats Injected with P. multocida

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Abstract

To determine effects of *Bdellovibrio bacteriovorus* on some serum chemistry parameters, and evaluate its ability to regulate *in vivo* damage and control pathogen activity, twelve Sprague Dawley rats were injected subcutaneously, once daily, with 1 x 10^8/ml *B. bacteriovorus* (ATCC™ 1534) in saline and some serum chemistry parameters measured. Another set of 12 Sprague Dawley rats were also injected once daily with 10^8/ml of the pathogen *P. multocida*. Further, 12 other rats were injected with 1 x 10^8/ml each of *B. bacteriovorus* and then with *P. multocida*. All injections were for 168 hours. A group of 12 rats were injected intramuscularly once, at collection of blood samples, with 2 mg/kg of Ketamine Hydrochloride, used as anaesthesia. A final group of 12 rats, not injected with any of the bacteria or anaesthetic served as controls. Blood was collected from all rats for analysis by cardiac puncture. Though there were instances when serum chemistry concentrations were higher on injections with both *B. bacteriovorus* and *P. multocida*, compared with rats injected with only *P. multocida* or the controls, findings showed that *B. bacteriovorus* injected into rats which had previously been injected with *P. multocida* led to lower levels of alanine aminotransferase, aspartate aminotransferase and creatinine when compared with concentrations in rats injected with only *P. multocida*. Mortality was reduced by 88% in rats injected with *P. multocida* and *B. bacteriovorus* compared with those injected with only *P. multocida.* It is concluded that *B. bacteriovorus* effectively mitigated damage caused by *P. multocida* in rats.

Key words: *B. bacteriovorus* (ATCC™ 1534), *P. multocida*, Alanine, Aspartate, Creatinine, Rats, *in vivo*

Introduction

Bdellovibrio are bacteria only relative recently discovered. They are Gram-negative, tiny vibroids measuring between 0.3 - 0.5μm by 1.4 - 2.5 μm in size. They exhibit motility by polar flagella which are single and sheathed with dampened waveforms, characteristic of the genus. The interest about them is in their predatory nature, as they attack and prey on other Gram-negative bacteria. They have an array of degradative enzymes, with which they create pores in the host cell walls and access the periplasm. They use their prey's cytoplasmic contents as nutrients for growth and reproduction, and finally burst the host cell envelopes, eventually causing host cell deaths (Kilpatrick, 1999).

In addition, this organism can be used as *in vivo* living controls of many pathogenic Gram-negative microorganisms and inspiration for design of antimicrobial agents (Lambert *et al.* 2006). Sockett and Carey, (2004) predict that in the near future, *Bdellovibrio* may have antimicrobial therapeutic applications in medical science. This is because they prey on a wide variety of Gram-negative hosts, possess highly desirable qualities of a good microbial control agent, and also bacteria do not offer resistance to them (Ferris and Clesceri, 1971; Koval and Bayer, 1997; Scholl *et al.,* 2005).

Materials and Methods

Experimental Procedure

Sixty, sixteen week old Sprague Dawley albino rats, kept at ambient temperature of about 25 ^{o}C (± 5 ^{o}C), relative humidity of 55% (± 5%), and supplied feed and water *ad libitum* were used.

They were treated in the following manner: a group of 12 rats were injected once, subcutaneously, every 24 hours, for 168 hours, with 1 ml of 1 x 10^{8}/ml *B. bacteriovorus* (ATCC 15364) to determine its effects on rats' serum chemistry parameters. To determine similar effects of pathogen, 12 rats were also subcutaneously injected once 24 hourly with *Pasteurella multocida* from the typed collection of the National Veterinary Research Institute (NVRI), Vom, Plateau State, for 168 hours. A control group of 12 rats was injected once, intra-muscularly, with 2 mg/kg of Ketamine Hydrochloride, and then bled, shortly after the drug took effect. A second control group was made up of 12 rats not injected with *P. multocida*, *B. bacteriovorus* or Ketamine Hydrochloride.

For *in vivo* effects of *B. bacteriovorus* on pathogen, another set of 12 rats were subcutaneously injected with *P. multocida* followed by injection with 1 ml (1 x 10^{8} PFU/ml) *B. bacteriovorus* (ATCC 1534) suspension once daily for 168 hours. After this period of treatment, each group of rats were also bled. Fur over the sternum area overlying the heart was thoroughly cleaned with tincture of iodine, and one millilitre of blood withdrawn by cardiac puncture.

