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Plasmid Screening among *E. Coli* Isolates from Abattoir Wastewater in Bauchi- Nigeria

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Abstract

A study on the multiple antibiotic resistance patterns and plasmid screening of some strains of *Escherichia coli* isolated from abattoir wastewater was carried out. Isolation and characterization of *E. coli* was carried out from 150 samples of the wastewater, using standard procedures. Antibiotic susceptibility testing and plasmid curing were done on the strains. Out of 150 samples screened only 18 (12%) *E. coli* were recovered. Among the various classes of antibiotics tested, high resistance was found with augmentin (77.7%), followed by amoxicillin, streptomycin and septrin with 61.1%, each, and gentamicin and chloramphenicol each with 55.5% respectively. Ciprofloxacin was the most potent with 83.3% susceptibility. Twelve (66.6%) of the isolates showed multiple antibiotic resistance. Plasmid-mediated resistance was identified in most of the isolates. This study has revealed the emergence of multidrug plasmids-mediated resistance among *Escherichia coli* in abattoir wastewater in Bauchi State Nigeria.

Keywords: *Plasmid screening, E. coli, Wastewater, Abattoir*

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Background to the Study

Antibiotics have been a vital public health tool since the discovery of penicillin in 1928, saving the lives of millions of people around the world. Today, however, the emergence of drug resistance in bacteria is reversing the miracles of the past eighty years, with drug choices for the treatment of many bacterial infections becoming increasingly limited, costly, and, in some cases, nonexistent (Center for Disease Control and Prevention, 2014).

Abattoirs in developing countries are generally less developed compared with the situation for case in point in Europe and US (Chukwu, 2008). They can be modern or very simple but many of them disregarding type may amount to a threat to human wellbeing because of insanitary conditions (Verheijen, 1996). Abattoir wastewater potentially contaminated with microbial pathogens harmful to humans and animals are principal recipient of enteric bacteria with multiple antibiotic resistance (Prescott *et al.,* 1999), and an important site for horizontal gene transfer, by containing nutrients and high concentration of microorganisms (Barberio, 2001). Approximately 61 % of the known human pathogens in the world are zoonotic (Taylor *et al.,* 2001). *Escherichia coli* is an example of zoonotic bacteria that can cause diseases in humans and can be present in high levels in abattoir waste (Adeyemi and Adeyemo, 2007). The presence of pathogenic enteric microorganisms in aquatic environments can be a source of disease when 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, 2010) and detected the presence of pathogens that are known causes of diarrheal diseases and a possible hazard to human health in the abattoir waste and water contaminated by abattoir waste (Benka-Coker and Ojior, 1995; Abiade-Paul, 2005; Nwanta, 2010). It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste to new locations (Adeyemi and Adeyemo, 2007). There is a concern that abattoir waste may provide an environment in which antibiotic resistance factors can spread to sensitive bacteria, and then there may be an increased possibility of transfer of resistance factors to humans.

Materials and Methods

Study Site

The study was carried out in Bauchi State, Nigeria. It is located on the latitude: 10.3098, Longitude: 9. 8452 I I I N 10 18 35, E 9 50 43. Waste effluents from the abattoirs are used for irrigation in agriculture. The abattoir has a daily slaughter of approximately 60 cattle and 40 goats and sheep. 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 raw effluent untreated wastewater were collected from the abattoirs in sterile 200ml glass bottles and were transported to Bayero University Kano (BUK) Microbiology laboratory in an ice cooler box for analysis. All samples were analyzed within 24 hours (Svanström, 2014).

Isolation of Bacterial Isolates

The *Escherichia coli* were isolated using standard procedures (Oluwole *et al.,* 2011). Characterization of the isolates was based on the gram stain, morphological and cultural as

well as biochemical characteristics. The pure colonies were further sub-culture and stored on nutrient agar slant for further analysis (Svanström, 2014).

