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Experimental and Mathematical Model for the Study of the *In-vitro* Susceptibility of Antimicrobial Agents against *Escherichi coli* Isolates Obtained from Abattoir Centres in Ikorodu, Lagos, Nigeria

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Authors' contributions

This work received the collaboration of all authors. Author AAA designed the model, contributed to the introduction and discussions. Author MJO designed the methodology protocol and contributed to the discussion and corrections of the manuscript. Author AOR carried out the statistical analysis and assisted in the model design. All authors read, discussed and approved the final manuscript.

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ABSTRACT

Escherichia coli (E. coli) cause a variety of intestinal and extra-intestinal infections, such as urinary tract infection, diarrhea, meningitis, peritonitis and septicemia. *E. coli* still remains the leading bacterium that lives in the digestive tract of humans and animals. A total of 66 *Escherichia coli* isolates obtained from faeces and effluents from abattoir were examined for their susceptibility to 11 antimicrobial agents. Pathogenic *E. coli* can be released with the animal wastes coming from slaughter houses into the environment, where they can persist and cause infections. In this study we have evaluated the sensitivity to antibiotics by *E. coli* strains isolated from several types of

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infected faeces and effluents. Strains of *E. coli* were identified based on their cultural and biochemical characterisation. The antibiotic sensitivity test was performed using the disc diffusion method according to standards of the Clinical and Laboratory Standard Institute. High resistance levels (≥60) were detected against antimicribial agents like septrin and tetracycline. Moderate resistances (between 40-59%) were detected against chloramphenicol, augmentin, ampicillin, cotrimoxazole and streptomycin. Ciprofloxacin (87.9%), nitrofurantoin (80.3%), gentamicin (77.3%) and amoxycillin (62.1%) were highly sensitive to the *E. coli* strains. This work presents a four compartmental model, which describes the interactions of infections with *Escherichia Coli* in the in-vitro study, to gain more insight into the in-vitro analysis of the model formulated. The classical threshold for basic reproduction number R_0, is obtained: if $R_0 < 1$, the *E. coli* will clear/die out of the population agar medium that was considered but if $R_0 > 1$, then the *E. coli* will grow and persist within the agar medium. We also determine the local and global stability of the *E. coli* model in the in-vitro, using the comparison theorem. Our result shows that when the threshold parameter is less than unity, the disease – free equilibrium will be asymptotically stable and unstable at thresholds greater than unity. Numerical simulations were carried out using maple software to show the effects of treatment on the *E. coli* model. The result showed the pronounced effect of treatment which gives more insight about the *E. coli* model which was in great accord with the investigational analysis of the experimental work.

Keywords: Escherichi coli isolates; antimicrobial drugs; mathematical model and reproduction number.

1. INTRODUCTION

There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. *Escherichia coli* (*E. coli*), is a common bacterium that has been studied intensively by geneticists because of its small genome size, normal lack of pathogenicity and ease of growth in the laboratory in-vitro. The bacterium (*E. coli)* originally known as Bacterium coli, belongs to the Family Enterobacteriaceae and was first isolated and characterized in 1885 by the German scientist and pediatrician Theodore Escherich. *Escherichia coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals, it's a prokaryotic living organism without a nuclear membrane, it reproduces rapidly and inhabits the intestinal tract of humans and animals. Though most *E. coli* are harmless, some are known to be pathogenic, causing both severe intestinal and extra intestinal diseases in man [1]. When these organisms are eliminated into the environment together with faeces, it contaminates water, soil and food [2-3]. *Escherichia coli* is a serious infectious disease associated with high rates of mortality and morbidity [4].

The different pathogenic *E. coli* are characterized by particular genes associated with their virulence. Many enteric infections caused by *E. coli* are transmitted by inter-human contacts such as those caused by Entero-invasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAggEC) or Enteropathogenic *E. coli* (EPEC) [5-6]. Those ascribed to Enterotoxigenic *E. coli* (ETEC) or Shiga toxin-producing *E. coli* (STEC), are primarily transmitted to humans through the consumption of contaminated food and water [7-8]. Shiga toxin-producing *E. coli* cause a wide range of human diseases, including mild-to-severe diarrhoea to haemorrhagic colitis (HC) and the life-threatening haemolytic uremic syndrome (HUS) [8] and are characterised by the production of potent cytotoxins. STEC have gained increasing global concern as food-borne pathogens worldwide [8-10] and are the only diarrheagenic *E. coli* pathogroup with an ascertained zoonotic origin, with ruminants being regarded as the main animal reservoir [11-12]. *E. coli* associated with an inter-human circulation; represent a leading cause of diarrhoea, with high mortality rates, in developing countries [5]. *E. coli* O157:H7 infection can result in hemolytic uremic syndrome and accounts for thousands of cases of severe food borne illness in the United States each year [13]. *E. coli* O157:H7 can infect humans from a wide variety of sources; however, the most common source of exposure and subsequent infection is contaminated food. Poorly cooked, tainted ground beef and other bovine food products have often been implicated as the primary source of infection in outbreaks [14-17].