Before collection of blood samples, all experimental rats were observed for pathological, physiological and physical signs of disease, including mortality.

Determination of Bacterial Pathogenicity

Test bacterium was serially diluted in physiological saline. The count of bacteria in each dilution was determined using McFarland's standard. Six animals each were subcutaneously injected with the dilutions of inocula. Mortality was considered a sign of infection. Live animals were sacrificed at 168 hours for gross anatomical lesions/histology, and the LD_{50} calculated according to the method of Reed-Muench (Saganuwan, 2015).

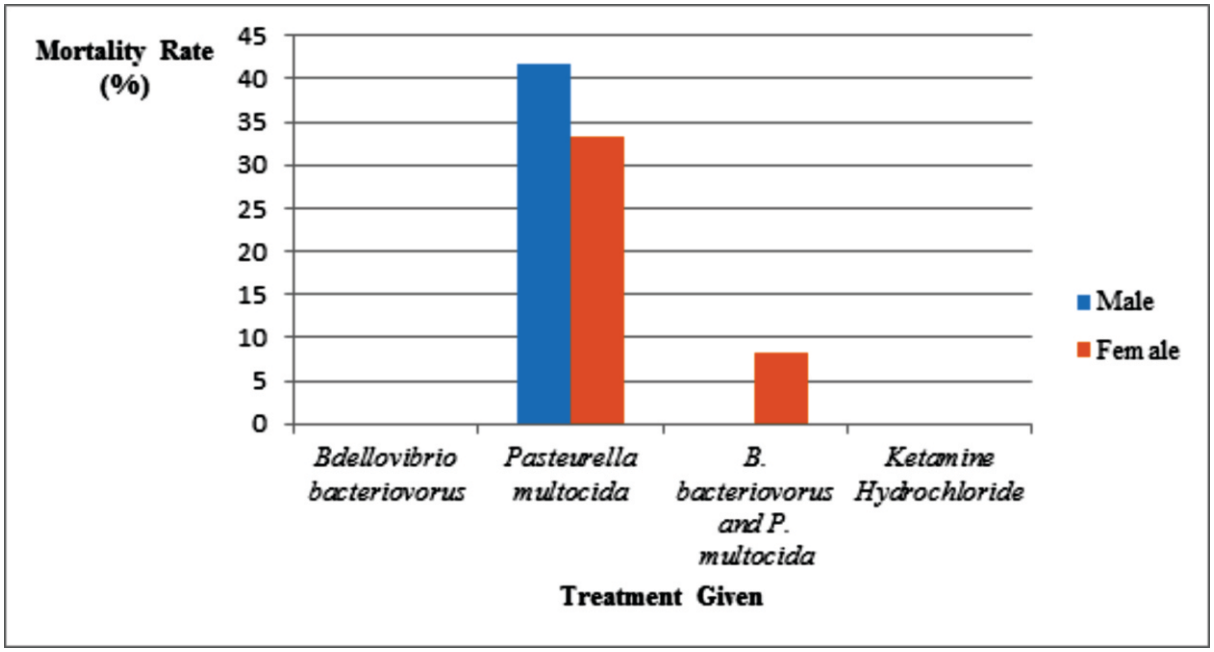
Determination of Serum Chemistry Values

Blood serum chemistry values were determined automatically using the Cobas® 111 (Asia Biomedicals - India) Auto-analyser system. Serum chemistry indices measured were creatinine (CRT) concentration, Aspartate aminotransferase (AST) concentration and Alanine aminotransferase (ALT) concentration.

Results

Mortality Rates

Nine rats (5 male and 4 female), totaling a mortality of rate 75% was recorded in rats challenged with *P. multocida.* In rats injected with both *P. multocida* and *B. bacteriovorus,* 1 (8.3%) female rat died (Figure 1). After injecting rats with both *P. multocida* and *B. bacteriovorus*, the drop in mortality rate was 88.8% when compared with rats injected with *P. multocida*.



