

## EVALUATING THE SENSITIVITY OF *PSEUDOMONAS AERUGINOSA*, *ESCHERICHIA COLI* AND *SALMONELLA TYPHI* TO VARIOUS BRANDS OF AMPICILLIN AND AMOXICILLIN AVAILABLE IN NIGERIA

E.E. Anomohanran, U.B. Owhe-Ureghe and D.A. Ehwareme

Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria

Received 2014-10-04; Revised 2014-10-13; Accepted 2014-10-18

Funding: This article was funded from personal funds

Competing Interests: The authors have declared that no competing interests exist

### ABSTRACT

The failure of antimicrobial agents in the treatment of some ailments has become a great concern to health care practitioners. This could be as a result of low quality drugs, sneaked into the market by those who fake them, thus, this study was carried out to evaluate the sensitivity of various brands of Amoxicillin and Ampicillin on clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The test organisms were clinical bacterial isolates obtained from clinical samples from four hospitals/laboratories in Delta State, Nigeria. The antibiotics were subjected to two fold serial dilution method to determine the Minimum Inhibitory Concentration (MIC) from which the sensitivity of the isolates to the various brands of the antibiotics was determined. The result showed that the sensitivity of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* to the various brands of Amoxicillin are 54, 62 and 68% respectively. The result also revealed that the sensitivity of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* to Ampicillin are 64, 64 and 70% respectively. The result further indicates that a mean sensitivity of the isolates to Amoxicillin was 61%, while the mean sensitivity to Ampicillin was obtained as 66%. The study has therefore established the need for a routine evaluation of antibiotics and other pharmaceuticals in the Nigerian markets.

**Keywords:** Amoxicillin, Ampicillin, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*

### 1. INTRODUCTION

*Pseudomonas aeruginosa* is an opportunistic gram negative bacilli and one of the most important causes of nosocomial infections, especially in patients with burns, cystic fibrosis and neutropenia or as part of the normal flora of the human skin.

When the host is immunocompromised, this opportunistic bacterium can quickly colonize and infect the burn and wound sites. Since *Pseudomonas aeruginosa* can rapidly spread from the wounds into other organs via the blood stream as a result of a number

of virulence factors, the clinical outcome in these patients can lead to sepsis which is frequently fatal.

*Escherichia coli* is also gram-negative, facultative anaerobic and non-sporulating bacteria (Iroha *et al.*, 2009). The organism grows at 37°C but some laboratory strains can multiply at 49°C. Growth can be driven by aerobic or anaerobic respiration. *Escherichia coli* is notorious for causing serious and even life threatening complication in humans, which include infections of the urinary tract, wound, endotoxin induced shock, meningitis (especially in neonates), bacteraemia and diarrhoea (Pearson, 2007).

**Corresponding Author:** E.E. Anomohanran, Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria

*Salmonella typhi* is related to *Escherichia coli* being members of the family Enterobacteriaceae. It is known to ferment glucose and mannose but does not ferment lactose or sucrose (Prescott *et al.*, 2005). It is often pathogenic for humans or animals when ingested. Infections cause by *Salmonella typhi* includes enteric fevers (typhoid fever), Bacteremia with focal lesions, enterocolitis (diarrhoeal disease), paratyphoid fever or abdominal cramps (Santos *et al.*, 2001; Tortora *et al.*, 2002; Prescott *et al.*, 2005).

Ailments which are caused by *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* bacteria are commonly treated using antibiotics such as ampicillin and amoxicillin. Ampicillin is a beta-lactam antibiotic with trade names as Omnipen, Polycillin and Principen. They are generally used in the treatment of infections caused by bacteria. These infections include ear infections, bladder infections, pneumonia and gonorrhoea. The drug is also employed in the treatment of skin and skin-structure infections, intro-abdominal infections and gynaecological infections (Adnan *et al.*, 2013). The antibiotic is known for its ability to penetrate the gram positive bacteria and in some cases the gram negative ones. Ampicillin is noted for its amino acids that help to attack the formed membranes of gram negative bacteria (Adnan *et al.*, 2013). It also works against the enzyme transpeptidase, which is a basic component needed by the bacteria to form the membranes that surround them.

