Antibiotic Susceptibility and Phenotypic Plasmid Screening Among *Escherichia coli* Isolated from Abattoir Wastewater in Bauchi State, Nigeria

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Abstract: This study highlighted the antibiotic susceptibility and the emergence of multidrug resistance plasmids among *Escherichia coli* in abattoir wastewater in Bauchi state Nigeria. Isolation and characterization of *E. coli* was conducted from 150 samples using standard procedures. Antibiotic susceptibility testing and plasmid curing were done on the strains. Of these samples screened only 18 (12%) E. *coli* were recovered. Antibiotic susceptibility testing showed high resistance to Augmentin (77.7%) with a clear diameter of inhibition ranging between 8.0±0.0 – 17.3±1.4 mm, followed by amoxicillin (8.0±0.0 – 17.3±1.4) mm, streptomycin (8.0±00 – 10.0±2.6) mm and septrin (8.0±0.0 – 10.3±0.3) mm, with 61.1%, each, and gentamicin (8.0±0.0 – 17.3±0.0) mm and chloramphenicol (8.0±0.0 – 11.0±2.0) mm, each with 55.5% respectively. Ciprofloxacin (18.0±0.0 - 40.0±0.5) mm, was the most potent with 83.3% activity. Multiple antibiotic resistance was examined in 12 (66.6%) of the isolates. After curing, antibiotic susceptibility testing most of the isolates were observed to harbor plasmid-mediated resistance. This study has revealed the emergence of multidrug plasmids mediated resistance among *Escherichia coli* in abattoir wastewater in Bauchi State Nigeria.

Key words: Abattoir, *Escherichia coli,* Plasmid, Resistance, Waste water

INTRODUCTION

Antibiotics have been a vital tool in public health since the discovery of penicillin in 1928, saving the lives of millions of people around the world. However, the emergence of drug resistance bacteria is reversing the miracles of the past; with drug choices for the treatment of many bacterial infections becoming increasingly limited, costly and in some cases nonexistent (Graf *et al*., 2018). Abattoirs in developing countries are generally less developed compared with those in developed countries such as Europe and USA (Chukwu, 2008). They can be modern or very simple but many of them may pose threat to human wellbeing because of insanitary conditions (Verheijen *et al.,* 1996). Abattoir wastewater is potentially contaminated with microbial pathogens that are harmful to humans and animals. These abattoir waste waters are principal recipients of enteric bacteria with multiple antibiotic resistance (Prescott *et al.,* 1999), and an important site for horizontal gene transfer because they contain nutrients and large number of microorganisms (Barberio *et al.,* 2001). Approximately 61 % of the known human pathogens in the world are zoonotic (Taylor *et al.,* 2001). *Escherichia coli* is a zoonotic bacteria that cause diseases in humans and can be present in high levels in abattoir waste water (Adeyemi and Adeyemo, 2007). The presence of pathogenic enteric microorganisms in aquatic environments can lead to emergence of disease especially when the water is used for drinking, recreational activities or irrigation. Several studies have discovered that abattoirs in developing countries have an unhygienic environment (Adeyemo, 2002; Nwanta *et al.,* 2010) and several pathogens that are known causes of diarrheal diseases were detected and cause possible health hazard to the peoples associated with the abattoir and to the community at large (Benka-Coker and

Ojior, 1995; Abiade-Paul *et al.,* 2005; Nwanta *et al.,* 2010). It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste environment to other environments (Adeyemi and Adeyemo, 2007).There is a concern that waste may provide an environment in which antibiotic resistance factors can spread to sensitive bacteria and between can increase the possibility of transferring the resistance factors to humans.

MATERIALS AND METHODS

Study site

The study was carried out in Bauchi State, Nigeria. Bauchi state is located on the latitude: 10.3098, Longitude: 9. 8452 I I I N 10 18 35, E 9 50 43. Waste effluents from abattoirs (Bauchi, Misau and Azare) are used for irrigation in agriculture. The abattoir has a daily slaughter of approximately 60 cattle, 40 goats and 40 sheeps. At the abattoirs, waste from the slaughtering process is washed out into a drainage channel to a body of rivers without any processing.

