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Antibiotic Resistance: An Investigation on Effectiveness of Antibiotics Treatment on Bacterial Growth

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Abstract

Background: The proclivity of bacteria resistance to antibiotics has led researchers to develop more interest in antibiotics efficiency in tackling bacterial infections. As part of the effort to finding final resolutions to antibiotics effectiveness, this study was conducted to ascertain the antibiotic sensitivity pattern of three bacterial strains, this study was carried out from March 2019 to June 2019, using different antibiotics such as Ampicillin (AMP), Tetracycline (TET), Erythromycin (ERY), Chloramphenicol (C) Cephalexin (CN), Doxycycline (DO) and Streptomycin (STR) on *E. coli*, *Staphylococcus albus* and *Bacillus megaterium* as part of a project. The potential antagonistic effects were observed in all the antibiotics but with different effects on bacteria strains. Some of the antibiotics were very effective in some bacterial strains and others were less effective. The results obtained evidence that tetracycline has more effects on *E. coli* and *Bacillus meg*, but less effective on *Staphylococcus*. The most effective antibiotic for *E. coli* was chloramphenicol while the least effective was erythromycin. For *Staphylococcus albus*, the investigation result found Cephalexin to be more effective, while the least effective was ampicillin. However, Doxycycline also appeared to be more effective on *Bacillus megaterium* compared to chloramphenicol.

Subject Areas

Science and Medicine, Biomedical, Diseases Treatment

Keywords

Antibiotic, Resistance, Infection & Treatment, Erythromycin, Cephalexin, Chloramphenicol

1. Introduction

Antibiotic has been the key to improving performance and tackling antimicrobial resistance in both human and other animals for many years. With the evolution of improved methods of disinfection and surgical asepsis, minimally traumatic surgical techniques and the use of antibiotics to prevent infection, the incidence of postoperative endophthalmitis rapidly decreased from 10% during the late 1800s to substantially less than 1% in the modern surgical era (Axenfeld, 1908) [1]. The advent of multidrug resistance among pathogenic bacteria is imperilling the worth of antibiotics, which have previously transformed medical sciences. The crisis of antimicrobial resistance has been ascribed to the misuse of these agents and due to unavailability of newer drugs attributable to exigent regulatory requirements and reduced financial inducements (Aslam *et al.*, 2018) [2]. Evidence has shown the beneficial effects of antibiotics used in many studies, however, despite their effectiveness, the issue of over prescription has dominated the media and in the medical field. According to Jun *et al.* (2017) [3], the efficacy of antimicrobials is influenced by many factors: 1) bacterial status (susceptibility and resistance, tolerance, persistence, biofilm) and inoculum size; 2) antimicrobial concentrations; 3) host factors (serum effect and impact on gut micro-biota). The commonly used antimicrobials to treat bacterial infections have both safety and side effects, but they are generally safe and tolerated in the short-term (Suleyman and Zervos, 2016) [4]. However, serious long-term adverse effects may occur, it is therefore crucial for antibiotic selection to be individualised based on source of infection. There should be implementation standard precautions and infections control measures, minimising unnecessary antibiotic exposure, and optimising treatment and duration with removal of source of infection are all essential mechanisms to prevent the widespread resistance and improved outcomes (Suleyman and Zervos, 2016) [4].

According to Dr. Bearison of American Society for Microbiology, researchers at the USDA-ARS have investigated the consequences of antibiotic exposure on *Salmonella*, including multidrug-resistant strains for many years. They posited that while antibiotics are important drugs to preventing and curing diseases in humans and animals, the drugs can also have unintentional side effects if not properly administered for use.

Also, evidence from various researchers have shown that additional understanding of the linkage between antimicrobial usages, bacterial status and host response will offer researchers a new insight into how microbes can be combated and promote the struggle for the design of antimicrobial treatment prescription that would reach better clinical outcomes (Jun *et al.*, 2017) [3].

The use of antibiotics will inevitably eradicate bacterial infections in human and in some animals when administered correctly. Antibiotics serve as growth promoter additives in animal feed (Nathan and Cars, 2014) [5]. Many antibiotics can be excreted via urine and faeces as unchanged and active species (Rosi-Marshall and Kelly, 2015) [6]. Antibiotics are natural, synthetic and semi-synthetic com-

pounds that show antimicrobial activities (Catteau *et al.*, 2018) [7]. Antibiotics are currently the most successful family of drugs used in treating microbial infections in humans and animals, with specific action on the target (Kumar *et al.,* 2019) [8]. The use of antibiotics in modern medicine has played a significant role in decreasing the spread of diseases, however, over prescription of the drug in the recent years has caused most strains to become resistance to treatment. An accelerated use of known antimicrobials for human, animals, and agriculture benefit revealed that regular and repeated use of antibiotics have negative implication to the environment and natural ecosystem (Nielsen *et al.*, 2018) [9]. Antimicrobial resistance (AMR) poses a serious global threat of growing concern to human, animal, and environment health. This is due to the emergence, spread, and persistence of multidrug-resistant (MDR) bacteria or "superbugs" (Davies and Davies, 2010) [10].