Mastitis is a disease affecting dairy cows, in recent decades, *E. coli* infections have been identified in an ever increasing proportion of mastitis cases [18]. Among the coliform bacteria, *E. coli* is the dominant pathogenic species in dairy cow mastitis [19-20]. The typical infection pattern for *E. coli* intramammary infection includes a clinically severe inflammatory response by the host with outcomes of either elimination of coliforms within 96 hours of the initial infection [21-22], or a deleterious outcome for the host including shock, sepsis and often death [23-24]. In addition to severe disease, several authors have reported persistent and clinically less severe *E. coli* infection [25-27].

The treatment of illnesses caused by this bacterium often requires antimicrobial therapy. Decision to use antimicrobial therapy depends on the susceptibility of the microorganism and the pharmacokinetics of the drug for achieving the desired therapeutic concentration at the site of infection and thus clinical efficacy [28]. Study has shown that the repeated and unsuitable use of antibiotics has led to an increasing rate of antimicrobial resistance [29].

Mathematical models gives an insight into the epidemiological spread of diseases and proffer solution with interventions which was able to be tested under a wide range of management conditions, thus allowing researchers to identify areas and processes vulnerable to disruption and offering specific management guidance. Our work aimed at studing the susceptibility of 11 antimicrobial agents and using mathematical models to describe the in-vitro analysis of an *Escherichia coli* which was sub divided into the, Susceptible, Infected, Infected-Treated and Removal respectively.This model was developed as a model that is capable of reflecting widespread bacterial growth and population dynamics in an agar medium. This will allow us to focus on the current and future efforts towards developing intervention strategies aimed at lowering the load of *E. coli* strains present in both human and mammals' food chain.

2. MATERIALS AND METHODS

2.1 Sampling Strategy

Two different sampling points was considered for the study: faecal samples from cows at an abattoir and effluents discharged from the abattoir itself to the water body close to the abattoir. A total of 100 faecal samples were collected from different abattoirs centres in Ikorodu, Lagos, Nigeria. The abattoirs were visited twice a week for a 4 weeks period. The effluents from the abattoirs were also collected. The effluents samples were collected twice a week for a 4 weeks period for a total of 50 samples. A total of 150 samples were collected and analysed.

2.2 Laboratory Procedure for Isolation and Identification of *E. coli*

2.2.1 Bacterial isolation

A MacConkey agar were inoculated by the collected swabs and incubated at 37°C for 24 hours. The organism ferments lactose to produce pink coloniees on the MacConkey agar. The representative colonies from the MacConkey agar were transfered to Eosin Methylene Blue agar and incubated at 37°C for 24 hours to observe the cultural characteristics [30-31]. The colonies were also transfered to sorbitol MacConkey agar to check if they can ferment sorbitol.

2.2.2 Biochemical tests

A lope full was taken from growing colony, inoculated into 10 ml nutrient broth then incubated at 37°C for 24 hours. The biochemical test used in the identification of *E. coli,* for the study are: Gram staining, indol production, methyl red test, voges-proskaur test, citrate utilization, urease activity, catalase production and motility were carried out using standard methods described by [32-36].

2.2.3 Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml of peptone water, incubated at 37°C for 4 hours. This actively growing bacterial suspension was then adjusted with peptone water so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) tetraoxosulphate (vi) acid (H_2SO_4). This turbidity is equivalent to approximately 1 x 10^8 colony forming units per ml (CFU/ml).

2.2.4 Antibiotic susceptibility testing

Susceptibility of organisms to different antibiotics were tested using disk diffusion method as described by Kirby-Bauer diffusion technique

[37-38] on freshly prepared Mueller Hinton agar and standardized by the method of National Committee for Clinical Laboratory Standard (NCCLS 2000) [39] using some selected antibiotics namely: Erythromycin (10 ug), Amoxicillin (30 ug), Ciprofloxacin (5 ug), Gentamicin (10 ug), Ampicillin (25 ug), Streptomycin (25 ug) etc. For each combination of the antibiotics and the bacterial strains, the experiment was performed in triplicate. The antimicrobial agent with a clear zone of inhibition of more than 17 mm was considered to be sensitive, while those that are less than 18mm are resistant.

2.3 Mathematical Model Formulation

We formulate the mathematical model that describes the interaction between *Escherichia coli* strain (A) in the in-vitro by considering the dynamical system of equation for the in vitro analysis population. The population is divided into four groups as Susceptible, Infected, Infected –Treated and Removal. So we assume that the population are constant. Our model also includes recovery rate upon treatment.

$$\left.\begin{aligned} S' &= \Lambda_h - \beta E_{IA} S - \mu S + r E_{RA} \\ E'_{IA} &= \beta E_{IA} S - (\mu + \sigma + \gamma) E_{IA} \\ E'_{TA} &= \gamma E_{IA} - (\mu + \delta) E_{TA} \\ E'_{RA} &= \delta E_{TA} - (\mu + r) E_{RA} \end{aligned}\right\} \quad (1)$$

For our dynamical equations, we define the following variables and parameters as follows:

r is the rate of loss of immunity, μ Natural Death rate and Λ_h is the recruitment rate of *E. coli*. β is the transmission probability /rate and δ is the recovery rate upon treatment with standard drug respectively. Thus the model is presented by the following ODE:

The assumptions for the model (2) are:

(i) It is a closed population (ii) No reproduction since model describes in the in vitro (iii) Some of the strain recovered, due to the drug and that were administered.