In the same vein, Amoxicillin is also a beta-lactam antibiotic used for treating bacteria infection. This drug is well easily absorbed by the human body and is usually sold as Amoxil, Dispermox and Alphamox. Amoxicillin works by preventing the synthesis of bacterial walls that will surround and protect them. Ampicillin and Amoxicillin are quite the same in basic composition. Amoxicillin works by cross linking the linear peptidoglycan polymer chains on the cell wall of the gram positive and negative bacteria. In other words, the antibiotic weakens the defences of the invading organisms that make a person sick. In effect, the function of both drugs is to prevent the membranes of the bacteria from fully forming so that the antibodies, other humoral substances and cells of the human immune system can penetrate them and remove them. It is established that bacteria by itself cannot survive inside the human body without the protective walls that surround it (Adnan *et al.*, 2013).

The three organisms considered in this study are very common among patients attending hospitals in the study area and have posed some level of challenges both to patients and clinicians. The physiological changes that occur in a sick person presents the clinicians the

opportunity to procure an effective antibiotic to be administered. The antibiotic therapy will be considered inappropriate if the empirical drug selected, the dose and method of administration are not efficient against the causative pathogen (Pachauri and Kataria, 2012; Adnan *et al.*, 2013). This is why this study is carried out to consider the various brands of Ampicillin and Amoxicillin in the Nigerian markets and determine their efficacy.

## 2. MATERIALS AND METHODS

### 2.1 Source and Collection of Clinical Specimen

Different clinical samples were collected from patients who attended Out Patient Department (OPD) of the Delta State University Health Centre Abraka, Central Hospital Warri, General Hospital Eku and Standard Diagnostic Centre Warri. The clinical samples include surgical wounds samples, sputum of patient with upper respiratory tract infection, burn wound, ear swabs from patients with ear infections, High Vaginal Swab (HVS) and mid stream urine. The samples were taken to the Microbiology Laboratory in Delta State University, Abraka, Nigeria where various microbiological and biochemical test were carried out to obtain the desired test organisms.

### 2.2. Determination of Zones of Inhibition

Isolates were exposed to different brands of Amoxicillin and Ampicillin using the agar well diffusion method in accordance with the recommendation of the National Committee for Clinical Laboratory Standards, (NCCLS, 2002). The area showing no obvious visible growth was taken as the zones of inhibition that can be detected with the unaided eye. Faint growths of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, were ignored. Isolates having a zone of inhibition equal to and greater than 12 mm ( $\geq 12$  mm) was taken to be sensitive, while the zone of inhibition less than 11 mm or equal to 11 mm was taken to be resistant ( $\leq 11$  mm) (Cheesbrough, 2002; Owhe-Ureghe, 2011).

### 2.3. Determination of Minimum Inhibitory Concentration (MIC)

The potency of a chemotherapeutic agent is usually expressed on the basis of the lowest concentration or minimal inhibitory concentrations of antimicrobial agent required to inhibit the visible growth of a particular bacterial isolate *in-vitro* after overnight incubation (Nnela and Cox, 1988; Darabi *et al.*, 2010). In determining the clinical isolates sensitivity to Amoxicillin and Ampicillin, the two-fold (Log) serial

dilution method were employed (Jazani and Babazadeh, 2008). The antimicrobial agent was prepared in accordance with the method of Wood and Washington (1995) and as stipulated in the National Committee for Clinical Laboratory Standards (NCCLS, 2002) guidelines.

Ten (10) sterile test tubes were arranged in a plastic rack for serial dilution and 1 mL of freshly prepared Mueller-Hinton broth was measured into the test tubes. This was followed by the addition of 1 mL of diluted antibiotic stock solution into the test tube and the combination mixed homogeneously. A freshly cultured isolates was mixed with 5 mL of peptone water and the mixture incubated at 35°C until it achieved the turbidity of the 0.5 McFarland standard. 0.1 mL of the mixture containing the known organisms was then introduced into the test tube containing the broth and the antibiotic. The combination was shaken vigorously to have a uniform result. A tube containing isolate without antibiotic and un-inoculated tube were used as a positive and negative control respectively. At the end of the 24 hours of inoculation, the lowest concentration at

which there was no visible growth in the tube was determined as the Minimum Inhibitory Concentration (MIC) (Wiegand *et al.*, 2008; Chayani *et al.*, 2009; Agboke and Esimone, 2011).