Antibiotics do not only affect the target population in which it was meant to treat, but also influence the non-target population with high toxicity impact (Grenni *et al.*, 2018) [11]. In many instances, antibiotic residues in the environment can influence the selection of microorganisms and promote the dissemination of antibiotic resistant bacteria and antibiotic resistance genes (Rizzo *et al.*, 2013) [12]. According to Berendonk *et al.*, (2015) [13], Zhou *et al.*, (2013) [14] and Seiler and Berendonk (2012) [15], heavy metals can pose a co-selection pressure for antibiotic resistance, which ultimately lead to the emergence of multiple resistance strains for heavy metals and antibiotics.

2. The Aim of the Experimental Trial

This study aimed to investigate and identify the resistance of *Staphylococcus albus*, *Bacillium megaterium* and *E. coli* to tetracycline, doxycycline, ampicillin, chloramphenicol, cephalexin, streptomycin and Erythromycin.

3. Material and Methods

The beginning of the trial started with risk assessment to ensure hazard control mechanism was in place. To avoid cross contamination of equipment, Bunsen burner and ethanol were used to disinfect the equipment.

During the trial, agar plates were prepared 24 hours prior to culturing the bacteria. 12 plates were labelled with each bacteria, date and time of the inoculation. The bacterial growth assays were inoculated into nutrient broth in agar plate and were left for 30 minutes to allow the bacteria to adapt and settle on the plates. Thereafter, the plates were stamped using Bio-Rad disks of 6.5 mm disks made from superior quality absorbent paper and impregnated with precise concentrations of antimicrobial agents' samples such as ampicillin (AMP), tetracycline (TET), erythromycin (ERY), chloramphenicol (C) cephalexin (CN), streptomycin (STR) and Doxycycline (DO). These were incubated at 25˚C for seven days. This same process was repeated three times during 8 weeks period. After each trial, work area and equipment were disinfected with virkon to eradicate

any bacteria residue in order to create a safer place for others using the lab for experiment or investigation.

During each trial, the zone of inhibition was calculated; using $A = \pi r^2$ and the results were recorded. Data were presented as mean and standard deviation as a cumulative trial. The data was manually calculated to determine statistical significance at $p < 0.05$. The closer the value is to 0.00, the better, showing minimum significance difference between the mean of each bacterium. The values showing higher numbers specify that there is a significance difference between the values used to produce the mean and the mean itself.

For an effective documentation of the result accuracy, reliability and validity in this trial, the time was organised to allow proper check of the bacteria growth and data collation. A contingency plan was put in place in case there is any short of equipment and materials required to carry out the trial.

4. Experimental Data Design

Several indicators were used to measure antibiotic use in each trial. The most commonly used metric was the design of the dosage put in the plate. The investigator ensures the dosage in each trial was equal as a control measure to avoid overdose that may influence the results. However, there were several limitations on the uses as occasionally, the antibiotics disks developed a problem that could not be resolved the same day, but an alternative was used to ensure the timescale was met. It is though believed that specific combinations of drugs would reduce drug resistant bacterial infections. As presented in **Table 1**, the sample treatments with the antibiotics have different inhibition zones. Negative results were achieved in some of the trials; probably due to the bacteria being resistance to treatment or due to contaminated equipment, systematic or sample errors. However, the repeated experiment ensures the minimization of any error during the trial.

During the first trial, *E. coli* was treated with seven different antibiotics, however, erythromycin shows very low inhibition zone. Data suggests the potential inhibitory effect of ampicillin, tetracycline, chloramphenicol, cephalexin, and doxycycline on the bacterial infections.

The result shows that different antibiotics have different inoculum effects on the growth and selection of resistance of the same strain. However, a small inoculum effect was observed for some of the antibiotic like erythromycin and a significant inoculum effect for chloramphenicol on *E. coli*. The researcher sees the need to put recommendation forward on the future research of the effectiveness of chloramphenicol use for *E. coli* due to its effectiveness in this trial.

The trial result (**Figure 1**) reveals chloramphenicol, a promising drug that can reduce the rate of *E. coli* spread and resistance to treatment. Many antibiotic resistances have become a major global public health problem (Aslam *et al.*, 2018) [2]. Therefore, chloramphenicol is of unique interest for a variety of reasons (Hahn, 1967) [16]. It is the first antibiotic to be completely synthesised by methods of organic chemistry and is still the only antibiotic that is industrially produced by chemical synthesis rather than by fermentation (Hahn, 1967) [16].

Table 1. Mean of inhibition zone of different treatments of bacterial species.