Sample collection and preparation

A total of 150 samples of untreated wastewater were collected from the abattoirs in sterile 200ml glass bottles and transported in an ice container and to Microbiology laboratory, Bayero University Kano (BUK) for analyses. All samples were analyzed within 24 hours.

Isolation of *Escherichia coli*

Escherichia coli were isolated using standard procedures (Oluwole *et al.,* 2011). Characterization of the isolates was done based on Gram's reaction, morphological and cultural characteristics as well as biochemical reactions (indole, Simon citrate, MRVP, TSI etc). Pure colonies were further sub-cultured and stored on nutrient agar slant for further analysis (Svanström, 2014).

Antibiotics Susceptibility Test

Disc diffusion test was carried out to determine the susceptibility of the isolates to selected antibiotics as described by Igwe *et al.,* (2013). The following antibiotic discs were used: chloramphenicol (30µg), augmentin (30µg), amoxicillin (30µg), ciprofloxacin (10µg), gentamicin (10µg), septrin (30µg) and streptomycin (30µg).

Plasmid Curing

Plasmid curing was carried out in order to determine the location (plasmid borne or chromosomal) of the drug resistance markers as described by Ojo *et al.,* (2014). The curing analysis of the isolates was performed using $0.1mgmL^{-1}$ of acridine orange. Isolate were grown for 24h at $37^{o}C$ in Mueller-Hinton broth containing $0.1mgmL^{-1}$ acridine orange. The broth was agitated to homogenize the content and a loopful of the broth medium was inoculated on Mueller Hinton Agar (MHA) plates and antibiotics sensitivity testing was carried out. Presence of zone of inhibition on MHA was indicative of plasmid-mediated resistance (plasmid cured) while absence or lower zone of inhibition on MHA was indicative of chromosome-mediated (plasmid not cured).

Data Analysis

Data comparison was performed using the one way ANOVA ($p< 0.05$) in the statistical package for Social Science Statistical Program, SPSS software version 16.0 (SPSS © Inc., Chicago, Illinois).

RESULTS

A total of 18 *E. coli* were isolated from 150 water samples. The *E. coli* isolates were all Gram negative rods, motile, indole positive, citrate negative as well as produce gas on TSI**.**

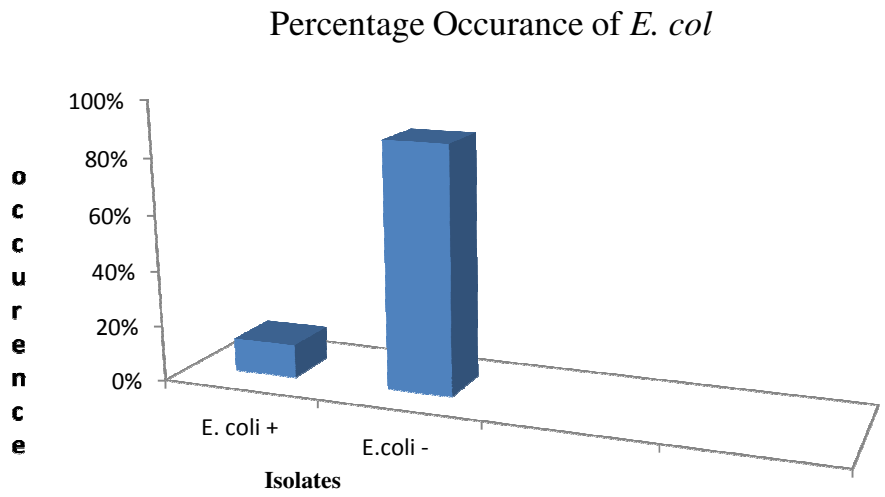

Figure1: Occurrence of *E. coli* isolates from abattoir wastewater

The AST was carried out using 30µg Augmentin disk recommended by Clinical Laboratory Standard Institute (CLSI, 2008). The CLSI break points for *E. coli* interpretive criteria for Augmentin were use to describe the isolates as Augmentin sensitive and Augmentin resistant.