Antibiotics Susceptibility Test

Antimicrobial disc diffusion tests were carried out as previously described by Igwe *et al,*(2013); Hadley, (2002); Cowan and Steel, (1993); Cheesbrough, (2000), and standardized by the method of National Committee for Clinical Laboratory Standards, (NCCLS, 2003). The following antibiotic discs were used: chloramphenicol (30µg), augmentin (30µg), amoxicillin (30µg), ciprofloxacin (10µg), gentamincin (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 (Ojo *et al.,* 2014).

The curing analysis of the isolates was performed using 0.1mgmL-1 of acridine orange as described by (Ojo *et al.,* 2014). Isolate were grown for 24h at 370C 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 were culture on Mueller Hinton Agar (MHA) plates and antibiotics sensitivity testing was carried out as previously described. 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), Rasool *et al.* (2003) and Yah. (2014).

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* isolates were obtained from 150 water samples. The *E. coli* isolates are all Gram negative rods, motile, indole positive, citrate negative as well as produce gas on TSI.

Table 1 Occurrence of *E. coli* isolates from abattoir wastewater

Total No. of Samples	**No (%) of *E. coli* Positive**	**No (%) of *E. coli* negative**
150	18 (12%)	132(88%)

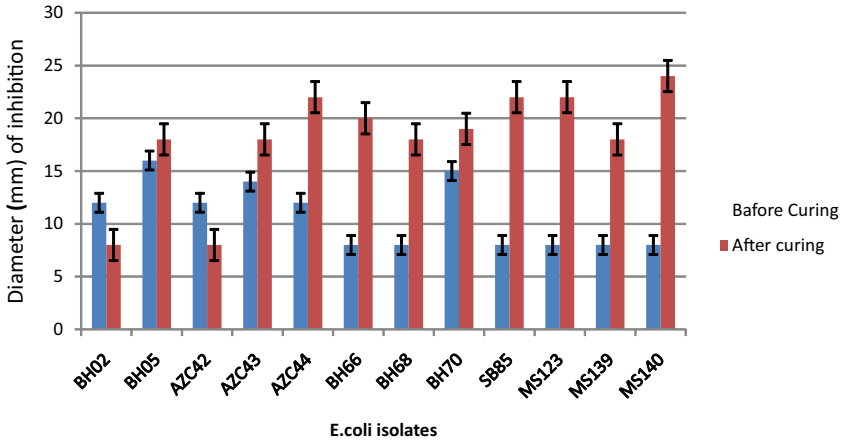

Figure 1: Antibiotics susceptibility testing (AST), to Augmentin of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueller Hinton Agar (MHA) plate covered with the required inoculums and a 30µg Augmentin disk was laid on the surface. The plates were incubated at 370C for 24hours. The CLSI break points for *E.coli* interpretive criteria for Augmentin was use to describe the isolates as augmentin sensitive and augmentin resistant. This procedure was carried out before and after curing experiments of the isolate.

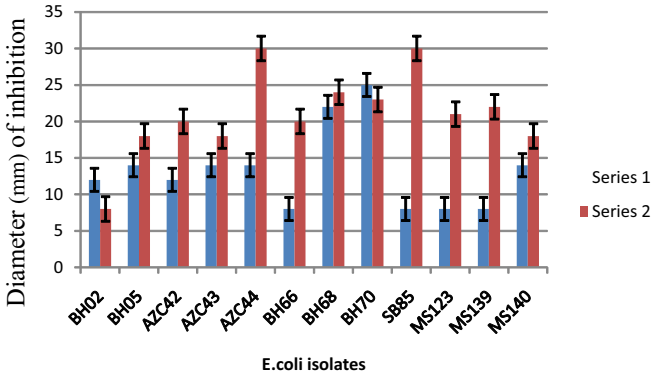

Figure2: Antibiotics susceptibility testing on Amoxicilin of the MDR isolates before and after curing

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueler Hinton Agar (MHA) plate covered with the required inoculums and a 30µg Amoxicilin disk was laid on the surface. The plates were incubating at 370C for 24hours. The CLSI break points for *E.coli* interpretive criteria for Amoxicilin was use to describe the isolates as Amoxicilin sensitive an Amoxicilin resistant. This procedure was carried out before and after curing experiments of the isolate.