Inhibition zone of Bacteria strains					
Antibiotics	**Bacteria strains**	**1st trial**	**2nd trial**	**3rd trial**	**Mean average**
STR	*E. coli*	2.01	1.53	1.13	1.56
	Bacillus megaterium	2.54	2.01	2.01	2.19
	Staphylococcus albus	2.01	1.13	1.54	1.56
AMP	*E. coli*	2.83	0.5	1.13	1.49
	Bacillus megaterium	3.8	2.54	0	3.17
	Staphylococcus albus	1.54	0.79	0	1.16
TET	*E. coli*	2.01	0	0	2.01
	Bacillus megaterium	3.14	0	0	3.14
	Staphylococcus albus	0	0	0	0
ERY	*E. coli*	1.33	0	0.5	0.91
	Bacillus megaterium	2.54	2.54	0	2.54
	Staphylococcus albus	2.01	2.01	2.01	2.01
C	*E. coli*	3.8	2.01	1.54	2.45
	Bacillus megaterium	2.01	1.53	2.01	1.85
	Staphylococcus albus	2.01	2.01	2.01	2.01
CN	*E. coli*	3.14	0.79	0.79	1.57
	Bacillus megaterium	2.54	2.01	2.54	2.36
	Staphylococcus albus	3.14	2.54	3.14	2.94
DO	*E. coli*	0	0	1.54	1.54
	Bacillus megaterium	0	4.52	3.8	4.16
	Staphylococcus albus	0	2.01	0.5	1.25

Trends in mean of the effects of different antibiotics such as ampicillin (AMP), tetracycline (TET), streptomycin (STR) erythromycin (ERY), chloramphenicol (C) cephalexin (CN) and doxycycline (O) on *E. coli*.

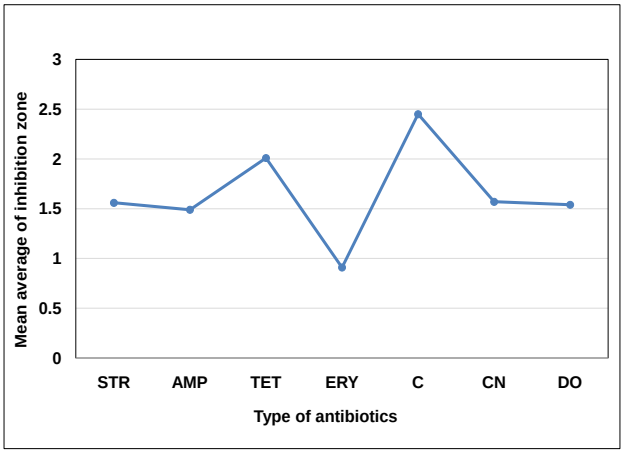


Figure 1. Effects of antibiotics on *E. coli* (Zone of inhibition (cm^2). Trends in mean of the effects of different antibiotics such as ampicillin (AMP), tetracycline (TET), streptomycin (STR) erythromycin (ERY), chloramphenicol (C) cephalexin (CN) and doxycycline (O) on *E. coli*.

Several authors such as Brock (1961) [17], Davis and Feingold (1962) [18], Gale (1963) [19], Hahn (1964) [20], Goldberg (1965) [21] and Vazquez (1966) [22] have provided evidence on advancement in knowledge of the mechanism of action for chloramphenicol. Chloramphenicol is a widely used antibiotic with nitro and chlorine substituents which is resistant to traditional biological treatments (Nie *et al*., 2014 [23] and Pilehvar *et al*., 2012) [24]. Bio-electrochemical system is an efficient method for Chloramphenicol wastewater treatment because it removes the chlorine substituent and transforms the nitro group into amino substituent (Liang *et al*., 2013 [25]; Yan *et al*., 2019 [26], Guo *et al*., 2017 [27]; Sun *et al*., 2013 [28]; Yan *et al*., 2018 [29] and Wang *et al*., 2011) [30].

It is therefore absolutely vital to exploit more benefit of chloramphenicol's effectiveness treatment of *E. coli*. Potrykus and Wegrzyn (2011) [31], once state that an *E. coli* strain (strain CM2555) bearing the chloramphenicol acetyltransferase (*cat*) gene was found to be sensitive to chloramphenicol, when it was tested on cat gene. According to Brock, (1961) [17], chloramphenicol has been known to inhibit the growth of bacteria, and its mode of action is thought to be by inhibition of protein synthesis. Lacks and Gros (1960) [32] also found that the initial rate of incorporation of radioactive amino acids into RNA in *E. coli* treated with chloramphenicol was decreased by 80%.