### 3. RESULTS

The clinical isolates used in this study were exposed to amoxicillin and ampicillin to determine their inhibitory ability. The concentration of the antimicrobial agent at which the organisms were inhibited was determined and presented as shown in **Figs. 1 and 2**. **Figure 1** shows the MIC for amoxicillin while **Fig. 2** shows the MIC for ampicillin.

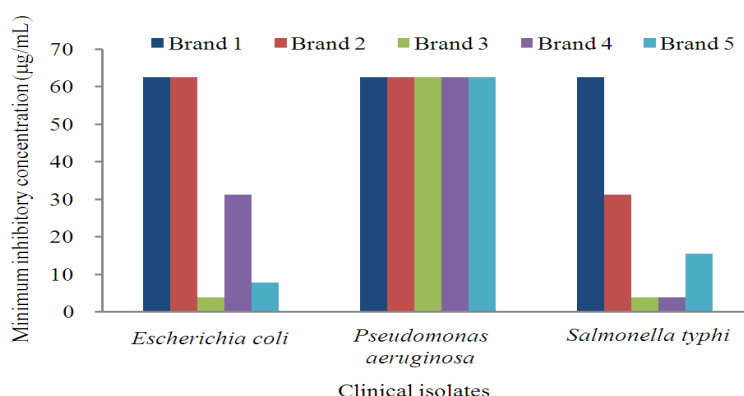
The number of bacterial isolates that were sensitive to the various brands of Amoxicillin after inoculation for 24 hours is presented in **Table 1**. On the other hand, the number of isolates that were sensitive to the brands of Ampicillin is as shown in **Table 2**. These values shown in **Table 1 and 2** are used to determine the rate of sensitivity of the test isolates to both antibiotics.

**Table 1.** The number of sensitive bacteria isolates to Amoxicillin

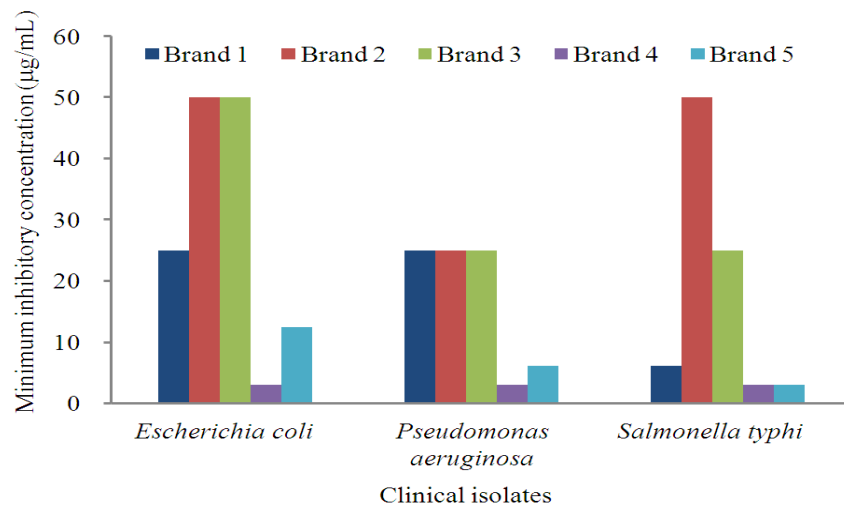
Organism	Number of isolates sensitive to amoxicillin				
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5
<i>Escherichia coli</i> (n = 10)	2	1	9	6	9
<i>Pseudomonas aeruginosa</i> (n = 10)	4	3	6	8	10
<i>Salmonella typhi</i> (n = 10)	5	2	8	10	9

