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ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM INDIGENOUS SLAUGHTER CHICKEN IN NAIROBI, KENYA

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ABSTRACT

Background: **Indigenous chicken, which end-up being consumed by humans, are normally raised free-range in villages; feeding from the ground which could easily be contaminated by substances, including bacteria, brought-in by flood water during heavy rains. The infected chickens may then serve as sources of the bacterial strains to humans who handle and/or consume them. If these bacteria are pathogenic to humans and resistant to antibiotics, it will be difficult to treat the resultant human diseases using the particular antibiotic(s).**
Objective: **To establish antibiotic susceptibility/resistance patterns of bacteria isolated from intestines of slaughtered indigenous chickens after heavy rains in Nairobi, Kenya.**
Design: **This was a cross-sectional study**
Subjects: **Bacterial isolates from chicken intestinal-content obtained from three slaughterhouses in Nairobi,**
Methodology: **Antibiotic susceptibility testing was carried out on *Escherichia*, *Staphylococcus* and *Streptococcus* using disc diffusion technique.**
Setting: **Laboratory testing at the University of Nairobi Bacteriology laboratory.**
Results: ***Escherichia*** **isolates were highly resistant to Ampicillin, Sulphamethoxazole and Amoxicillin at 100%, 93.3%, 93.3% respectively; 13.3% were resistant to Gentamycin, while all were susceptible to Ciprofloxacin.** ***Staphylococcus*** **isolates were resistant to Clindamycin at 73.3%, Tetracycline at**

46.7%, Chloramphenicol at 40%, but all were susceptible to Sulphamethoxazole and Erythromycin. *Streptococcus* isolates were resistant to Sulphamethoxazole, Clindamycin, Erythromycin, Tetracycline, Chloramphenicol at 93.3%, 86.7%, 60%, 60% and 53.3% respectively; the three isolates showed multidrug resistance.
Conclusion: **The study showed that antibiotic resistance is still a threat to the lives of animals/humans, if the resistance gets transferred to pathogenic bacteria it will be difficult to cure the disease caused by antibiotic resistant pathogens. It is therefore, recommended that, before prescribing antibiotics, antibiotic susceptibility testing should be done. Also, prudent usage and disposal of antibiotics is recommended in order to reduce development and transfer of resistance within and across bacteria.**

INTRODUCTION

In Kenya, poultry population is about 36 million; over 80% of which are of indigenous type (Justus *et al*., 2013); These indigenous chicken are kept under free-range system of management in villages where they scavenge for food with little or no supplementation (Kingori *et al*., 2010). Because of their feeding habit, the indigenous chickens pick different materials from the environment, which include: antibiotics, bacteria, insects, herbs, among others which should have been contaminated by bacteria with resistant genes (Justus *et al*., 2013). These chickens serve as a source of protein to humans in form of meat and eggs (Kingori *et al*., 2010).

By feeding from the environment, chicken can acquire resistant bacteria which are later transferred to humans following consumption of contaminated poultry meat. The resistant bacteria may in turn transfer resistant genes to other bacteria in the human body since gene transfer among bacteria has been demonstrated to occur easily (Kikuvi *et al*., 2007). The birds may also pick the antibiotic-resistance bacteria and free antibiotics from the soil or the water that they drink; these can increase the chance of inducing antibiotic resistance in their gut flora (Marshall and Levy, 2011; Kikuvi *et al*., 2007). Antibiotic resistance has been among the top global health challenges for a number of years now, and is becoming worse as the years go-by (Marshall and Levy, 2011; Fair and Tor, 2014). In cases of antibiotic resistance, the resultant food-borne or animal-acquired illnesses in humans will be less responsive to treatment with respect to the particular antibiotic(s) (Fair and Tor, 2014).

Environmental contamination could be from farmland, in form of manure or sludges containing bacteria (some of which could be antibiotic-resistant) and free antibiotics disposed by pharmacies, hospitals and/or patients (Burkhardt *et al*., 2005) K'oreje *et al*., 2016); from flooding, that play a very big role in disease outbreaks due to environmental destruction and their contribution towards spread of disease- carrying vectors, insects, a wide variety of herbs, among others (Opere, 2013); which may be harboring antibiotic-resistant genes, which will end-up being transmitted to bacteria that the birds were carrying originally. Although *Escherichia coli, Staphylococcus* and *Streptococcus,* organisms tend to exist as normal flora in intestinal tracts of different animals including chickens, most strains are opportunistic and can cause diseases (Aarestrup *et al*., 2000; White *et al*.,

2003). However, whether pathogenic or not, when they acquire antibiotic-resistance genes or create antibiotic-resistance pressure, they become a very big threat to animal and human health (Adelaide *et al.*, 2008).