Previous research also shows that a strain of *E. coli* is partially resistant to tetracycline (Franklin and Godfrey, 1965) [33]. However, this trial disproof this idea as the rate of treatment throughout the trial period was consistent. Tetracycline antibiotics are well known for their broad spectrum of activity, spanning a wide range of Gram-positive and -negative bacteria, spirochetes, obligate intracellular bacteria, as well as protozoan parasites. The first tetracyclines were natural products derived from the fermentations of actinomycetes (Grossman, 2019) [34]. When tetracycline was discovered in 1950s, their antimicrobial spectrum was broader than any other antibiotic known then (Dürckheimer, 1975) [35]. Tetracyclines are characterised by their exceptional chemotherapeutic efficacy against a wide range of Gram positive and Gram-negative bacteria (Dürcheimer, 1975) [35]. The main use of tetracycline is due to its effectiveness in infectious diseases caused by *E. coli* and *Haemophilus influenzae.* Tetracycline are inexpensive antibiotics which cannot be used to treat human but has been used extensively in the prevention and therapy of animal infections and at subtherapeutic levels in animal feed as growth promoters (Chopra and Robert 2001) [36]. Tetracycline prevents aminoacyl-tRNA to the ribosomal acceptor site (Chopra and Robert 2001) [36]. It plays an important role against a wide range of gram-positive and gram-negative bacteria in tropical organisms.

Bacillus megaterium can be infection to human; it is thought that understanding the antibiotic spectrum of these pathogens and their common mechanisms of antibiotic resistance is crucial. It is therefore crucial to understand the mechanisms associated with the emergence and distribution of the resistance of this infectious disease. Antibiotics which appear especially useful in the treatment of

Bacillus infections are clindamycin and vancomycin, to which clear majority of the strains are susceptible *in vitro*. Beta-lactam antibiotics, including the new cephalosporins and penicillin, are of little value in this setting (Sliman, R., Rehm, S. and Shlaes, D.M. 1987) [37].

This trial discloses positive use of doxycycline antibiotic on *Bacillus megaterium* bacteria. During the trial, it was observed that the zone of inhibition of doxycycline outweighed that of ampicillin, tetracycline, erythromycin, chloramphenicol, streptomycin and cephalexin. Ampicillin also had a significant effect on the bacteria and the least effective antibiotic was cephalexin. It is essential to note that some difficulties were encountered during the documentation of this data. The concerns were centred on data recorded for ampicillin. Two sets of results were recorded for the different trials. Some had a higher inhibition zone average, and others were either very low or zero which created doubt whether there were some anomalies in the data. The three repeated trials were used to avoid significant error that may have occurred.

The treatment of *Bacillus megaterium* using different antibiotics also produced different results based on the effectiveness of the antibiotics. Members of the *Bacillus genus* are generally found in soil and most of these bacteria have the ability to disintegrate proteins, namely proteolytic activity. It was reported that the resistance to cephalexin was due to how *Bacillus* synthesised cephalexin. An examination of the antibiotic resistance of the bacteria strains revealed the *Bacillus megaterium* was highly resistance to streptomycin and cephalexin.

According to Aslim *et al.* (2002) [38], *Bacillus megaterium strains* have an antagonistic effect on antibiotics. *Bacillus megaterium strain* can be used in certain biotechnological studies. *Staphylococcus albus* was also tested on the antibiotic ampicillin, tetracycline, erythromycin, chloramphenicol, cephalexin, streptomycin and doxycycline.

The results obtained from the use of streptomycin produced three values which were different; however, there was no significant difference between them (**Figure 2**). They were all in the range of 1 to 2 cm^2 and were valuable results when compared to the rest of the data. Ampicillin also shows two sets of results with numbers different from each other. Erythromycin unveils much accurate result with all recorded data closed to each other. Chloramphenicol data obtained during the trials suggest reliability and accuracy of the data.

In relation to the effectiveness of preventing *Staphylococcus* growth, cephalexin presents a more promising outcome. Three trials were carried out and two of the results manifest positive correlation. As for, doxycycline, the numbers were assumed not to be accurate as expected, due to significant difference between the inhibition zone in the different weeks. However, when ampicillin was compared with doxycycline, there was no significant difference. The average result for both had little effects on *Staphylococcus* growth. There was a large significant difference between cephalexin and erythromycin, despite both having significant effects on the treatment *Staphylococcus albus*.

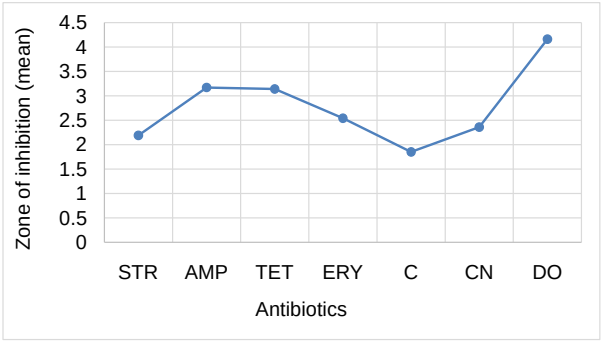


Figure 2. *Bacillus megaterium bacteria* (Zone of inhibition (cm^2). Trends in mean of the effects of different antibiotics such as ampicillin (AMP), tetracycline (TET), erythromycin (ERY), chloramphenicol (C) cephalexin (CN), streptomycin (STR) and doxycycline (O) on *Bacillus megaterium*.