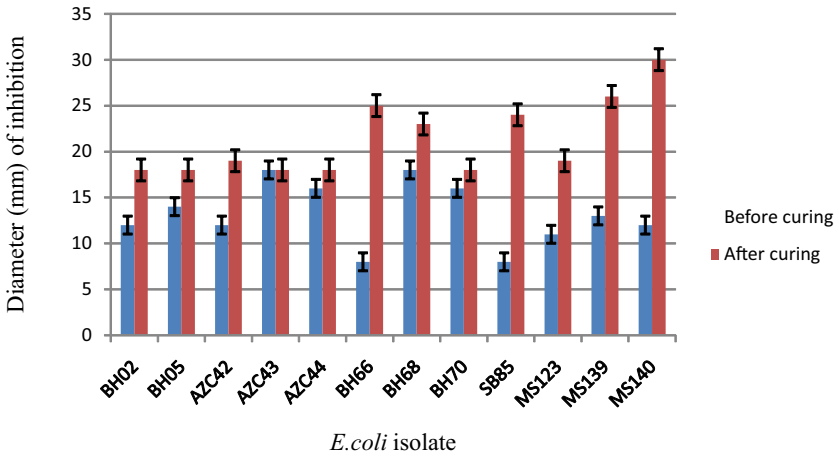

Figure 3: Antibiotics susceptibility testing on Gentamycin of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueller Hinton Agar (MHA) plate covered with the required inoculums and a loμg Gentamycin disk was laid on the surface. The plates were incubated at 370C for 24hours. The CLSI break points for *E.coli* interpretive criteria for Gentamycin were use to describe the isolates as Gentamycin sensitive and Gentamycin resistant. This procedure was carried out before and after curing experiments of the isolate.

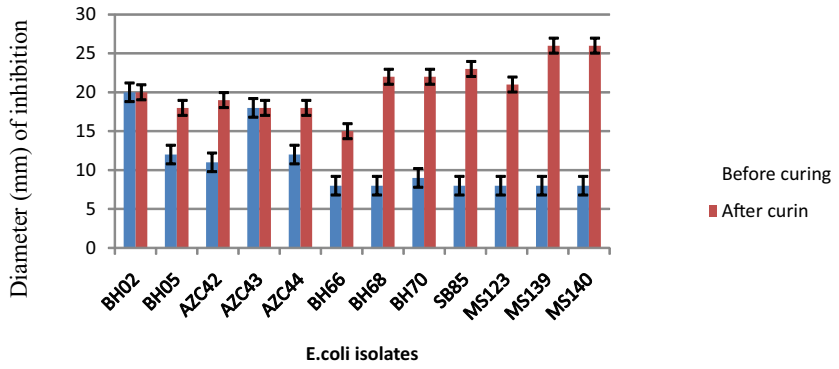

Figure 4: Antibiotics susceptibility testing to Streptomycin of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueller Hinton Agar (MHA) plate covered with the required inoculums and a 30μg Streptomycin disk was laid on the surface. The plates were incubated at 370C for 24hours. The CLSI break points for *E.coli* interpretive criteria for Streptomycin were use to describe the isolates as Streptomycin sensitive and Streptomycin resistant. This procedure was carried out before and after curing experiments of the isolate.