Antibiotics are essential for human and animal health, but need to be used cautiously, noting that food animals are important to human welfare (Chwarza and Anclab, 2001; Marshall and Levy, 2011). This study was conducted to establish antibiotic susceptibility/resistance patterns of three bacteria belonging to the genera *Escherichia*, *Staphylococcus* and *Streptococcus*, which were isolated from intestines of Kenyan indigenous chickens.

MATERIALS AND METHODS

Study design: A cross sectional study was carried out, where intestines of slaughtered indigenous chickens were bought from three different slaughterhouses (Kariokor, Burma and Kangemi) in Nairobi.

Sample collection and processing: One hundred and twenty (120) samples were collected; 40 from each of the three slaughterhouses. The intestines were placed in sterile universal bottles separately and transported in a cool box to the bacteriology laboratory, Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for processing. At the laboratory, bacterial isolation and characterization from chicken intestinal contents were done using standard bacteriological methods (Markey *et al.*, 2013). The three most isolated bacteria, which belonged to the genera *Staphylococcus, Streptococcus* and *Escherichia,* were used to test for antibiotic susceptibility/resistance to commonly used antibiotics in animals/human treatment.

Antibiotic susceptibility/ resistance testing: The *Staphylococcus spp, Streptococcus spp* and *Escherichia coli* isolates were tested for antibiotic susceptibility using disc diffusion method, using Mueller-Hinton (MH) agar (Oxoid, Basingstoke, United Kingdom), following the method given by the Clinical and Laboratory Standards Institute (CLSI, 2016). Five isolates of each species from each slaughterhouse were randomly chosen for testing, and the reference strains, ATCC 25923 *Staphylococcus aureus* and ATCC 25922 *Escherichia coli,* used to compare the results.

The following antibiotic discs from Oxoid company were used respectively for the isolates; guided by CLSI (2016): for *E. coli* isolates, the drugs used were Gentamycin (10µg), Amoxycillin (30µg), Ciprofloxacin (5µg), Sulphamethoxazole (23.75µg) and Ampicillin (10µg); for *Staphylococcus* and *Streptococcus* isolates, the drugs used were Sulphamethoxazole (23.75µg), Erythromycin (5µg), Clindamycin (2µg), Chloramphenicol (30µg) and Tetracycline (30µg).

After seeding the Muller Hinton plates with bacterial suspensions whose turbidity was adjusted to match that of 0.5 MacFarland nephelometer tube, respective antibiotic discs were placed on the seeded plates/agar and incubated at 37° C overnight prior to reading. Growth inhibition zones were then measured using a ruler, in millimeters, and results interpreted according to the guidelines provided by CLSI (2016).

Data analysis: The results were analyzed by Chi-square using Statistical Package for the Social Sciences (SPSS) to test the association between the antibiotic resistance among the isolates and with their respective slaughterhouse at p-value of 0.05.

RESULTS

E. coli isolates showed antibiotic resistance to Ampicillin, Sulphamethoxazole; Amoxicillin, Gentamycin and Ciprofloxacin at 100%, 93.3%, 93.3%, 13.3% and 0% respectively. Thus, all the tested *E. coli* isolates were susceptible to Ciprofloxacin but were resistant to more than 2 antibiotics. *E. coli* were not statistically significant to the antibiotics among the slaughterhouses with p- values of 0.6, 0.3 and 0.3 for Gentamycin, Sulphamethoxazole and Amoxicillin respectively. The tested *Staphylococcus* isolates were resistant to the tested antibiotics as follows: Clindamycin at 73.3%, Tetracycline at 46.7%, Chloramphenicol at 40%, Sulphamethoxazole at 0%, Erythromycin at 0%; 46.7% of the isolates showed resistance to two or more antibiotics and 13.3% were susceptible to all the antibiotics tested. Susceptibility/resistance of the tested *Staphylococcus* were not statistically significant to the antibiotics among the slaughterhouses; for Chloramphenicol and Clindamycin with p-value of 0.2 for both and to Tetracycline with p-value of 0.4.