2.4 Model Analysis

2.4.1 The Invariant Region of the system of equation (1)

Let: $N(t) = S(t) + E_{IA}(t) + E_{TA}(t) + E_{RA}(t)$ (2)

Differentiating and simplifying, we obtain at disease free.

$$N'(t) = S'(t) + E'_{IA}(t) + E'_{TA}(t) + E'_{RA}(t)$$

$$= \Lambda_h - \beta E_{IA} S - \mu S + r E_{RA} + \beta E_{IA} S - (\mu + \sigma + \gamma) E_{IA} + \gamma E_{IA} - (\mu + \delta) E_{TA} + \delta E_{TA} - (\mu + r) E_{RA}$$

$$= \Lambda_h - \mu (S(t) + E_{IA}(t) + E_{TA}(t) + E_{RA}(t)) - \sigma E_{IA}(t)$$

$$= \Lambda_h - \mu (N(t)) - \sigma E_{IA}(t)$$

At disease free $E_{IA}(t) = 0$

$$\Rightarrow N'(t) + \mu (N(t)) = \Lambda_h \quad (3)$$

Using integrating factor (3), we have

$$N(t) e^{\int \mu dt} = \int \Lambda_h e^{\int \mu dt} dt + k$$

$$N e^{\mu t} = \Lambda_h \int e^{\mu t} dt + k = \frac{\Lambda_h}{\mu} e^{\mu t} + kz$$

$N(t) = \frac{\Lambda_h}{\mu} + e^{-\mu \bullet t} k$, and at $t = \longrightarrow \infty$

We obtain

$$N(t) = \frac{\Lambda_h}{\mu} \quad (4)$$

2.4.2 Positivity of solutions

For the *Escherichia coli* strain model of equation (1) to be epidemiologically well posed, we need to show that all solution with non-negative initial conditions will remain non – negative, for all $t \geq 0$.

Theorem 1: Let: $\Phi = \Phi_C \subset R_+^{\ 4}$ with

$$\Phi_C = \left\{ \begin{aligned} &(S(t), E_{IA}(t), E_{TA}(t), E_{RA}(t)) \in R_+^{\ 4} \\ &: (S(t) + E_{IA}(t) + E_{TA}(t) + E_{RA}(t)) \leq \frac{\Lambda_h}{\mu} \end{aligned} \right\},$$

Then the solutions $(S(t), E_{IA}(t), E_{TA}(t), E_{RA}(t))$ of the system (1) are all positive $\forall t \geq 0$.

Proof: From the first differential equation of system (1),

$$\left.\frac{dS}{dt} \geq -[\beta E_{IA} + \mu]S \Rightarrow \frac{dS}{S} \geq -[\beta E_{IA} + \mu]dt\right\} \quad (5)$$

Integrating both sides

$$\int \frac{dS}{S} \geq -\int_0^t [\beta E_{IA} + \mu]dt \quad ,$$

To obtain

$$S(t) \geq Ke^{-\int_0^t [\beta E_{IA} + \mu]dt}$$

$$t = 0, \quad S(0) = Ke^0 = K = S(0)$$

Therefore $k = S(0)$

Hence

$$S(t) \geq S(0)e^{-\int_0^t [\gamma_j X_2 + \theta_j X_8 + (\delta + d)]dtt} \geq 0$$

$$\forall \quad t > 0 \quad (6)$$

Similar reasoning can be used for other differential equations of equation (1) hence, it follows that the *Escherichia coli* strain model is positive and bounded with a unique solution.

Using the next generation matrix operator techniques, which was describe by Diekmann et al. (2000), and subsequently analysed by Van den et al (2012), the basic reproduction number R_0 of the model equation (1) was obtained, which represent the spectral radius (ρ) of the next generation matrix, K i.e. $R_0 = \rho K$, where $K = FV^{-1}$.And the matrices of F (the new infection terms) with V (the transition terms) . It can be shown that is the largest (dominant) Eigen-value of FV^{-1} is, R_0, which are the basic reproduction numbers respectively.

$$F = \begin{bmatrix} \frac{\beta\Lambda_h}{\mu} & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad (7)$$

$$V = \begin{bmatrix} (\mu+\sigma+\gamma) & 0 & 0 \\ -\gamma & (\mu+\delta) & 0 \\ 0 & -\delta & (\mu+r) \end{bmatrix} \quad (8)$$

Thus, the basic reproduction number is given below as

$$\left. R_0 = \frac{\beta\Lambda_h}{\mu(\mu+\sigma+\gamma)} \right\} \quad (9)$$

2.4.3 Existence and stability analysis of disease-free Equilibrium, ε_0

The model has a disease-free equilibrium (DEF), which is obtained by setting the right-hand side of equation (1) to zero, given by

$$\varepsilon_0 : (S, E_{IA}, E_{TA}, E_{RA}) = \left(\frac{\Lambda_h}{\mu}, 0,0,0,\right) \quad (10)$$

Hence the infection – free equilibrium

$$\varepsilon_0 : (S, E_{IA}, E_{TA}, E_{RA}) = \left(\frac{\Lambda_h}{\mu}, 0,0,0,\right) \quad (11)$$

2.4.4 Local stability of the equilibrium

Theorem 2: The disease free equilibrium of system (1) is locally asymptotically stable if the trace $Tr(A) < 0, Det(A) > 0$, with all eigenvalues been negative, provided if $R_0 < 1$ otherwise unstable.