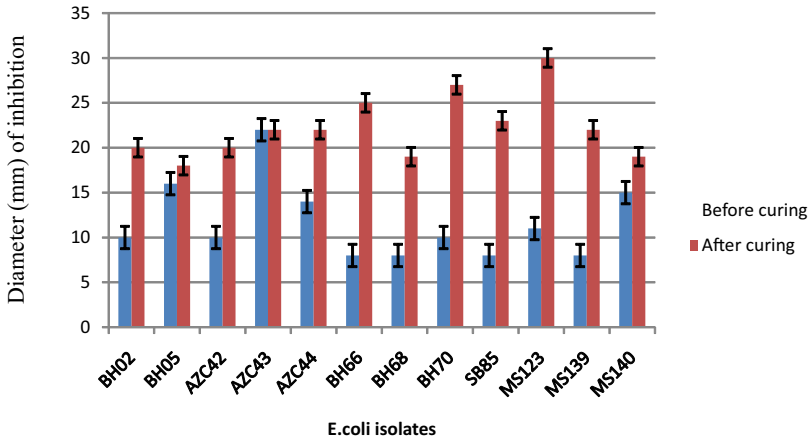

Figure 5: Antibiotics susceptibility testing to Septrin of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueler Hinton Agar (MHA) plate covered with the required inoculums and a 30µg Septrin disk was laid on the surface. The plates were incubated for 24hours at 370C. The CLSI break points for *E.coli* interpretive criteria for Septrin were use to describe the isolates as Septrin sensitive and Septrin resistant. This procedure was carried out before and after curing experiments of the isolate.

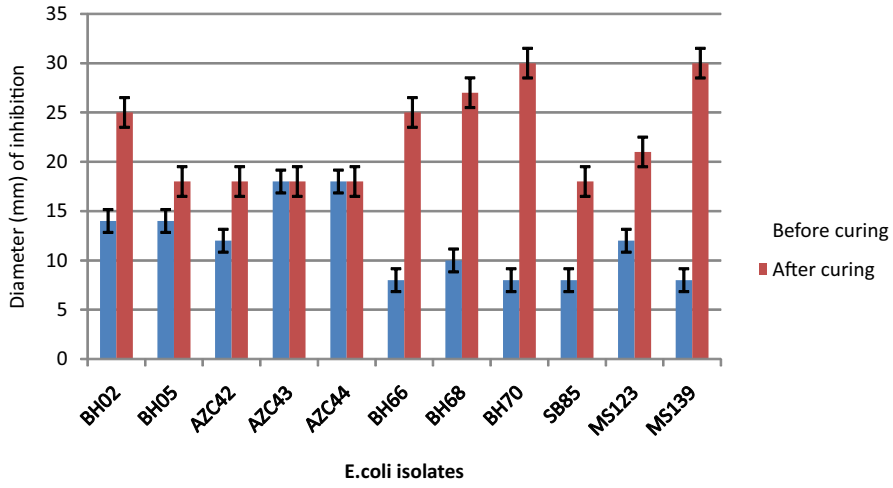

Figure 6: Antibiotics susceptibility testing to Chloramphenicol of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueler Hinton Agar (MHA) plate covered with the required inoculums and a 30µg Chloramphenicol disk was laid on the surface. The plates were incubated for 24hours at 370C. The CLSI break points for *E.coli*

interpretive criteria for Chloramphenicol were use to describe the isolates as Chloramphenicol sensitive and a Chloramphenicol resistant. This procedure was carried out before and after curing experiments of the isolate.