Streptococcus isolates were resistant to the tested antibiotics as follows: Sulphamethoxazole at 93.3%; Clindamycin at 86.7%; Erythromycin at 60%; Tetracycline at 60%; Chloramphenicol at 53.3%; 93.3% of the isolates showed resistance to more than two antibiotics, while 13.3% were resistant to all the antibiotics used. The Susceptibility/resistance test results for *Streptococcus* were not statistically significant to the antibiotics among the slaughterhouses; for Sulphamethoxazole, Erythromycin, Chloramphenicol and Tetracycline with p-values of 0.3,0.4,0.8 and 0.4 respectively. However, there was a significance difference in the susceptibility of *Streptococcus* to Clindamycin among the slaughterhouse with P-value of 0.01.

The prevalence of resistance of the isolates to the antibiotics for the various sites is shown by

Figure 3 for *E. coli* and Figure 4 for both *Staphylococcus* and *Streptococcus* isolates. *E. coli* reference strain, ATCC 25922, was resistant to Amoxycillin, Ampicillin and Sulphamethoxazole and highly susceptible to Ciprofloxacin and Gentamycin; while *Staphylococcus aureus* reference strain, ATCC 25923, was susceptible to all antibiotics tested. Table 1 Shows antibiotic resistance patterns and respective frequencies, per bacterial isolate for each antibiotic.

Table 1

Antibiotic resistance patterns and respective frequencies

Bacteria	Antibiotics tested	Frequency of resistance
E. coli (n= 15)	AMC	14 (93.3%)
	AMP	15 (100%)
	CIP	0 (0%)
	CN	2 (13.3%)
	RL	14 (93.3%)
Staphylococcus (n=15)	TE	7 (46.7%)
	DA	11 (73.3%)
	RL	0 (0%)
	E	0 (0%)
	C	6 (40%)
Streptococcus(n=15)	TE	9 (60%)
	DA	13 (86.7%)
	RL	14 (93.3%)
	E	9 (60%)
	C	8 (53.3%)

Where: n: number of isolates, CN: Gentamycin, AMC: Amoxycillin, RL: Sulphamethoxazole, AMP: Ampicillin, DA: Clindamycin, TE: Tetracycline, C: Chloramphenicol and E: Erythromycin.

Figure 1. Showing Multidrug resistance (MDR) frequencies per isolate

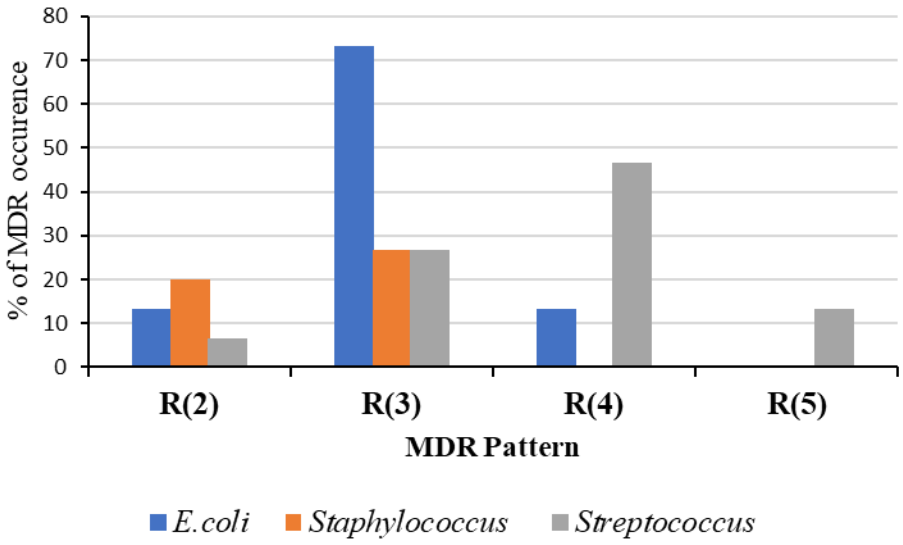

Figure 1. Showing Multidrug resistance (MDR) frequencies per isolate; where R (2) means resistant to 2 antibiotics, R (3), R (4), R (5) means resistant to three, to four, to five antibiotics respectively. The pattern of occurrence of multidrug resistance was as follows: For E. coli: AMC/AMP was at 6.7%; RL/AMP at 6.7%; AMC/RL/AMP at 73.3% and CN/AMC/RL/AMP at 15.3 %. Staphylococcus isolates showed MDR patterns as follows:

DA/C; DA/TE and DA/C/TE at 15.3 %, 6.7% and 26.7% respectively. For Streptococcus isolates, the MDR patterns were as follows: RL/DA at 6.7%; RL/TE/E at 6.7%; RL/TE/DA at 15.3 %; RL/DA/C at 6.7%; RL/DA/E/TE at 20%; RL/DA/E/C at 20%; R/DA/C/TE at 6.7% and RL/E/DA/TE/C at 15.3 %. Figure 2. Shows Multidrug resistance frequencies per slaughterhouse; Figure 3. Shows the plate with antibiotic inhibition pattern demonstrated by a Staphylococcus isolate.

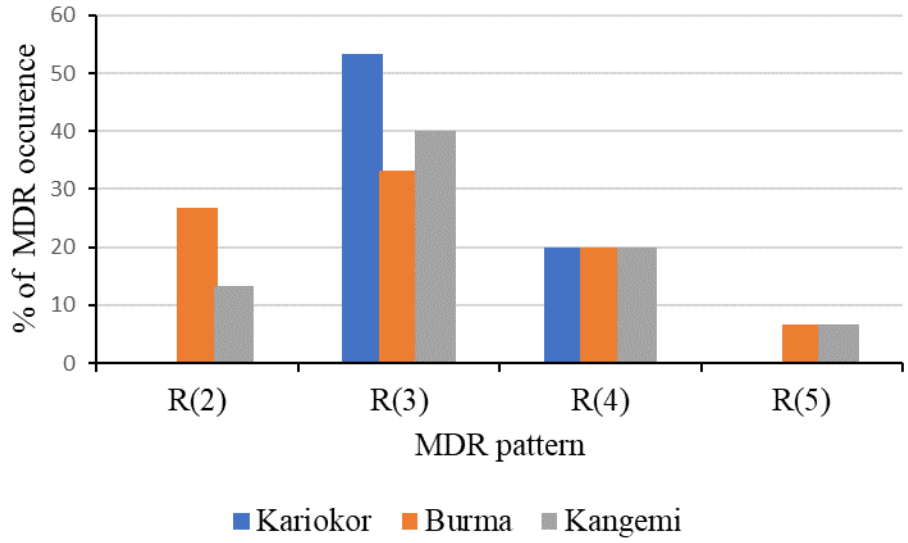

Figure 2. Showing Multidrug resistance frequencies per slaughterhouse. where R (2) means resistant to 2 antibiotics, R (3), R (4), R (5) means resistant to three, to four, to five antibiotics respectively.

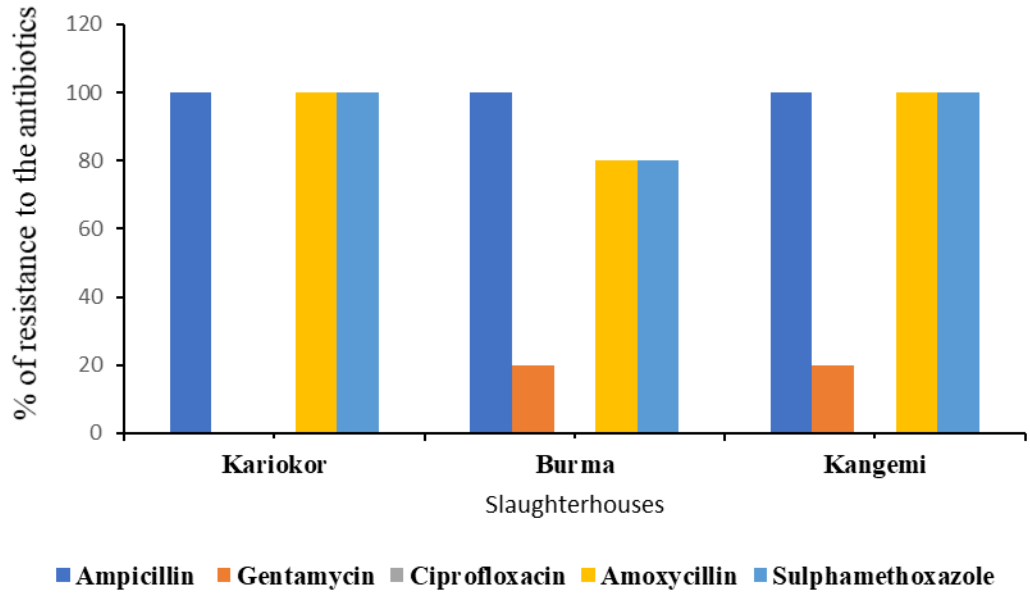

Figure 3. *Antibiotic* susceptibility results of E. coli isolated from different slaughter houses

