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Test of Randomness of Residuals for the Four-parameter Logistic model used in Obtaining the IC_{50} Value for *Allivum sativum* Methanolic Extract Against *Aeromonas hydrophila*

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ABSTRACT

Numerous publications ignore statistical diagnosing of the nonlinear model utilized, and the data might be nonrandom- an essential necessity for all of the parametric statistical evaluation approaches. In cases where the diagnostic tests demonstrate that the residuals reveal a pattern, then a variety of remedies for example nonparametric analysis or shifting to another model should cure the problem. The subject of this study is test for the randomness of the residual for the Four-parameter Logistic model used in obtaining the IC_{50} Value for *Allivum sativum* methanolic extract against *Aeromonas hydrophila* using the Wald–Wolfowitz runs test. The result shows that the number of runs was 10, the expected number of runs under the assumption of randomness was 5.8, indicating the series of residuals had adequate runs. As the p-value was greater than 0.05, the null hypothesis is not rejected demonstrating no substantial evidence that the residuals were nonrandom, and the residuals represent noise.

INTRODUCTION

Just about the most harmful fish pathogenic agents is *Aeromonas hydrophila*, a known reason behind motile aeromonad septicaemia (MAS) in numerous stream fishes as well as being thought to be caused by method of accidental scratches [1–5]. Instances of this ailment especially on fish species are documented in certain great deal places from the United states of America to south East Asia including Indonesia [6]. Species of fish influenced by the bacteria are numerous and include hybrid striped bass, channel cat fish, Goldfish (*Carassius auratus*), Tilapia (*Tilapia nilotica*), Snakehead fish (*Ophiocephalus striatus*), American eel (*Anguilla rostrata*), Carp (*Cyprinus carpio*), Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (*Oncorhynchus mykiss*) amongst them [7–11]. The bacterium is a Gram-negative rod-shaped and comes from the family Aeromonadaceae. It comes with a great specific polar flagellum that is unbelievably motile, plus it's found in diverse environment such as soil, in sewer, and also in brackish water. The bacterial virulence components consist of its capability to produce a number of tandem-like invasion on the bacterial system, which includes adhesions, the development of cytotoxins, enzymes like lipases, and the continuing development of a dense biofilm [12–15].

A previous study shows the inhibition of the bacterium *Aeromonas hydrophila* using solvent extracts from *Salvia officinalis* [16]. A nonlinear regression exercise using the four-parameter logistics equation gave the IC_{50} value of 21.92 mg/mL (95% confidence interval from 20.86 to 23.03). The method of mathematically fitting nonlinear curve using the ordinary least squares method relies heavily on the residuals for the curve to be normally distributed of equal variance (homoscedastic), and does not show autocorrelation [17–19]. Aside from this, an important consideration that has not been highlighted enough is that the residuals must be random. In order for randomness to be met we perform the Wald–Wolfowitz runs test [20] statistical diagnosis tests. The subject of this study is test for the randomness of the residual for the Four-parameter Logistic model used in obtaining the IC_{50} Value for *Allivum sativum* methanolic extract against *Aeromonas hydrophila* [21].

METHODOLOGY

Acquisition of Data

Data were acquired from **Figure 2** from the works of [21]. Initial outcomes demonstrated that the residuals followed the normally distribution.

Runs test

The runs test [22] was performed to the residuals of the regression in an effort to identify nonrandomness. This might identify an organized deviation of over or under estimation parts of the curve when utilizing a particular model [20]. The runs test compares the series of the residuals which are generally negative and positive. An excellent run is normally signified by an alternation or a balance number of the negative and positive residual values. The number of runs of sign is generally portrayed by means of a percentage of the maximum number feasible. The runs test computes the probability for the existence of way too many or an inadequate number of runs of sign. The existence of too many of a run sign might reveal the existence of negative serial correlation and the existence of too few runs might reveal a clustering of residuals with the exact same sign or the existence of systematic bias.