When compared the mean of the different bacteria strains with the antibiotics used, doxycycline had the highest mean value followed by ampicillin and tetracycline respectively. However, no result was obtained from tetracycline on *staphylococcus* treatment. The comparison of the data shows a clear correlation which tallied with the idea of Lee *et al.* (2010) [39] investigation. Antibiotic-associated signal can influence the resistance development in bacterial population (Lee *et al.*, 2010) [39]. Under an increasing concentration of norfloxacin, highly resistant *E. coli* population excreted indole as a signalling molecule to the susceptible population, which can cause susceptible strains to regulate the efflux pump expression (Lee *et al.*, 2010) [39]. However, there is no evidence that antibiotic can function as a signal, but can lead to the selection of bacteria that would produce signal, which, in turn, increased the MIC of the entire population (Andersson and Hughes, 2014) [40].

In this investigation, cephalexin was found to be an effective treatment for *Staphylococcus albus* (**Figure 3**). The study carried out by Rayner and Munckof (2006) [41] revealed that *Staphylococcal* infections are a common and significant clinical problem in medical practice. Most strains of *Staphylococcus albus* are now resistant to penicillin, and methicillin-resistant strains of *S. albus* (MRSA) are common in hospitals and are emerging in the community. This investigation result agrees with report of Rayner and Munckof. New antibiotics such as linezolid and quinupristin or dalfopristin have been revealed to have a good anti-staphylococcal activity. However, the cost to purchase the drug is expensive (Rayner and Munckof, 2006) [41]. The resistance of *staphylococcus* to tetracycline in the trial unveils a greater value than all the antibiotics used. Compared to erythromycin, there was a significant difference 2.94 > 2.27 which disclosed that patients will respond faster to tetracycline than erythromycin.

This research study evidence indicates that *E. Coli* manifest resistant to erythromycin due to the inhibition zone lower than 1 cm^2, while *Staphylococcus albus* and *Bacillus megaterium* have larger zone of inhibition (**Figure 4**). A general analysis of this data table disclosed that doxycycline, ampicillin and tetracycline

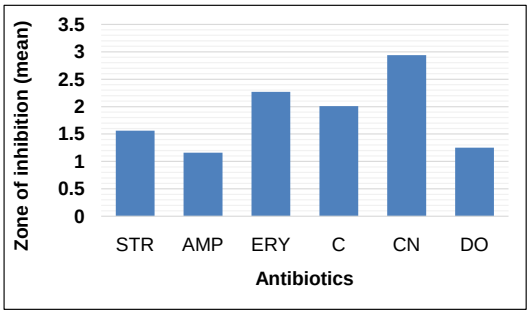


Figure 3. *Staphylococcus albus* (zone of inhibition (cm^2). Trends in mean of the effects of different antibiotics such as ampicillin (AMP), tetracycline (TET), erythromycin (ERY), chloramphenicol (C) cephalexin (CN), streptomycin (STR) and doxycycline (O) on *Staphylococcus*.

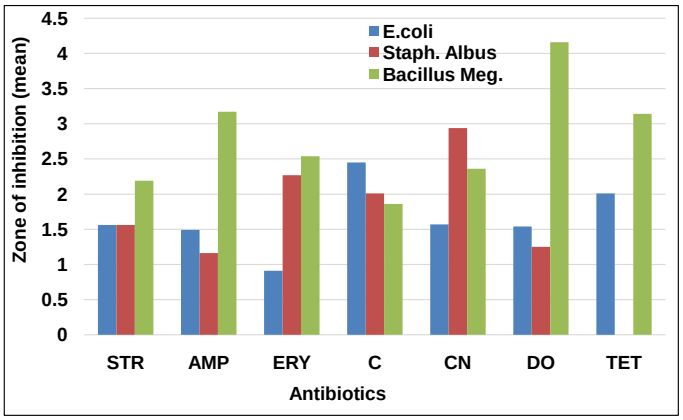


Figure 4. Comparison of different antibiotics on bacteria strains. Relationship between antibiotics: ampicillin (AMP), tetracycline (TET), erythromycin (ERY), chloramphenicol (C) cephalexin (CN), streptomycin (STR) and doxycycline (O) and bacteria strains: *Staphylococcus*, *E. coli* and *Bacillus megaterium*.

have more effects on *Bacillus meg*. Cephalexin and Tetracycline also play significant effects on *E. coli.* The most effective antibiotic depends on the bacteria, but chloramphenicol proof to have a relatively large zone of inhibition on all three bacteria investigated in this trial. The most effective antibiotic for *E. coli* in this investigation appears to be chloramphenicol, while the least effective is erythromycin. For *Staphylococcus albus*, the most effective antibiotic is cephalexin, while the least effective is ampicillin. The most effective antibiotic for *Bacillus meg* is doxycycline while the least effective is chloramphenicol despite its effectiveness in most strains.