**Table 2.** The number of sensitive bacteria isolates to Amoxicillin

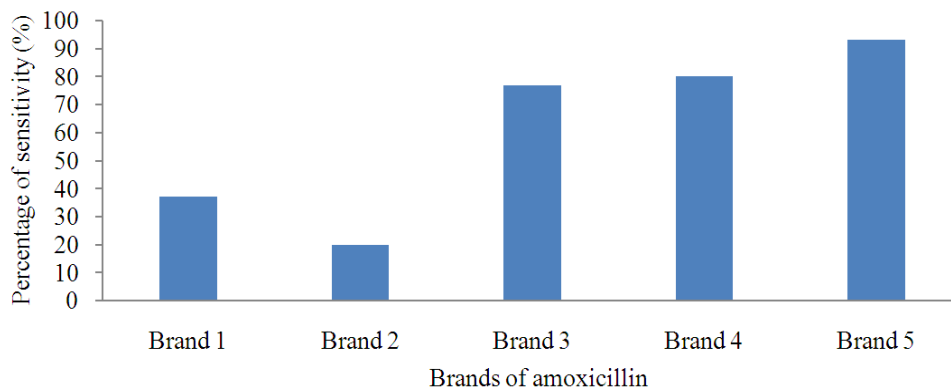
Organism	Number of isolates sensitive to amoxicillin				
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5
<i>Escherichia coli</i> (n = 10)	8	4	3	8	9
<i>Pseudomonas aeruginosa</i> (n = 10)	8	3	2	10	9
<i>Salmonella typhi</i> (n = 10)	10	5	2	10	8



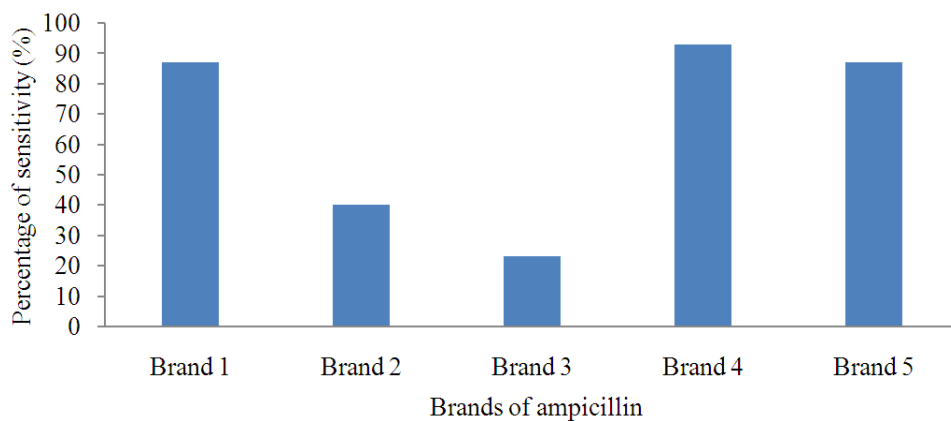
**Fig. 1.** Minimum Inhibitory Concentration (MIC) of amoxicillin for test isolates



**Fig. 2.** Minimum Inhibitory Concentration (MIC) of ampicillin for test isolates



**Fig. 3.** Percentage sensitivity of various brands of amoxicillin against test isolates



**Fig. 4.** Percentage sensitivity of various brands of ampicillin against test isolates

The rate of sensitivity of the clinical isolates to the various brands of Amoxicillin is presented as shown in **Fig. 3** while the rate of sensitivity of the clinical isolates to the various brands of Ampicillin is presented as shown in **Fig. 4**.

#### 4. DISCUSSION

A total of three hundred clinical isolates made up of 100 isolates each of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were subjected to Amoxicillin and Ampicillin drugs using the two-fold serial dilution technique. The result, as shown in **Fig. 1** indicated that a dose of  $62.50 \mu\text{g mL}^{-1}$  inhibited the growth of the organisms when exposed to Brand 1. For Brand 2, a dose of  $62.50 \mu\text{g mL}^{-1}$  inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*, while a dose of  $31.25 \mu\text{g mL}^{-1}$  inhibited *Salmonella typhi*. A dosage of  $3.90 \text{ g mL}^{-1}$  inhibited the growth of *Escherichia coli* and *Salmonella typhi* for Brand 3 while  $62.5 \mu\text{g mL}^{-1}$  inhibited the growth of *Pseudomonas aeruginosa*. The application of Brand 4 to the isolates showed an inhibitory dose of 31.25, 62.50 and  $3.90 \mu\text{g mL}^{-1}$  for *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* respectively. Brand 5 inhibited the growth of the isolates by a dose of 7.81, 62.50 and  $15.62 \mu\text{g mL}^{-1}$  respectively.