Proof:

The Jacobian matrix of equations (4) at the disease-free equilibrium point ε_O is

$$|J(E_0) - \lambda I| = 0$$

$$|J(E_0) - \lambda I| = \begin{vmatrix} -\mu-\lambda & \frac{\beta\Lambda_h}{\mu} & 0 & 0 \\ 0 & \frac{\beta\Lambda_h}{\mu} - (\mu+\sigma+\gamma) - \lambda & 0 & 0 \\ 0 & \gamma & -(\mu+\delta)-\lambda & 0 \\ 0 & 0 & \delta & -(\mu+r)-\lambda \end{vmatrix} = 0 \quad (12)$$

$$|J(E_0)-\lambda I| = \begin{vmatrix} -\mu-\lambda & \frac{\beta\Lambda_h}{\mu} & 0 & 0 \\ 0 & (\mu+\sigma+\gamma)[R_0-1]-\lambda & 0 & 0 \\ 0 & \gamma & -(\mu+\delta)-\lambda & 0 \\ 0 & 0 & \delta & -(\mu+r)-\lambda \end{vmatrix} = 0 \quad (13)$$

Clearly the eigenvalues of $J(E_0)$ are all negative:

$\lambda_1 = -\mu, \ \lambda_2 = -(\mu + r)$, are negative

While the remaining two eigenvalues are obtained from the 2×2 matrix of the $|J(E_0)-\lambda I| = 0$ must satisfy this two condition of **Routh-Hurwitz** criterion that is (i) $Tr(A) < 0$ (ii) $Det(A) > 0$

Let $A = \begin{pmatrix} (\mu+\sigma+\gamma)[R_0-1] & 0 \\ \gamma & -(\mu+\delta) \end{pmatrix}$

And taking the trace of A that is $Tr(A)$

We have:

$$Tr(A) = (\mu+\sigma+\gamma)[R_0-1] - (\mu+\delta)$$
$$= (\mu+\sigma+\gamma)[R_0-1] - (\mu+\delta) < 0,$$

whenever $R_0 < 1$.

Thus: $Tr(A) < 0$, which satisfied (i).

(ii) The determinant of A , must be positive that is

$$Det(A) = \begin{vmatrix} (\mu+\sigma+\gamma)[R_0-1] & 0 \\ \gamma & -(\mu+\delta) \end{vmatrix}, \quad \text{If}$$
$$= (\mu+\sigma+\gamma)[R_0-1](-(\mu+\delta)) > 0$$
$$R_0 < 1$$

Thus by **Routh-Hurwitz** condition the disease free equilibrium E_0 is locally asymptotically stable since (i) $Tr(A) < 0$ (ii) $Det(A) > 0$ and all eigenvalues are negative.

2.4.5 Global stability of the disease free equilibrium

Theorem: 3: The *E. coli* Free equilibrium ε_0 of system (1) is globally asymptotically stable if $R_0 < 1$ and unstable if $R_0 > 1$. Proof:

Using comparison theorem as implemented in [12], which the rate of change of the infected compartment of equation (1) can be written as

$$\begin{pmatrix} E'_{IA} \\ E'_{TA} \\ E'_{RA} \end{pmatrix} \le (F-V)\begin{pmatrix} E_{IA} \\ E_{TA} \\ E_{RA} \end{pmatrix} - F_i \begin{pmatrix} E_{IA} \\ E_{TA} \\ E_{RA} \end{pmatrix}$$

Where F and V remain their original meaning, and according to Castillo–Chavez and song (2004). All Eigen – values of matrix (F-V) are real and negative.

Thus, we obtain

$$\begin{pmatrix} E'_{IA} \\ E'_{TA} \\ E'_{RA} \end{pmatrix} \le \begin{pmatrix} \left(\frac{\beta\Lambda_h}{\mu} - (\mu+\sigma+\gamma)\right) & 0 & 0 \\ \gamma & -(\mu+\delta) & 0 \\ 0 & \delta & -(\mu+r) \end{pmatrix} \begin{pmatrix} E_{IA} \\ E_{TA} \\ E_{RA} \end{pmatrix} \quad (14)$$

Then the corresponding eigenvalues of $(F-V)$ are all negative i.e

$$\begin{pmatrix} (\mu+\sigma+\gamma)(R_0-1)-\lambda & 0 & 0 \\ \gamma & -(\mu+\delta)-\lambda & 0 \\ 0 & \delta & -(\mu+r)-\lambda \end{pmatrix} = 0 \quad (15)$$

Simplifying to get

$\lambda_1 = -\mu - r$, is negative,

While the remaining two eigenvalues are obtained from the 2×2 matrix of the $|J(E_0)-\lambda I| = 0$, which must satisfied the two

condition of **Routh-Hurwitz** criterion that is (i) $Tr(A) < 0$ (ii) $Det(A) > 0$

Let $A = \begin{pmatrix} (\mu+\sigma+\gamma)(R_0-1) & 0 \\ \gamma & -(\mu+\delta) \end{pmatrix}$

And taking the trace of A that is $Tr(A)$, we have

$Tr(A) = (\mu+\sigma+\gamma)(R_0-1) - \mu - \delta < 0$,

whenever $R_0 < 1$.