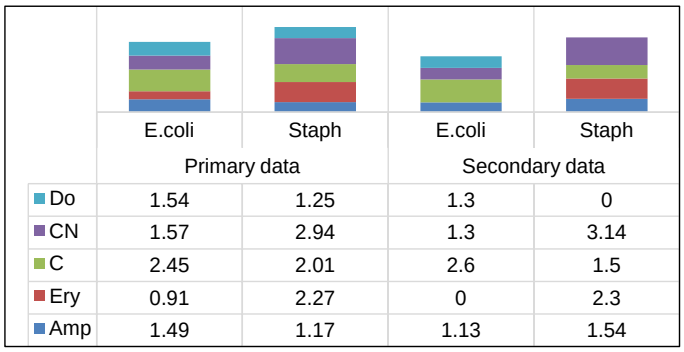
A remarkable less significant difference was observed in the resistance pattern of *staphylococcus* to cephalexin and erythromycin. This reveals that data isolates of *staphylococcus* epidermidis was resistant to cephalexin and erythromycin. However, the result obtained in the primary data did not indicate significant difference in the treatment. The outcome of this result was supported by the secondary data. The authors did not find significant difference between primary and secondary data. Evidence from primary and secondary data could not find

any significant difference on the effectiveness of doxycycline on *staphylococcus* and *E. coli*, which is a clear indication that this investigation result is supported by the secondary data (**Figure 5**). On the effectiveness of the antibiotics, doxycycline seems to be effective in the treatment of *E. coli* as both data revealed high zone of inhibition. Cephalexin seems to be more effective in *staphylococcus* treatment.

Ampicillin did not show promising results on effective treatment of *E. coli*. However, ampicillin is a beta-lactam antibiotic that attacks Gram-positive and some Gram-negative bacteria (Lawrence and Anthony, 2013) [43]. The amino group in ampicillin allows it to penetrate the outer membrane of Gram-negative bacteria. It will then become an inhibitor of transpeptidase, which is needed for bacterial cell wall formation, and eventually leads to cell disintegration (Anderson and Hughes, 2014) [40]. With the appearance of antibiotic-resistant bacteria, increasing numbers of infections are causing huge losses to both economic concerns and social resources over recent decades, and this has become a global problem (Zhou and Wang, 2013) [14]. Global antibiotic resistance shows no signs of decline, though it may perhaps shift direction (Aslam, 2018) [2].

Many findings suggest that inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of infections more unlikely (Kolár *et al.,* 2001 [44] and Rasheed *et al.,* 2014 [45]). This investigation reveals that understanding the mechanism in which bacteria evolve to antibiotics will provide valuable machinery that will help develop a more efficient and rapid transfer of antibiotic resistant bacteria.

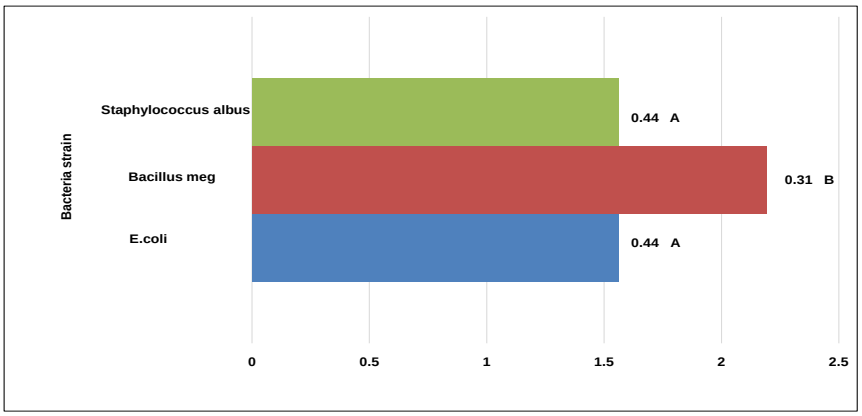
A research team led by a distinguished Professor of microbiology and molecular genetics of plant, soil and microbial sciences, James Tiedje of Michigan State University state very clearly that, in the fight against the rise of antibiotic resistance bacteria, researchers need to understand the use of antibiotic more efficiently. The team stress that tracking the source of antibiotic resistance is quite complicated because of antibiotic use, which increases the occurrence of widespread resistance (MSU, 2016) [46].