Alanine aminotransferase (ALT) measurements

ALT values in *B. bacteriovorus* injected rats remained unchanged (72 U/L) after 168 hours of once daily injection. However, ALT concentration was higher than in *P. multocida* injected rats, which was 70 U/L. ALT concentration was even lower (45.6 U/L) in rats injected with both *P. multocida* and *B. bacteriovorus* (Table 1).

Statistically, *post hoc* analysis showed ALT concentration differed significantly between rats injected with *B. bacteriovorus* and un-injected controls. Also, ALT concentration differed significantly between rats injected with *P. multocida,* and those injected with both *P. multocida and B. bacteriovorus*.

Table 1: ALT Concentration in Rats Injected with *B. bacteriovorus*, *P. multocida* and with *B. bacteriovorus* and *P. multocida*

	Treatment Given				
Organism/substance Injected *(Group)*	Value *(U/L)(a)*	*B. bacteriovorus* and *P. multocida*	Value *(U/L) (b)*	Difference *(U/L) (a-b)*	(Percent Change)
B. bacteriovorus	72.00		72.00	0	-
P. multocida	70.00	-	45.60	24.40	(34.9)
Ketamine Hydrochloride	94.40	*B. bacteriovorus* and *P. multocida*	94.40	0	-

Key: *N* = 12 for each group; - = Not Applicable; Control rats ALT value = 27 U/L

Aspartate Aminotransferase (AST) measurement

In *B. bacteriovorus* injected rats, AST concentration also remained unchanged, (231 U/L), throughout the period of experiment. However, rats injected with *P. multocida* had higher AST concentration, (347 U/L), than rats injected with *B. bacteriovorus* and with both *B. bacteriovorus* and *P. multocida*, (165. 3 U/L). All values were however higher than control rats'.

Multiple comparisons of AST concentration showed significant differences between rats injected with *B. bacteriovorus* and un-injected controls. Also, AST concentrations differed significantly between rats injected with *P. multocida* only and those injected with both *P. multocida* and *B. bacteriovorus.*

Table 2: AST Concentration in Rats Injected with *B. bacteriovorus*, *P. multocida* and with *B. bacteriovorus* and *P. multocida*

Treatment Given					
Organism/substance Injected*(Group)*	Value *(U/L) (a)*	*B. bacteriovorus + P. multocida*	Value *(U/L) (b)*	Difference *(U/L) (a-b)*	(Percent Change)
B. bacteriovorus	231.00	-	231.00	0	-
P. multocida	347.00	*B. bacteriovorus* and *P. multocida*	165.30	181.70	(52.40)
Ketamine Hydrochloride	336.70	-	336.70	0	-

Key: *N* = 12 for each group; - = Not Applicable; Control Rats AST value = 104 U/L

Creatinine (CRT) measurement

CRT concentration remained unchanged, (1 mg/dL), in all rats inoculated with *B. bacteriovorus,* which were however higher than concentrations in rats injected with *P. multocida*, (0. 50 mg/dL) and those injected with both *P. multocida* and *B. bacteriovorus*, (0. 46 mg/dL) (Table 3). In all cases, CRT concentrations were higher than controls.

Post hoc multiple comparisons showed that CRT differed significantly between *B. bacteriovorus* inoculated rats and un-injected control rats. Similarly, CRT concentrations differed significantly between all rats injected with *P. multocida* and control rats not injected. In Ketamine Hydrochloride injected controls, CRT concentrations were also significantly different from the un-injected rats.

Table 3: CRT Concentration in Rats Injected with *B. bacteriovorus*, *P. multocida* and with *B. bacteriovorus* and *P. multocida*

S/no		Treatment Given				
	Organism/substance Injected *(Groups)*	Valu e *(mg/dL)(a)*	*B. bacteriovorus* and *P. multocida*	Value *(mg/dL) (b)*	Difference *(mg/dL) (a-b)*	(Percent Change)
1.	*B. bacteriovorus*	1.00	-	1.00	0	-
5.	*P. multocida*	0.50	*B. bacteriovorus* and *P. multocida*	0.46	0.04	(8.00)
7.	Ketamine Hydrochloride	0.82	-	0.82	0	-

Key: *N* = 12 for each group; - = Not Applicable; Control rats CRT value = 0. 4 mg/dL

Discussion

The high drop in mortality rate by 88.0% on injecting rats with both *B. bacteriovorus* and *P. multocida* signalled the great moderation effect of the predator on pathogen. A similar outcome was observed by Willis *et al.* (2016) who injected Zebrafish larvae with *Shigella flexneri* alongside *B. bacteriovorus* and found effective *in vivo* predation in Zebrafish on *S. flexneri* by *Bdellovibrio*, which led to reduced pathogen concentration and a drop in host mortality.