Thus : $Tr(A) < 0$, which satisfied (i)

(ii) The determinant of A, must be positive that is

$$Det(A) = \begin{vmatrix} (\mu+\sigma+\gamma)(R_0-1) & 0 \\ \gamma & -(\mu+\delta) \end{vmatrix}$$
$$= -(\mu+\delta)(\mu+\sigma+\gamma)(R_0-1) > 0$$

Provided, if $R_0 < 1$.If thus by **Routh-Hurwitz** condition the disease free equilibrium ε_0 is globally asymptotically stable (GAS) since (i) $Tr(A) < 0$ (ii) $Det(A) > 0$ and all eigenvalues are negative. (Globally asymptotically stable),

Then by **Routh-Hurwitz** condition we have that the (DFE) is globally asymptotically stable (GAS), for $R_0 < 1$.

2.4.6 Local asymptotic stability of endemic equilibrium ε^*

Observe that system (1) have the endemic equilibrium point

$\varepsilon^* = \left(S^*, E^*_{IA}, E^*_{TA}, E^*_{RA}\right)$

Such that, $S^* = \dfrac{\mu+\sigma+\gamma}{\beta} = \dfrac{\Lambda_h}{\mu R_0}$

$$E^*_{IA} = \frac{-(\mu+\delta)(\mu+r)\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]}$$

$$E^*_{TA} = \frac{-(\mu+r)\gamma\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]}$$

$$E^*_{RA} = \frac{-\gamma\delta\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]}$$

The Jacobian matrix of (1) at ε^* is

$$j(\varepsilon^*) = \begin{pmatrix} -\beta E_{IA} - \mu & -\beta S & 0 & r \\ \beta E_{IA} & \beta S & 0 & 0 \\ 0 & \gamma & -(\mu+\delta) & 0 \\ 0 & 0 & \delta & -(\mu+r) \end{pmatrix} \qquad (16)$$

At endemic equilibrium, we have (16) reduces to

$$J\left|\varepsilon^* - \lambda I\right| = 0$$

$$= \begin{pmatrix} \beta \dfrac{(\mu+\delta)(\mu+r)\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]} - \mu & -\beta\dfrac{\Lambda_h}{\mu R_0} & 0 & r \\ -\beta \dfrac{(\mu+\delta)(\mu+r)\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]} & \beta\dfrac{\Lambda_h}{\mu R_0} & 0 & 0 \\ 0 & \gamma & -(\mu+\delta) & 0 \\ 0 & 0 & \delta & -(\mu+r) \end{pmatrix} = 0 \qquad (17)$$

And the eigenvalues of (17) is obtained as

$\lambda_1 = -\mu - \beta \dfrac{(\mu+\delta)(\mu+r)\Lambda_h(R_0-1)}{R_0\left[r\gamma\delta - (\mu+\sigma+\gamma)(\mu+\delta)(\mu+r)\right]}$, Provided if $R_0 > 1$

$\lambda_1 = -\beta\dfrac{\Lambda_h}{\mu R_0}$, whenever $R_0 > 1$

While the remaining two eigenvalues are obtained from the 2×2 matrix of the $\left|J(E_0) - \lambda I\right| = 0$, which must satisfied the two condition of routh-hurwirts criterion that is (i) $Tr(A) < 0$ (ii) $Det(A) > 0$.

Let $A = \begin{pmatrix} -(\mu+\delta) & 0 \\ \delta & -(\mu+r) \end{pmatrix}$, and taking the trace of A that is $Tr(A)$, we have

$Tr(A) = -(\mu+\delta) - (\mu+r) < 0$, thus: $Tr(A) < 0$, which satisfied (i).

(ii) The determinant of A , must be positive that is

$$Det(A) = \begin{vmatrix} -(\mu+\delta) & 0 \\ \gamma & -(\mu+r) \end{vmatrix} = -(\mu+\delta) \times -(\mu+r) > 0 \quad (18)$$

Which is positive thus by Routh-Hurwirts condition the Endemic equilibrium point (EEP) ε^* is locally asymptotically stable (LAS) since

(i) $Tr(A) < 0$ (ii) $Det(A) > 0$ and all Eigen values are negative.

Then by **Routh-Hurwitz** condition we have that the (EEP) is locally asymptotically stable (LAS), for $R_0 > 1$. It follows that (18) will have two real negative roots if $R_0 > 1$, hence all eigenvalues of (17) are real and negative if $R_0 > 1$ implying that endemic equilibrium point is locally asymptotically stable. The foregoing discussion is summarized as follows:

Theorem 4: The endemic equilibrium point (EEP) ε^* of system (1) is locally asymptotically stable if $R_0 > 1$ otherwise unstable.