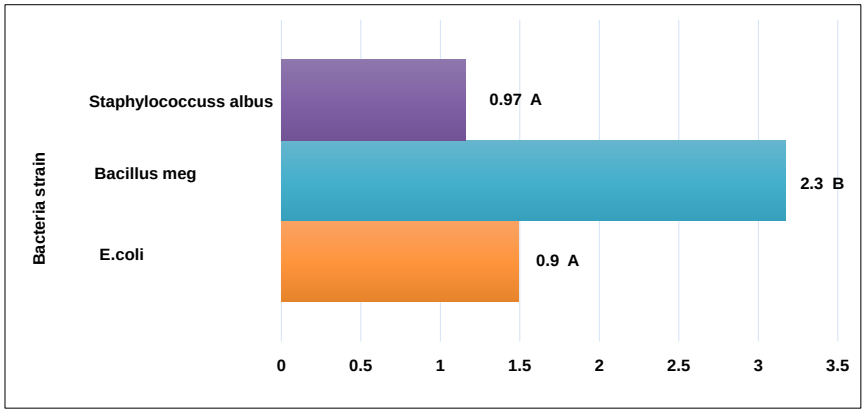
	E.coli	Staph	E.coli	Staph
	Primary data		Secondary data	
Do	1.54	1.25	1.3	0
CN	1.57	2.94	1.3	3.14
C	2.45	2.01	2.6	1.5
Ery	0.91	2.27	0	2.3
Amp	1.49	1.17	1.13	1.54

Figure 5. Comparison of primary data and secondary data on *E. coli* and *Staphylococcus* using antibiotics: ampicillin (AMP), erythromycin (ERY), chloramphenicol (C) cephalexin (CN) and doxycycline (O). Secondary data source: Sani R. A., Garba S. A. and Oyewole O. A. (2012) [42].

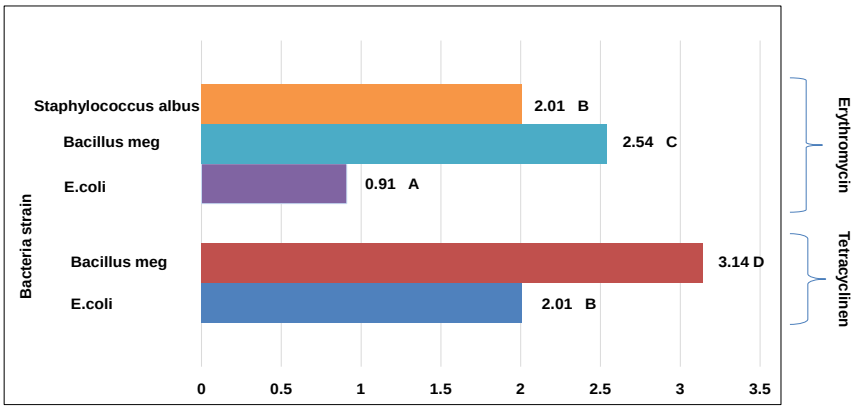
Standard deviation was used in this study to test the accuracy of the result in order to ascertain the correlation between them. The data detailed in Figure 6 shows significant different between the results and the mean average. A clear indication of minimal error is represented with smaller values. The higher values indicate high significant difference between the values used to produce the mean.



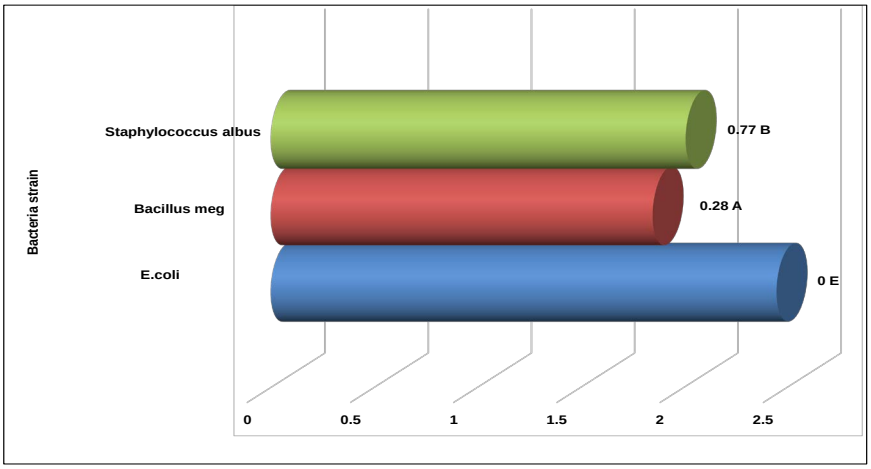
Evaluation of the effectiveness of streptomycin use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 0.40$).



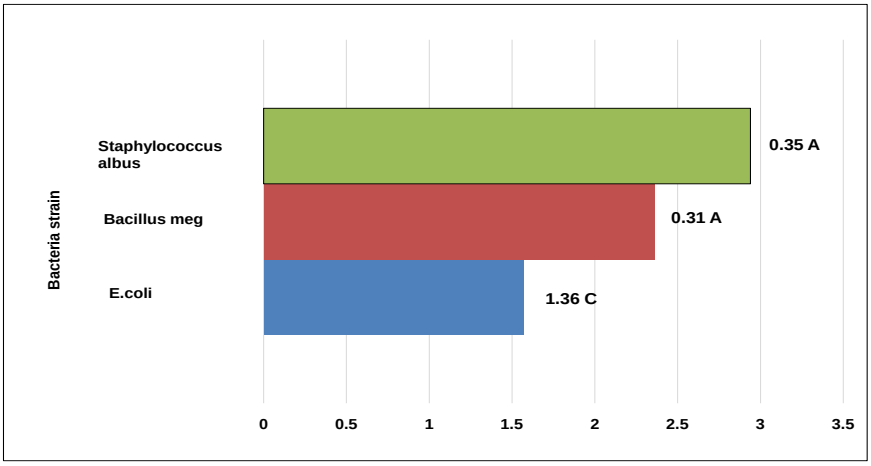
Evaluation of the effectiveness of ampicillin use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 1.4$).