The test statistic is

H_0= the sequence was produced randomly
H_a= the sequence was not produced randomly

$$Z=\frac{R-\bar{R}}{sR} \qquad \textbf{(Eqn. 1)}$$

Where Z is the test statistic, $\bar{R}$ is the expected number of runs, R is the observed number of runs and sR is the standard deviation of the runs. The computation of the values of $\bar{R}$ and sR (n_1 is positive while n_2 is negative signs) is as follows;

$$\bar{R}=\frac{2n_1.n_2}{n_1+n_2}+1 \qquad \textbf{(Eqn. 2)}$$

$$s^2R=\frac{2n_1.n_2(2n_1.n_2-n_1-n_2)}{(n_1+n_2)^2(n_1+n_2-1)} \qquad \textbf{(Eqn. 3)}$$

As an example

Test statistic: $Z = 3.0$
Significance level: $\alpha = 0.05$
Critical value (upper tail): $Z_{1-\alpha/2} = 1.96$
Critical region: Reject H_0 if $|Z| > 1.96$
If the test statistic value (Z) is larger than the critical value, then a rejection of the null hypothesis at the 0.05 significance level is made hinting that the sequence was fashioned in a nonrandom manner.

RESULTS

Fitting of a statistical model may be clinically diagnosed precisely using assessments which use residuals. Residuals are the contrast between an expected and observed quantity value of a specific mathematical model. The general rule would be that a poor model will show a bigger difference between the predicted and observed values.

Runs test

From **Table 1**, the number of runs was 10, the expected number of runs under the assumption of randomness was 5.8, indicating the series of residuals had adequate runs. The z-value indicates how many standard errors the observed number of runs is below the expected number of runs, the corresponding p-value indicate how extreme this z-value is. The interpretation is the same as other o-values statistics. If the p-value is less than 0.05 then the null hypothesis that the residuals are indeed random can be rejected. Since the p-value was greater than 0.05, therefore the null hypothesis is not rejected indicating no convincing evidence of non-randomness of the residuals and they do represent noise.

Table 1. Runs test for randomness.

Runs test	Residual data set
observations	5
below mean	4
above mean	6
no of runs	10
E(R)	5.800
var(R)	2.027
stdev(R)	1.424
Z-value	-0.562
p-value	0.287

The runs test is an important tool to detect nonrandomness in nonlinear regression based on the residuals [22]. The runs test could detect systematic deviation of the curve such as over or under estimation of the sections when using a specific model. The runs test looks at the sequence of the residuals that are usually positive and negative. A good run is usually signified by alternating or a balance number of positive and negative residual values. The number of runs of sign is usually expressed in the form of a percentage of the maximum number possible [20].

In time-series regression models, the runs test is also utilized as a technique to test for the presence of autocorrelation. To be precise, simulation studies using Monte Carlo have shown that the runs test produces distinctly asymmetrical error rates in the two tails suggesting that the use of runs test for autocorrelation detection might not be robust and the Durbin-Watson method would be the method of choice to assess autocorrelation [23]. Previous similar studies based on looking at the randomness of the residuals justify the method use in this study. For instance the use of the Baranyi-Roberts model in fitting an algae growth curve which shows adequacy in the statistics [24]. the Buchanan-three-phase model used in the fitting the growth of *Paracoccus* sp. SKG on acetonitrile [25] and *Moraxella* sp. B on monobromoacetic acid (MBA) [26]. In the arena of biosorption, the residuals for the Sips and Freundlich models utilized in modelling the isotherm of lead (II) uptake by alginate gel bead were found to be adequate based on the runs test [27].

CONCLUSION

The subject of this study is test for the randomness of the residual for the Four-parameter Logistic model used in obtaining the IC_{50} Value for *Allivum sativum* methanolic extract against *Aeromonas hydrophila* using the Wald–Wolfowitz runs test. The result shows that the number of runs was 10, the expected number of runs under the assumption of randomness was 5.8, indicating the series of residuals had adequate runs. As the p-value was greater than 0.05, the null hypothesis is not rejected demonstrating no substantial evidence that the residuals were nonrandom, and the residuals represent noise.

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