3. RESULTS

3.1 Bacterial Isolation and Identification

E. coli was isolated from 150 fecal samples after 24 hours incubation. The growing bacterial colonies appeared as round small, bright pink on MacConkey agar, while on Eosin Methylene Blue agar they appeared as small round colonies with green metallic sheen. They are gram negative bacteria, the result of the biochemical tests used in the identification of the organism (*E. coli) are* shown in Table 1 below:

Table 1. Biochemical tests used for the identification of isolated *E. coli* organism

Biochemical test	Result
Beta galactosidase	+
Citrate utilization	-
Hydrogen sulphide test	-
Indol production	+
Lysine decarboxylase	+
Motility	+
Urease	-
Voges-proskaur test	-

+ represent positive test and – represent negative result

Table 2. Antimicrobial sensitivity and resistance profiles of *Escherichia coli* isolated from abattoir centres

Antimicrobial agent	Concentration (µg)	Sensitivity (%)	Resistant (%)
Septrin	30	26 (39.4)	40 (60.6)
Gentamicin	10	51 (77.3)	15 (22.7)
Chloramphenicol	30	39 (59.1)	27 (40.9)
Augmentin	30	38 (57.6)	28 (42.4)
Ciprofloxacin	10	58 (87.9)	8 (12.1)
Amoxycillin	30	41 (62.1)	25 (37.9)
Tetracycline	30	22 (33.3)	44 (66.7)
Ampicillin	25	29 (43.9)	37 (56.1)
Nitrofurantoin	30	53 (80.3)	13 (19.7)
Cotrimoxazole	30	35 (53.0)	31 (47.0)
Streptomycin	25	38 (57.6)	28 (42.4)

Sensitive = Zone diameter of bacterial inhibition of ≥ 18 mm, Resistant = Zone diameter of bacterial inhibition < 18 mm

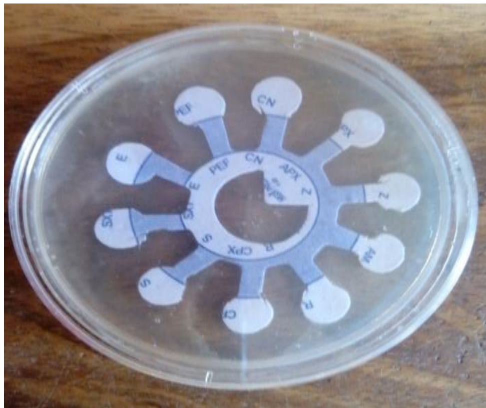

Fig. 1. Sensitivity of *Escherichia coli* strains to Multi drug disc

Fig. 2. Sensitivity of *Escherichia coli* strains to Multi drug disc

Fig. 3. Sensitivity and Resistance of *Escherichia coli* strain to Multi drug disc

Fig. 4. Sensitivity and Resistance of *Escherichia coli* strain to Multi drug disc

Fig. 5. Resistance of *Escherichia coli* strain to Multi drug disc

3.2 Numerical Simulation of the Mathematical Model

The numerical simulations were carried out to back our experimental result using maple 16 software.

4. DISCUSSION

Out of the 150 specimens collected, 66 (44%) *E. coli* isolates were identified. *E. coli* isolates are frequent contaminants of food of animal origin, and in this study, this microorganism was

recovered from 150 tested arbattoir wastes and its effluent samples; in addition, most of the isolates showed a multi-resistant properties.

Of the 66 *E. coli* isolates tested, all were resistant to one or more antimicrobial agent. Resistance to tetracycline was the most common finding (66.7%), followed by resistance to septrin (60.6%), ampicillin (56.1%), augumentin and streptomycin (42.4%). Several studies have shwon the resistance of *E. coli* to different antimicrobial agents. In the study carried out by Momtaz et al. [40], nine strains (15.78%) were resistant to a single antimicrobial agent and 11 strains (19.29%) showed resistance to two antimicrobial agents. Multi-resistance was found in 64.91% of *E. coli* strains. Their results indicate that all isolates harbour one or more of antibiotic resistance genes. Umolu et al. [41] study shows that very high resistance levels (>75%) were detected against tetracycline, augmentin and amoxicillin while nitrofurantoin and ofloxacin recorded the least resistance levels of 6% and 19% respectively among the isolates. Lietzau et al. [42] reported 15.7% and 19.4% prevalence of ampicillin resistance in women and men, respectively, while 10% and 15% of all isolates were resistant to cotrimoxazole and doxycycline respectively. In agreement with the above mentioned studies, our results confirmed large percentage of antibiotic sensitivity to *E. coli* with ciprofloxacin (87.9%), nitrofurantoin (80.3%) and gentamycin (77.3%).

The results from this study provide further evidence that pathogenic *E. coli* can contaminate the environment as a result of the discharge of untreated abattoir faeces and effluent. Additionally, the lack of enforcement of good hygiene practices may ease the release and persistence of multiple pathogenic of *E. coli* strains in the abattoir environment, making such a setting a unique favourable environment for bacteria to bacteria interaction and exchange of genetic material possibly leading to the emergence of new pathogenic strains with shuffled virulence features.

Figs. 6 and 7 shows that the transmission probability β has a great impact on the susceptible agar / *E. coli* free, because as the transmission parameter increases, the number of susceptible populations reduces which lead to the increase in the number of infected agar with *E. coli*, even when the treatment rate (standard drug) is taking to be at constant rate. However, if $R_o > 1$, Fig. 6 support our analytical result that