The use of ampicillin against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* as shown in **Fig. 2** indicated a required dose of 25.00, 25.00 and  $6.25 \mu\text{g mL}^{-1}$  respectively to inhibit the growth when exposed to Brand 1. However, a dose of 50.00, 25.00,  $50.00 \mu\text{g mL}^{-1}$ ; 50, 25,  $25 \mu\text{g mL}^{-1}$ ; 3.13, 3.13,  $3.13 \mu\text{g mL}^{-1}$  and 12.50, 6.25,  $3.13 \mu\text{g mL}^{-1}$  are required to inhibit the growth when exposed to Brand 2, Brand 3, Brand 4 and Brand 5 respectively.

The result of Amoxicillin indicates that a lower dose of Brand 3, Brand 4 and Brand 5 is required to inhibit the growth of *Escherichia coli* and *Salmonella typhi* when compared to other brands against the isolates. The use of Ampicillin recorded a higher dose of Brand 2, Brand 3 against *Escherichia coli* and *Salmonella typhi* while other brands indicated a low inhibitory concentration.

The result presented in **Fig. 3** showed that Brand 5 (93%) showed greater sensitivity of the clinical isolate. This is followed by Brand 4 (80%), Brand 3 (77%), Brand 1 (37%) and Brand 2 (20%). The result shown in **Fig. 4** indicates that Brand 4 (93%) showed

greater inhibition to the clinical isolates. This is followed by Brands 1 and 5 (87%), Brand 2 was 40% while Brand 3 was 23%.

**Figure 5** shows that 54 and 64% of *Escherichia coli* were sensitive to Amoxicillin and Ampicillin respectively. This means that 46% of *Escherichia coli* were resistant to the use of Amoxicillin, while 36% were resistant to Ampicillin. The result also shows that 38% of *Pseudomonas* were resistant to Amoxicillin, while 36% were resistant to Ampicillin. *Salmonella typhi* showed a resistance of 32% to amoxicillin and 30% to Ampicillin. This indicates that 61% of the test organisms were sensitive to Amoxicillin, while 66% were sensitive to Ampicillin. On the average, 64% of the isolates were sensitive to the antibiotics used in this study. This finding agrees with similar work carried out by Jazani and Babazadeh (2008) where they evaluated the sensitivity of *Pseudomonas aeruginosa* isolates to Ciprofloxacin. Their study showed that 50% of the isolates tested were resistant to ciprofloxacin.

This study has thus indicated that the efficacies of Amoxicillin and Ampicillin sold in the Nigerian market differ depending on their brands. Reasons which could be adduced for this difference ranges from the fact that the active ingredients in these antibiotics may be less than what is specified on the drug. The sensitivity of the antibiotic could also result from the deterioration of the active ingredients due to storage condition. One other factor which could be responsible is the resistance developed by the organism itself.

This study was not able to determine the factors which may have resulted in the non efficacies of some brands of the same type of antibiotic investigated.

#### 5. CONCLUSION

This study, which is aimed at determining the sensitivity of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* to various brands of amoxicillin and ampicillin was carried out using the two fold serial dilution method. The result showed that the sensitivity of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* to the various brands of Amoxicillin were 54, 62 and 68% respectively. The result also showed that the sensitivity of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* to Ampicillin were 64, 64 and 70% respectively. The



results of this study have shown that the efficacies of Amoxicillin and Ampicillin were 61 and 66% respectively. This study has therefore established the need for a routine evaluation of antibiotic in Nigerian markets, with the aim of detecting batches that have deteriorated or are of substandard.

## 6. ACKNOWLEDGEMENT

The authors would like to express their appreciation to the management and staff of Standard Diagnostic Centre, Warri; Delta State University Health Centre and Baptist Hospital, Eku for providing the clinical samples and the staff of Microbiology Laboratory, Delta State University, Abraka for assisting in the area of isolation of the various organism.

## 7. ADDITIONAL INFORMATION

### 7.1. Funding Information

This article was funded from personal funds.

### 7.2. Author's Contributions

**E.E. Anomohanran:** This author was involved in the collection of the clinical isolates, conducting of the experimental work and writing of the introduction, materials and methods, conclusion and the formatting of the references.

**U.B. Owhe-Ureghe:** This author was involved in the experimental work, the analysis of the results and the discussion of the results.

**D.A. Ehwareme:** This author was involved in the conduct of the experimental work and the write up of the result.

### 7.3. Ethics

The authors wish to state that this article conforms to the ethical standards specified by the American Journal of Agricultural and Biological Sciences.