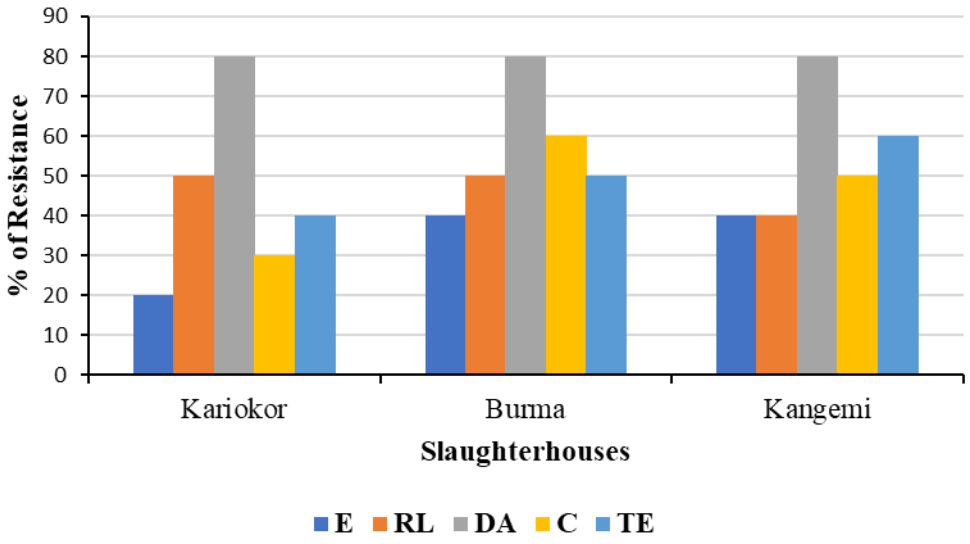

Figure 4. Antibiotics resistance of Staphylococcus and Streptococcus isolates with proportion to the Slaughter houses.

***Legend**: **E**: Erythromycin, **RL**: Sulphamethoxazole, **DA**: Clindamycin, **C**: Chloramphenicol and **TE**: Tetracycline.*

DISCUSSION

There is an increase in intensive use of antibiotics in poultry farming for treatment of bacterial infection and maintenance of healthy and reproductive birds (Yang *et al.*, 2004). In this study, *E. coli* showed high resistance to Ampicillin and Amoxicillin (100% and 93.3% respectively), yet it has been documented that these drugs are not used intensively in Kenya (Adelaide *et al.*, 2008). This means that there could be other factors that may have led to increase in resistance or the drugs are being used secretly (not recorded); which has resulted in bacteria developing respective resistance. Other factors which could have induced resistance to the antibiotic(s) include: exposure to improperly disposed left-over drugs in the environment (Khan *et al.*, 2013) or in rivers (Kimosop *et al.*, 2016), which could have precipitated development of the resistance in bacteria contact bacteria that came into contact with the drugs or the environment/rivers could have been contaminated with already- resistant bacterial strains.

Sulphonamides such as Sulphamethoxazole (to which *E. coli* and *Streptococcus* isolates showed resistance of 93.3%) are being used in prophylaxis of coccidiosis and different bacterial infections in Kenya (Adelaide *et al.*, 2008); this may have been the cause of increasing resistance to this antibiotic by the tested *E. coli* and *Streptococcus*. It has also been documented that resistance to Sulfonamides can be as a result of ribosomal mutation in the chromosomal gene *rpsl* or by enzymatic modification of the drug (Guerra *et al.*, 2003). However, *Staphylococcus* isolates from the study areas were 100% susceptible to this drug, which shows that the organisms are still susceptible to the antibiotic. Sulphonamides being effective drugs in treatments of bacterial infection for both Gram positive and Gram negatives in both animals and humans (Fair and Tor, 2014), it is a relief that they can

be still used in controlling Staphylococcal infections, since the organisms seem to be still susceptible.