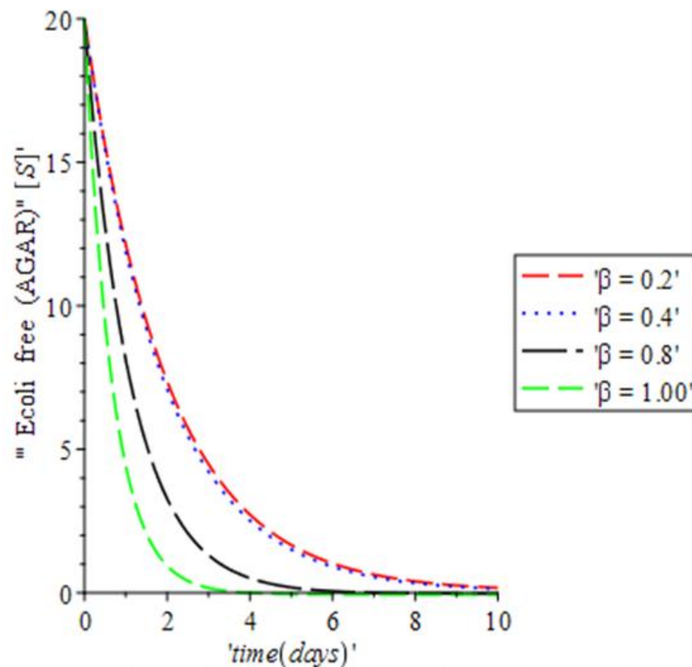

Figure 6: The graph of the Ecoli free (AGAR) population for different values of β

the *E. coli* persist in the agar medium population, thus, the observed increase in the infected *E. coli* population with the normal agar medium (*E. coli* free susceptible) declining.

Fig. 8 support our claim about the analytical result that if $R_o < 1$ the total population is stable. The administration of the standard drugs (treatment rate) i.e. γ , as shown in Fig. 8

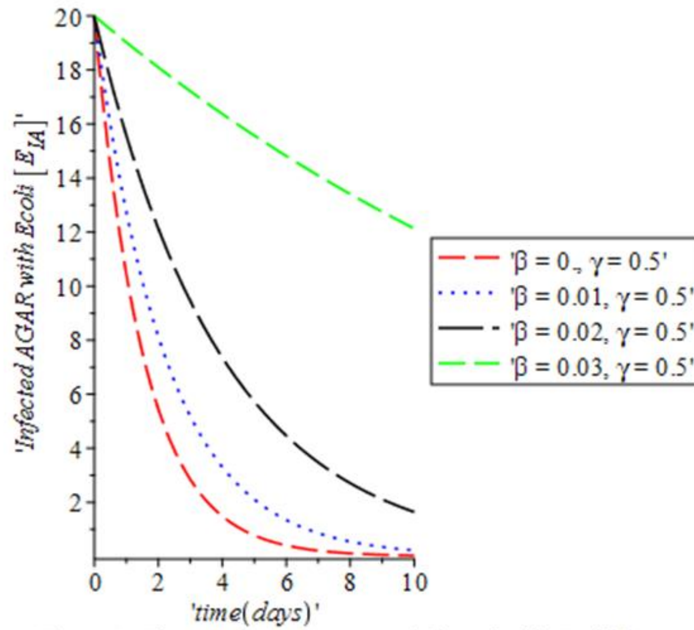

Figure 7: The graph of the Agar, infected with Ecoli for different values of β,taking γ constant

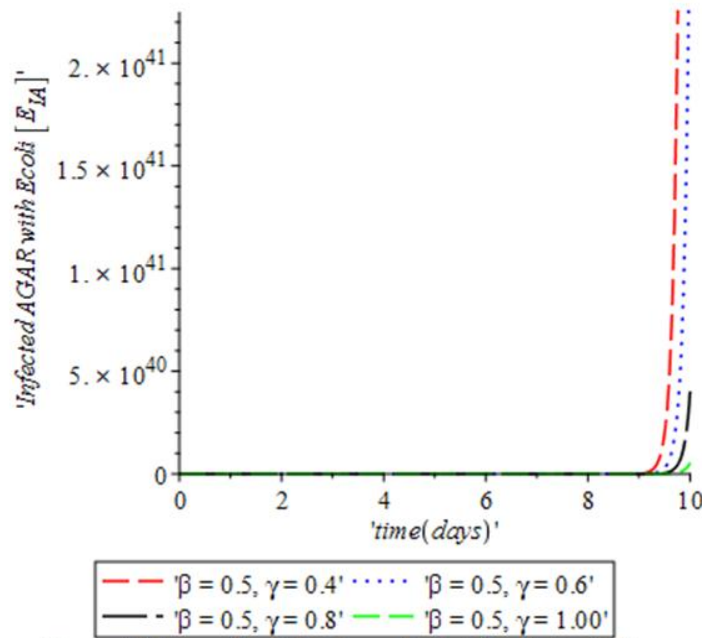

Figure 8: The graph of the Agar, infected with Ecoli for different values of γ,taking β constant

revealed that the infected *E. coli* will move to the treatment class E'_{TA}, while the infected E. coli decreases. The effectiveness of the standard drugs which suppress the *E. coli* were considered in Fig. 9, Fig. 10 respectively. Fig. 11 shows some Agar that was infected with *E. coli,* recovered upon treatment to the susceptible population of the agar medium.