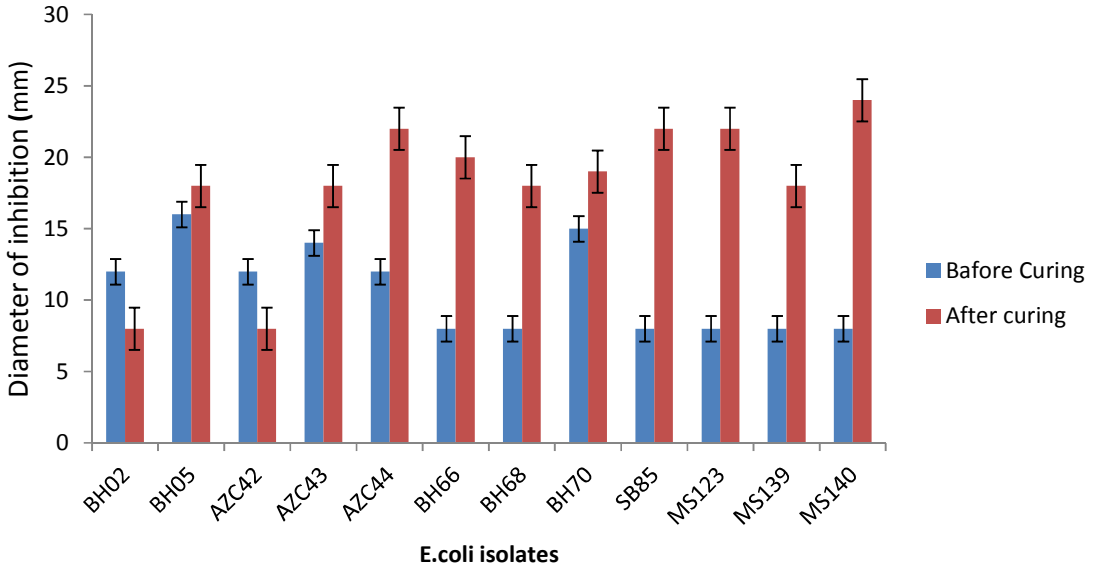

Figure 2: Antibiotics susceptibility test (AST) to Augmentin on the isolates before and after curing

The AST was carried out using 30µg Amoxicillin disk as recommended by Clinical Laboratory Standard Institute (CLSI, 2008). The CLSI break points for *E. coli* interpretive criteria for Amoxicillin were used to describe the isolates as Amoxicillin sensitive and Amoxicillin resistant.