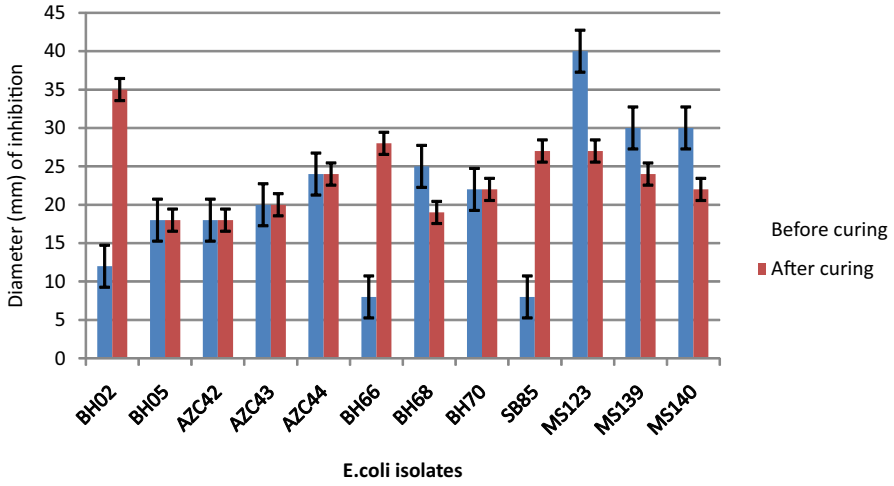

Figure 7: Antibiotics susceptibility testing to Ciprofloxacin of the MDR isolates before and after curing.

The AST was carried out as recommended by Clinical Laboratory Standard Institute (CLSI formerly NCCLS), disk diffusion test was adopted. The entire surface of Mueller Hinton Agar (MHA) plate covered with the required inoculums and a 30µg Ciprofloxacin disk was laid on the surface. The plates were incubated for 24hours at 370C. The CLSI break points for *E.coli* interpretive criteria for Ciprofloxacin were use to describe the isolates as Ciprofloxacin sensitive and Ciprofloxacin resistant. This procedure was carried out before and after curing experiments of the isolate.

Discussions

The detection of *E. coli* in these waters and its occurrence heighten public health concern about these waters that are used by local farmers for the irrigation of commercial crops (tomatoes, lettuce, cabbage, onions, spinach, sugarcane etc).

All the 18(100%) *E. coli* strains isolated were resistant to most of the antimicrobial agents tested. This result is similar to that obtained by Daini and Adesemowo (2008); Hughes *et al.* (1981) and Marquez *et al.*, (2008) who isolated 45 antibiotic resistant bacteria from wastewater samples. Twelve (66. 6%) of these isolates showed multiple resistances to the antimicrobial agents used. The frequency of susceptibility to ciprofloxacin was the highest (83. 3%), while sensitivity to augmentin (22. 2%) was the lowest. Resistance to high level of antibiotics has been ascribed in most instances to the presence of plasmids (Barker, 1999; Diani, 2006; Sherley, 2004). Ash *et al* (2002) also reported high levels of resistance in gram-negative bacteria in rivers in the United States. All the multidrug resistant strains were resistant to augmentin.

The antibiotic susceptibility in 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 resultantly 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 statiscal analysis shows significant difference at ($p<0.05$) for the isolates.

Conclusion

Escherichia coli isolated from these surface water 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 and plasmid were observed. Loss of plasmids due to treatment with acridine orange correlated with loss of resistance to antibiotics, suggesting that the observed multidrug resistance was plasmid-mediated. The occurrence of plasmid-mediated multidrug resistance in bacteria in these surface waters heightens the public health concern. The study showed a need for a continuous pollution monitoring programme of the surface waters in Nigeria.

Recommendations

This study has highlighted the emergence of multidrug resistance plasmids among Escherichia coli in abattoir wastewater in Bauchi state Nigeria. The uncontrolled use of antibiotics has contributed largely to this situation. Thus the government should make considerable effort to establish an antibiotic policy for the country.

To reduce the risks and to minimize the possible transmission to humans and animals in the environment of the MDR *E. coli* pathogens however, it is suggested that the following preventive measures are introduced at the abattoir:

1. Faeces and other abattoir waste be collected and destroyed or made non-hazardous instead of being excreted into the drainage channel.
2. Minimize the availability for scavenging animals to feed from the drainage channel for example by covering the same with a grid or using a closed piping system. To minimize the availability for scavengers would reduce the possibility of spread of pathogens from the drainage channel to other areas by the means of animals.
3. It is also advisable to have a continuously running treatment facility that minimizes the amount of bacteria in the effluent water before discharge into the nearby channel.

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