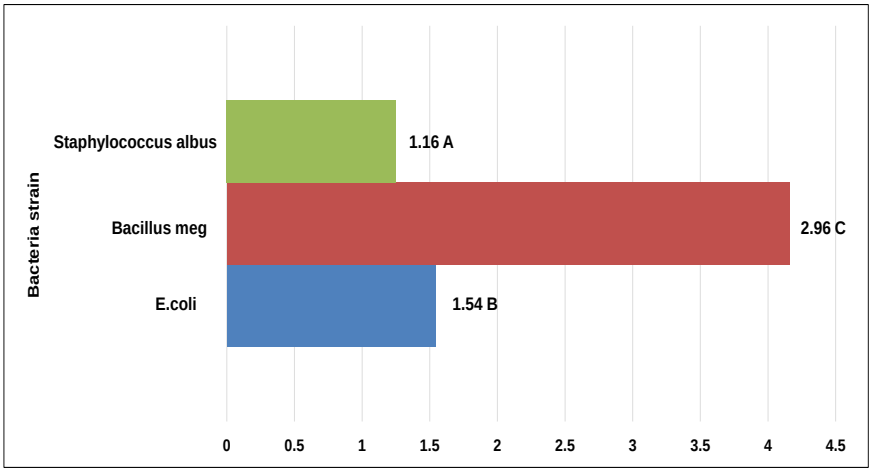
Evaluation of the effectiveness of tetracycline use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 2.1$).



Evaluation of the effectiveness of chloramphenicol use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 0.35$).



Evaluation of the effectiveness of cephalexin use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 0.67$).



Evaluation of the effectiveness of doxycyclin use on bacteria. Data followed by Unequal Letters Significantly Different ($p \leq 0.05$; researcher's t-test, $s^2 = 1.9$).

Figure 6. A test for significant difference of the various treatments.

Those with zero mean shows that all data values were equal to the mean. Taking example from the use of streptomycin to treat the three bacteria strains, significant difference was not found between *Staphylococcus* and *E. coli*. The standard deviation of both was 0.44 each from the mean. However, on the effectiveness of treatment, streptomycin was more effective in the treatment of *Bacillus megaterium*.

5. Conclusions

Antibiotic resistance poses serious threat to human and animal health globally if not used correctly (Berendonk *et al.,* 2015 [13] and Misra *et al.,* 2017 [47]). Important measures are required to reduce the risks posed by antibiotic resistance genes that occur in the environment. Evidence of this trial confides that further research is encouraged to investigate the key contributing factors to antibiotic resistance.

Understanding the influence of antibiotics on multidrug-resistant bacteria is critical to the proper selection and prudent use of antibiotics, while minimising potential collateral consequences (Science Daily, 2017) [48]. The world is currently at alert of the challenge of simultaneous expanding appropriate access to antimicrobials, while restricting inappropriate access, particularly to expensive, newer generation antimicrobials (Laxminaravan *et al*., 2016) [49].

The outcome of this investigation revealed that further experiments with bacterial strains will provide deeper understanding of bacterial resistance. This study found that antibiotics play significant role in restricting the further invasion of microbes in the body despite some strains being resistance to their treatments. The trial findings suggest that bacterial resistance has been widespread infections that require an immediate attention to prevent it from becoming serious menace in all parts of the world. Early treatment—antibacterial therapy is indeed necessary and should be promptly initiated. However, inadequate use of antibacterial (e.g., doses that are too low, therapy ended prematurely) is a major factor for the selection of resistant strains (Sani R. A., Garba S. A. and Oyewole O. A., 2012) [42]. In a view to tackling the growing problem of antibiotics resistance, a global action plan was endorsed at the World Health Assembly in May 2015 (WHO, 2015) [50]. One of the plan's 5 objectives is to improve awareness and understanding of antibiotic resistance through effective communication, education and training. Antibiotic treatment is one of the main approaches of modern medicine which is used to tackle infections. The "golden era" of antibiotics ranged from the 1930s to 1960s that gave rise to many antibiotics (Aslam, 2018) [2].

There is evidence that numerous attempts have been made to delineate the diverse aspects of antibiotic resistance and researches are still ongoing to with the aim to producing new drugs, however, a principally coordinated campaign is lacking, particularly at the political level worldwide to support researchers' onus of increasing our knowledge on antimicrobial resistance (AMR) threat on global population.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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