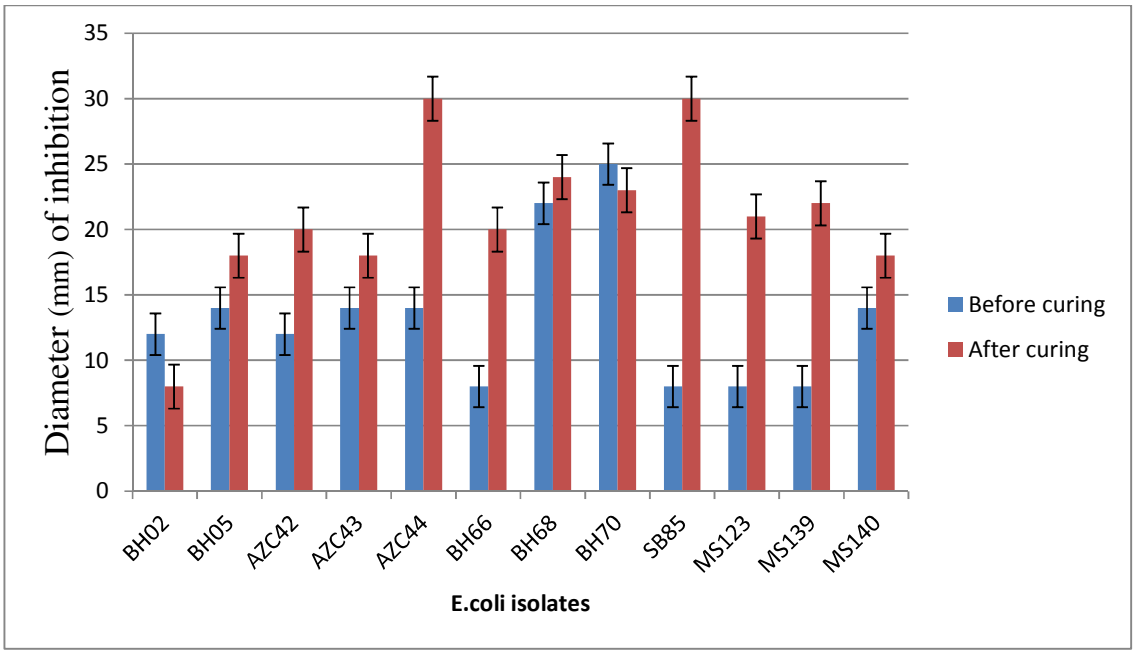

Figure 3: Antibiotics susceptibility testing on Amoxicillin on the isolates before and after curing

The AST was carried out using l0µg Gentamycin as recommended by Clinical Laboratory Standard Institute (CLSI, 2008). The CLSI break points for *E. coli* interpretive criteria for Gentamycin were use to describe the isolates as Gentamycin sensitive and Gentamycin resistant.

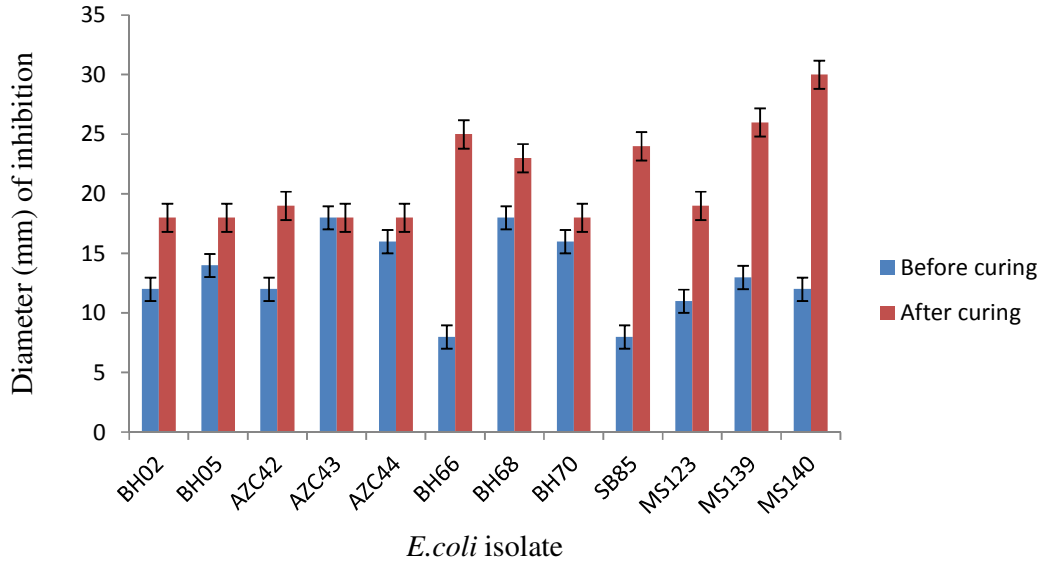

Figure 4: Antibiotics susceptibility testing on Gentamycin on the isolates before and after curing.

The AST was carried out using 30µg Streptomycin disk as recommended by Clinical Laboratory Standard Institute (CLSI, 2008). The CLSI break points for *E. coli* interpretive criteria for Streptomycin were use to describe the isolates as Streptomycin sensitive and Streptomycin resistant.