The presence of resistance to Chloramphenicol by *Staphylococcus* isolates (40%) and *Streptococcus* isolates (53.3%) is note-worthy because the use of this antibiotic in Kenya was outlawed since 2005 (Adelaide *et al.*, 2008). Resistance can occur through different means; it is documented that there may be an induced resistance to Chloramphenicol due to the presence of certain chemicals such as acetate among others (Rosner, 1985). It has been proven that resistance to aminoglycoside (Chloramphenicol in this case) can be due to enzymatic inactivation such as chloramphenicol acetyl- transferases (Cat) produced due to *cat* genes found in the bacteria, which are capable of transferring acetyl groups to the C1 and C3 positions of the chloramphenicol molecule and make the derivative chloramphenicol molecule unable to inhibit bacterial protein biosynthesis (Chwarza and Anclab, 2001).

Erythromycin and Tetracycline, among other antimicrobials, are widely used in poultry production for treatment of staphylococcal and other bacterial infections (White *et al.*, 2003); Tetracycline is also widely used as growth and production promoter in chickens. In this study, tested *Staphylococcus* isolates were resistant to Erythromycin and Tetracycline at 0% and 46.7% respectively; *Streptococcus* isolates were resistant to the drugs at 60% each. Since these drugs are widely used, there is possibility of resistance development towards them; however; the fact that *Staphylococcu*s isolates from the same study areas were 100 % susceptible to Erythromycin points to another possibility of it being a result of intrinsic factor(s). The study done by Aarestrup *et al.* (2000) in Denmark has, however, demonstrated resistance of *Staphylococcus* organisms to Erythromycin at 24%; it also demonstrated resistance of the organisms to Sulphamethoxazole at 19%.

In this study *Streptococcus* isolates showed higher resistance to antibiotics compared to *Staphylococcus* isolates; they showed high resistance to Sulphamethoxazole, Clindamycin and Tetracycline at 93.3%; 86.7% and 80% respectively. This resistance can be as a result of carrying R-plasmid by *Streptococcus* isolates tested or due to genetic composition of *Streptococcus organisms* (Burdett *et al.*, 1982). These organisms also showed resistance to Erythromycin at 60%. It has been documented that resistance to Erythromycin by *Streptococcus* organisms is mainly through two modes: target site modification and active efflux (Giovanetti *et al.*, 2002).

In this study, there were cases of multidrug resistance (to more than one antibiotic). *E. coli* isolates showed multidrug resistance at 100%; *Staphylococcu*s isolates at 46.7% and *Streptococcus* isolates at 93.3%, with respect to the drugs used; there is a possibility that the resistances were combined; borne on a single plasmid (Tosini *et al.*, 1998). *E. coli* results are similar to those of Salehi and Bonab (2006) in Iran which also found 100% multi-drug resistance in *E. coli* isolates. White *et al*. (2003) reported multidrug resistance at 21% in *Staphylococcus aureus* isolated from chickens in North- Eastern Georgia.

It has been shown that any bacterium, whether pathogenic or not, can be involved in transfer of antibiotic resistance genes; as long as they are carrying the respective resistance gene(s) (Chwarza and Anclab, 2001). If the transfer is to a pathogenic bacterium, it will be impossible to treat the resultant infection(s) using the particular antibiotic. The three

bacterial species tested for antibiotic susceptibility/resistance from intestinal contents of the indigenous chickens, were variously resistant to the tested antibiotics; some of them showing multiple resistance. This highlights the possibility of the chickens serving as sources of pathogenic bacteria and/or resistance genes to other chickens and humans. It is, therefore, recommended that, before dispensing an antibiotic, one ascertains its efficacy by carrying out antibiotic susceptibility testing to avoid selective selection of resistance in bacteria, measures must, therefore, be taken to prevent/reduce development of antibiotic resistance. One of these measures is creating awareness on the ills of antibiotic resistance and the factors that lead to its development, among people; this will contribute towards reduction of indiscriminate usage and disposal of antibiotics. It is also recommended that policy makers come up with guidelines on reduction of environmental contamination; effective policies must be formulated that will safeguard proper usage of antibiotics; for example: a ban should be enforced on usage of antimicrobials as food additives/growth promoters; also, on over-the-counter buying of drugs. There is also need for a policy on effective ways of disposing remaining or expired drugs.

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