AST, CRT and ALT concentrations in experimental rats injected with only *B. bacteriovorus* were all higher than control values. While the specific mechanism that led to this observation is unclear, it has been documented that *B. bacteriovorus* has no significant or harmful effect on eukaryotic animal cells and tissues (Tuyul, 2006). It is therefore unlikely that this could been as a result of damage to rats' tissues or organs.

The decrease in AST concentration after injecting rats with both *Bdellovibrio* and *P. multocida,* in contrast to the higher AST levels when only *P. multocida* was injected suggests that *B. bacteriovorus* may have lessened the pathogenicity of *P. multocida*. According to Nagy (1984) and Moss *et al*. (1987), AST is widely distributed in hepatic, cardiac, kidney and muscle tissue, with high serum levels found in diseases of these tissues. It is possible that *Bdellovibrio* may have effectively predated *in vivo* on *P. multocida* and reduced its population to levels that they were unable to cause great damage to these tissues of the rats. Attesting to the feasibility of this, Hobley *et al*. (2006) demonstrated by mathematical modelling that *Bdellovibrio* is capable of effective *in vivo* predation in the presence of cellular debris and decoys within living systems.

Regarding the lower serum ALT concentrations in rats injected with both *Bdellovibrio* and *P. multocida* compared with those injected with only *P. multocida,* Moss *et al.* (1987) reported that ALT concentration predicts

disease and degeneration in tissues, and that ALT being a liver-specific enzyme is therefore used in assessing liver integrity and function during disease. Given that it has been previously determined by Cheville and Rimler (1989) that subcutaneously injecting a protein from cultures of *P. multocida* (Type D) caused acute and chronic hepatic toxicity in rats, it could be reasoned that the lower ALT levels in rats injected with both *B. bacteriovorus* and *P. multocida* may have been due to its control by *Bdellovibrio* inoculation.

The finding that all rats injected with both *B. bacteriovorus* and *P. multocida* had consistently lower values of creatinine concentrations compared with values from rats injected with only *P. multocida,* was a highly positive outcome as Dominick and Rimler (1986) and Riischoff *et al*. (1987) had previously determined that *P. multocida* toxins produce significant damage to liver and kidneys of rats. Also according to Lamb *et al*. (2005), Lamb *et al*. (2006) and Miller (2009), the assay of creatinine in serum or plasma is the most commonly used test to assess renal function as a rise in blood creatinine levels indicates marked damage of the nephrons.

Therefore, highly lowered levels of this indicator in rats injected with both *Bdellovibrio* and *P. multocida*, by comparison higher in rats injected with only *P. multocida*, leads to the conclusion that given the similar treatments received by all rats, the only difference(s) being the injections of *Bdellovibrio*, any effect (positive or negative) must have been due to its presence.

Despite the foregoing, a pertinent question would be, if *Bdellovibrio* inoculations had such desirable impact on *P. multocida*, why then were there higher levels of ALT, AST and CRT concentrations in some rats injected with both *B. Bacteriovorus* and *P. multocida*, as opposed to those injected with only pathogen, signifying higher levels of tissue damage in the former?

Roth *et al*. (2003**)** reported that every treatment regimen, even the very well established and extensively researched protocols, often has idiosyncratic cases and occurrences, where such treatments may not be as effective as envisioned, result in unexpected outcomes or may even fail. It can be pointed out that even when average concentrations of serum chemistry indices were found to be higher in rats injected with *Bdellovibrio* and *P. multocida*, some individual rats still had lower concentrations than found in the *P. multocida*-only injected rats. Thus the differences in certain serum chemistry parameters may have been due to variations in individual rat physiologies.

Conclusion

It was found that *B. bacteriovorus* had overall positive impact on adverse pathogen effect on serum chemistry indices in the rat animal model. It is hoped that as more is discovered on the potential of this unique bacterium and its yet untapped potential, soon it could be used as a viable and safe alternative to antibiotic use in human and veterinary medical practice.

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