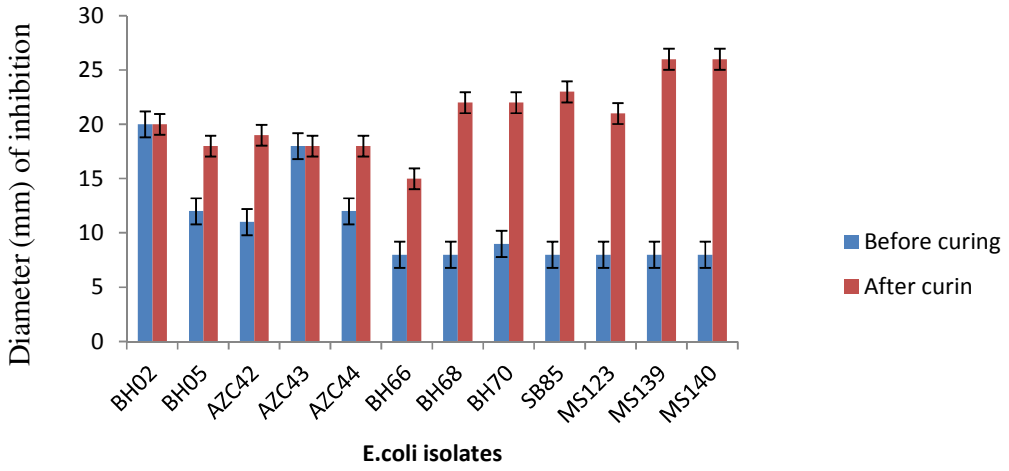

Figure 5: Antibiotics susceptibility testing to Streptomycin on the isolates before and after curing

The AST was carried out using 30µg Septrin disk as recommended by Clinical Laboratory Standard Institute (CLSI,2008). The CLSI break points for *E. coli* interpretive criteria for Septrin were use to describe the isolates as Septrin sensitive and Septrin resistant.

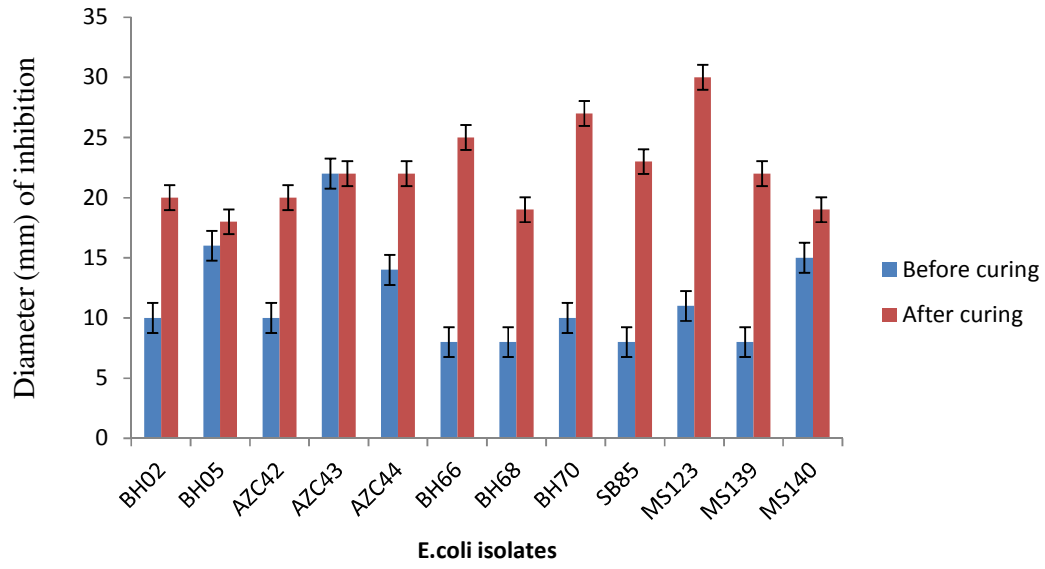

Figure 6: Antibiotics susceptibility testing to Septrin on the isolates before and after curing.