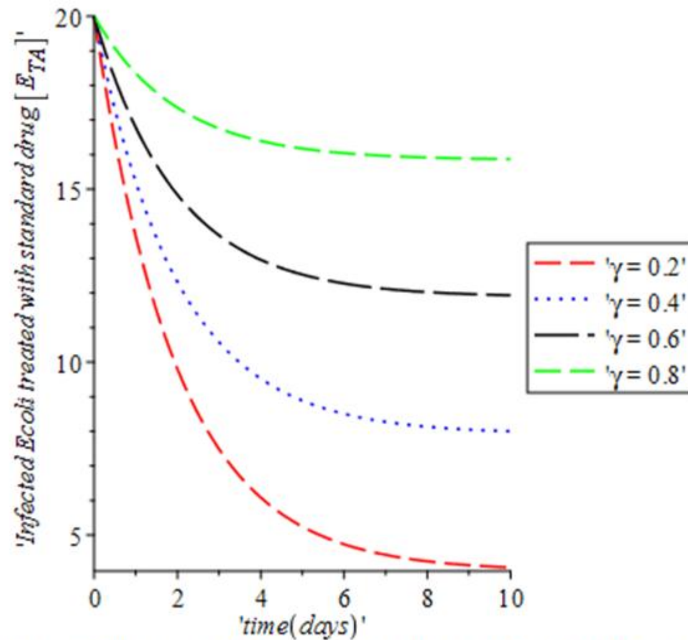

Figure 9: The graph of the treated ecoli population for different values of γ

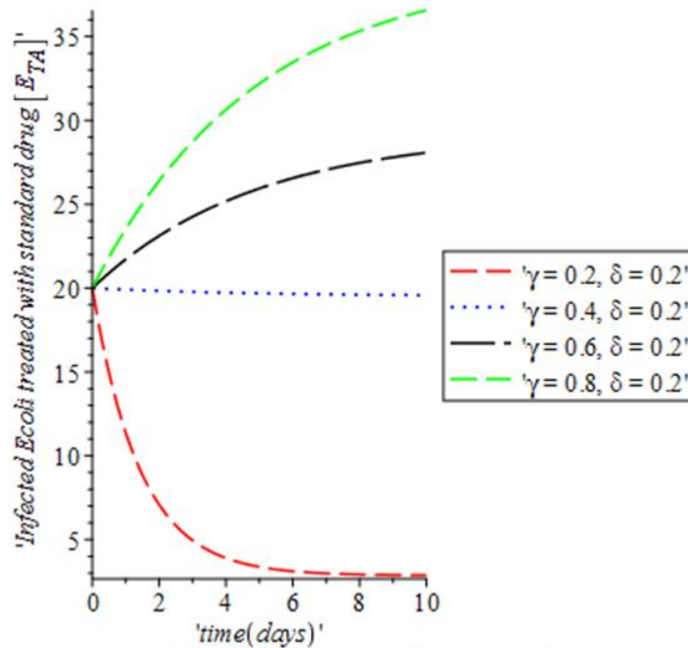

Figure 10: The graph of the treated ecoli population for different values of γ taking the recovery rate δ constant

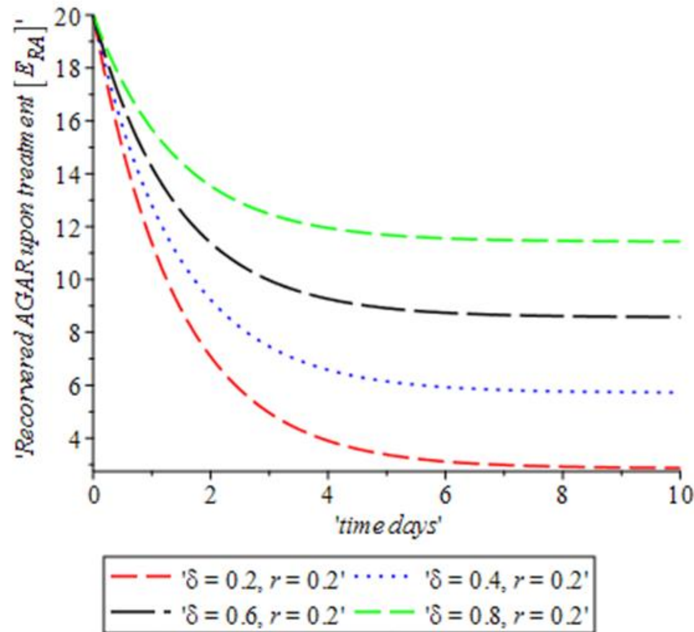

Figure 11: The graph of recorvered agar upon treatment for different values of δ taking the recovery rate r constant

The analytical result is in perfect agreement with our experimental result that the standard drugs was able to suppress the *E. coli* to an acceptable level which accounts for the increase observed in the recovered *E. coli* population as shown in Fig. 11.

An experimental and mathematical model that considered the in-vitro analysis of *Escherichia Coli* with standard drugs was considered and studied in this work. It was also established that the *E. coli* in the agar medium population was cleared out if the associated basic reproduction $R_0 < 1$ while the *Escherichia Coli* persist in the population if $R_0 > 1$. This research revealed that the standard drug gave good suppressive property in suppressing the *Escherichia Coli* growth. The isolated *E. coli* strains resist antinicrobial agents due to their resistant gene.

5. CONCLUSION

The study shows that a total of 66 *Escherichia coli* isolates were obtained from faeces and effluents from abattoir centres in Ikorodu, Lagos, Nigeria. Ciprofloxacin, nitrofurantoin, gentamicin and amoxycillin were highly sensitive to the *E. coli* strains while high resistance levels were detected against septrin and tetracycline. Moderate resistances were detected against chloramphenicol, augmentin, ampicillin, cotrimoxazole and streptomycin. The experimental work has been supported by the mathematical model.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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