## 8. REFERENCES

Adnan, S., D.L. Paterson, J. Lipman and J.A. Robert, 2013. Ampicillin/sulbactam: Its potential use in treating infections in critically ill patients. *Int. J. Antimicrobial Agents*, 42: 384-389. DOI: 10.1016/j.ijantimicag.2013.07.012

Agboke, A.A. and C.O. Esimone, 2011. Antimicrobial evaluation of the interaction between methanol extract of the lichen, *Ramalina Farinacea* (Ramalinacea) and Ampicillin against clinical isolates of *Staphylococcus aureus*. *J. Med. Plants Res.*, 5: 644-648.

Chayani, N., S. Tiwari, G. Sarangi and B. Mallick, 2009. Role of azithromycin against clinical isolates of family enterobacteriaceae: A comparison of its minimum inhibitory concentration by three different methods. *Ind. J. Med. Microbiol.*, 27: 107-110. DOI: 10.4103/0255-0857.45361

Cheesbrough, M., 2002. *Medical Laboratories Manual for Tropical Countries*. 1st Edn., Butterworth Kent, London, pp: 418.

Darabi, A., D. Hocquest and M.J. Dowzicky, 2010. Antimicrobial activity against *Streptococcus pneumoniae* and *Haemophilus influenzae* collected globally between 2004 and 2008 as part of the tigecycline evaluation and surveillance trial. *Diagnostic Microbiol. Infect. Dis.*, 67: 78-86. DOI: 10.1016/j.diagmicrobio.2009.12.009

Iroha, I.R., G.O. Ezeifeke, E.S. Amadi and C.R. Umezurike, 2009. Occurrence of extended spectrum B-Lactamase, producing resistant *Escherichia coli* and *Klebsiella pneumoniae* in clinical isolates and associated risk factors. *Res. J. Biol. Sci.*, 4: 588-592.

Jazani, N.H. and H. Babazadeh, 2008. Evaluation of the sensitivity of *Pseudomonas aeruginosa* clinical isolates to ciprofloxacin. *J. Biol. Sci.*, 8: 486-489. DOI: 10.3923/jbs.2008.486.489

NCCLS, 2002. Performance standards for antimicrobial susceptibility testing; Twelfth informational supplement. National Committee for Clinical Laboratory Standards, Wayne.

Nnela, K.S. and K.L. Cox, 1988. Potency deterioration of benzyl penicillin chloramphenicol and tetracycline. *Annual Rev. Med. Microbiol.*, 121: 166-172.

Owhe-Ureghe, U.B., 2011. Antibiotics susceptibility patterns of bacterial species isolated from fresh sea fishes sold in markets in Warri, Delta State, Nigeria. *Niger. J. Sci. Environ.*, 10: 260-269.

Pachaury, R. and A.K. Kataria, 2012. Evaluation of antimicrobial efficacy against *E. coli* isolates of different serogroups obtained from diarrhoeic neonates of calves, kids and lambs. *Media Peternakan*, 35: 175-178.

- Pearson, H., 2007. The dark side of *E. coli*. *Nature*, 445: 8-9. DOI: 10.1038/445008a
- Prescott, M.L., J.P. Harley and D.A. Kelvin, 2005. *Microbiology*. 6th Edn., McGraw Hill Company Bostan USA, pp: 992.
- Santos, R.L., S. Zhang, R.M. Tsohis, R.A., Kingsley and L.G. Adams *et al.*, 2001. Animal models of *Salmonella* infections: Enteritis versus typhoid fever. *Microbes Infect.*, 3: 1335-1344. DOI: 10.1016/S1286-4579(01)01495-2
- Tortora, G.J., B.R. Funke and C.L. Case, 2002. *An Introduction to Microbiology: Benjamin Curmmings*. 1st Edn., New York, pp: 887.
- Wiegand, I., K. Hilpert and R. Hancock, 2008. Agar and broth dilution methods to determine the Minimal Inhibitory Concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3: 163-175. DOI: 10.1038/nprot.2007.521
- Wood, G.L. and J.A. Washington, 1995. *Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods*. In: *Manual of Clinical Microbiology*, Murray, P.R. (Ed.), ASM Press, Washington DC., ISBN-10: 1555810861, pp: 1327-1341.