The AST was carried out using 30µg Chloramphenicol disk as recommended by Clinical Laboratory Standard Institute (CLSI, 2008). The CLSI break points for *E. coli* interpretive criteria for Chloramphenicol were use to describe the isolates as Chloramphenicol sensitive and a Chloramphenicol resistant.

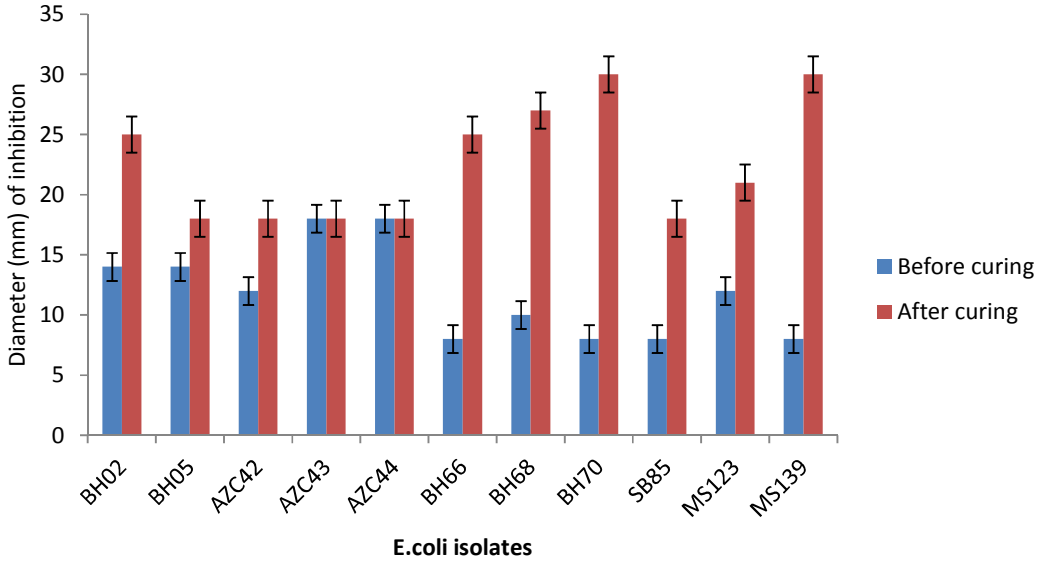

Figure 7: Antibiotics susceptibility testing to Chloramphenicol on the isolates before and after curing.

The AST was carried out using 30µg Ciprofloxacin disk as recommended by Clinical Laboratory Standard Institute (CLSI,2008). The CLSI break points for *E. coli* interpretive criteria for Ciprofloxacin were use to describe the isolates as Ciprofloxacin sensitive and Ciprofloxacin resistant.

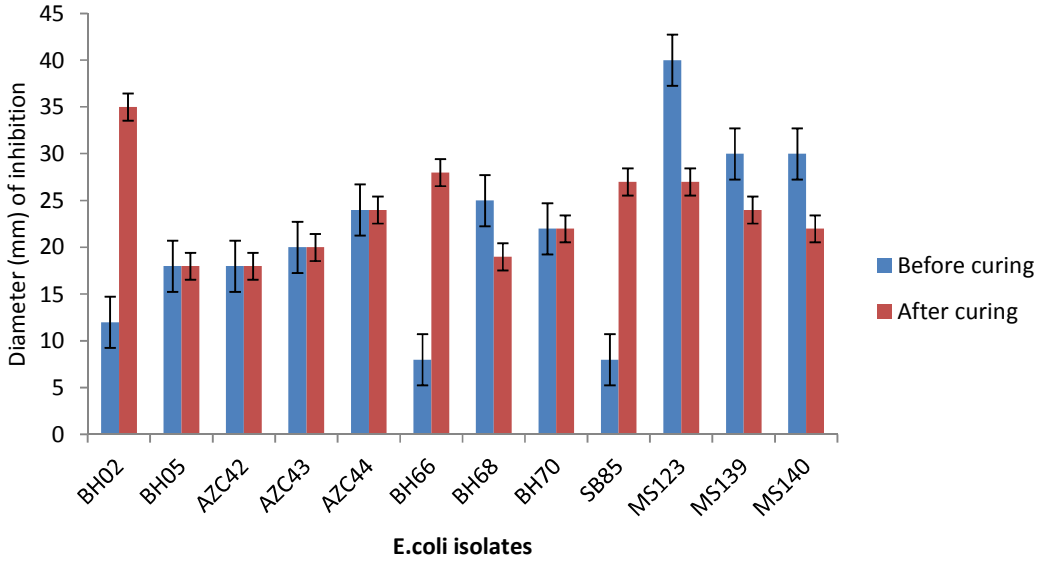

Figure 8: Antibiotics susceptibility testing to Ciprofloxacin on the isolates before and after curing.

DISCUSSION

The results show that the presence of *E. coli* in waste waters could be of public health concern most especially to local farmers who used these waters for irrigation of commercial crops such as tomatoes, lettuce, cabbage, onions, spinach, sugarcane etc. presence of *E. coli* in waste waters and the occurrence of multi-drug resistant pathogens have been ascertain by various researchers including (Pignato et al. 2009; Graf et al. 2018; Jorge, Silva, and Domingues 2016).

The present study revealed that all the 18 (100%) of *E. coli* from waste water were resistant to most of the antimicrobial agents tested. This result is similar to the result obtained by Hughes *et al*. (1981); Daini and Adesemowo (2008) and Marquez *et al*., (2008) who isolated 45 antibiotic resistant bacteria from wastewater samples. Twelve (66. 6%) out of the 18(100%) isolates showed multiple resistances to the antimicrobial agents used.

Resistance to high level of antibiotics has been ascribed in most instances to the presence of plasmids (Barker, 1999; Sherley *et al.*, 2004 Diani *et al.*, 2006). Ash *et al* (2002) also reported high levels of resistance in gram-negative bacteria in rivers in the United States. The antibiotic susceptibility of water isolates showed that higher levels of resistance existed among the isolates. This agrees with the findings of (Idika, 1999), who studied *Vibrio cholera* isolates during an outbreak of cholera in Lagos in 1997 and reported that the isolates from water were resistant to augmentin and gentamicin.

In determining the mechanism of resistance to antibiotic by *E. coli* isolates, plasmid curing assay was conducted. The assay revealed that most antibiotics resistant *E. coli* isolated in this study were plasmid-mediated since 88. 8% of the isolates showed zones of inhibition (cured) when tested against the selected antibiotics. While 11.1% showed no zone of inhibition (plasmid not cured) indicating chromosomal – borne resistance gene. The screening of the isolate with acridine orange suggest that the resistance makers were stably lost, which is in line with previous studies that says loss of plasmids correlated with loss of resistance (Ojo *et al.,* 2014). The statistical analysis shows significant difference at ($p<0.05$) for the isolates.

CONCLUSION

Escherichia coli isolated from these sources were found to be resistant to augmentin, gentamicin, amoxicillin and other commonly used antibiotics. Higher levels of resistance were observed in the isolates; multidrug resistance were observed. Loss of plasmids due to treatment with acridine orange correlated with loss of resistance to antibiotics. This suggests that the observed multidrug resistance was plasmid-mediated. The occurrence of plasmid-mediated multidrug resistance in bacteria in these waters heightens the public health concern.

RECOMMENDATIONS

The study suggested that, there is a need for a continuous pollution monitoring programme of the wastewaters in Nigeria. The uncontrolled use of antibiotics has contributed largely to the spread of multidrug resistance. However, government should make considerable effort to establish an antibiotic policy in the country. In order to reduce the risk and minimize possible transmission of MDR *E. coli* pathogens to humans and animals in the environment.

Furthermore; fecal and other abattoir waste soul be decontaminated or made non-hazardous instead of being excreted directly into the drainage channel, this minimize the possibility of spreading pathogens from the drainages to other areas. Further studies can be conducted to identify the nature and size of the plasmid dictating the mechanism of drug resistance in *E. coli*.

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