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Potential Involvement of Plasmids on Antibiotic Resistance and Virulence in *Escherichia coli* from Select Abattoirs in Aba Metropolis, Nigeria

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ABSTRACT

Plasmids are extra-chromosomal genetic materials which confer some attributes such as resistance to antibiotics, chemicals and synergy formation among the bacteria. Abattoir plays a vital role to man by serving a link for the inflow of animal protein to the food chain but can also serve as a hotspot for the influx of pathogenic bacteria such as *Escherichia coli* with antibiotic and virulent traits. This study aimed at exploring the potential involvement of plasmids on antibiotic resistance and virulence in *E. coli* from three select abattoirs in Aba Metropolis, Nigeria. Fifty (50) *E. coli* isolates collected from soil, effluent, butchers' palms, animals, table/slab and knives were phenotypically identified using cultural characteristic growth pattern of the organism on Eosin Methylene Blue (EMB) and biochemical test. Using plasmid elimination technique, plasmids from the bacterial isolates were cured using acridine orange while virulence traits of protease formation, hemolysis and biofilm formation were carried out using skim milk agar, blood agar and Congo red agar respectively and the antibiotic susceptibility testing was carried out using the Kirby Bauer method. The rate of resistance in the uncured bacteria isolates ranged from 82.7% to 100% in uncured isolates which was similar to results obtained following plasmid curing. Plasmid curing had a minimal effect on resistance rates as only 1% to 2% observed change were observed in majority of the antibiotics tested (91.7%) but greater than 5% decrease (6.2%) was observed only with respect to a single antibiotic (ofloxacin). Same slight change was recorded in Multiple Antibiotic Resistance (MAR) indices of the bacteria: 79.6% to 71.4% (index 1), 18.4%-23.5% (index 0.92) and 2%-5.1% (0.83). Same trend was observed in the studied virulence traits of 30% to 46% before curing and 24% to 34% after curing. Only 5% reduction took place in the isolates except for hemolysin production which reduced by 10%. These finding indicate minimal role of plasmids in antibiotic resistant *E. coli* with virulent traits from select abattoirs in Aba metropolis of Nigeria.

Keywords: Abattoir, *E. coli*, Plasmid, Virulence Traits, Curing, Antimicrobial Resistance.

Introduction

Though normally innocuous bacteria found as normal flora in the gastrointestinal tract of various warm-blooded animals, *Escherichia coli* are still regarded as an important pathogen. Antibiotic resistance and virulence characteristics are specific traits which often could differentiate pathogenic from non-pathogenic strains. These traits have however been described in a variety of *E. coli* from different sources (Otokunefor *et al*., 2020; Ajuga *et al.,* 2021; Perewari *et al*., 2022; Adewuyi-Oseni *et al*., 2023; Olorunleke *et al*., 2024, Kleist *et al*., 2025) some of which are not directly associated with clinical disease in man.

One possible explanation for this is the presence of the genes encoding for these traits on mobile genetic elements such as plasmids, integrons and transposons. This would enable dissemination of such traits between strains and could be a potential public health menace, negatively impacting eradication and control strategies. Of these mobile genetic elements however, plasmids are commonly occurring and studied. They have been reported in over 50% of all known bacterial groups (Shintani *et al*., 2015; Domingues *et al*., 2025). Additionally, the effect of these plasmids on specific traits can simply be studied *in vitro* by evaluating for the trait before and after plasmid curing which rids an isolate of plasmids present (Otokunefor *et al*., 2019).

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Understanding the role plasmids could play in antibiotic resistance and virulence is the first step in defining the public health threat of dissemination of characteristics linked with such plasmids. However, the number of studies in Nigeria that have set out to explore this are limited (Tsaku *et al*., 2017; Monarreez *et al.,* 2019; Agbagwa *et al.,*2022) explored the roles of plasmids in *Escherichia coli* in poultry litter and urine with respect to antibiotic resistance and found out that after curing, antibiotic resistance in the organisms reduced. Considering the dearth of information on this especially with respect to our specific locale, this study therefore set out to explore the role of plasmids in antibiotic resistance and virulence traits of *Escherichia coli* isolated from select abattoirs in Aba metropolis, Nigeria.

Materials and Methods

Isolation and Identification of *Escherichia coli*

Fifty *Escherichia coli* were isolated from three select abattoirs: Old express abattoir 5.0748ºN, 7.3583ºE; Goodmorning market 5.4999ºN, 7.3752ºE and Nsulu abattoir 5.10405ºN, 7.37657ºE in Aba metropolis. The identities of the isolates were determined by first culturing of Eosin Methylene Blue agar. Isolates with *E. coli* characteristic colonies showing a green metallic sheen were then purified by sub-culturing to nutrient agar and standard biochemical tests (Indole test, Methyl Red (MR) test, Voges-Proskauer (VP), Urease, Citrate, Triple Sugar Iron (TSI), Oxidase, motility, Carbohydrate fermentation, Catalase.) used to determine the isolate identities (Cheesebrough, 2010).

Plasmid Curing

Plasmid curing of test isolates was carried out as previously described (Otokunefor et al., 2019) by growing the organism in nutrient broth containing 0.1 mg/ml of acridine orange for 24 hours at 37°C. Further testing of isolates was then carried out on isolates grown in both the presence (cured) and absence (uncured) of 0.1 mg/ml of acridine orange.

Antimicrobial Susceptibility Testing

The isolates were subjected to antibiotic susceptibility testing using the Kirby– Bauer disk diffusion method on Mueller–Hinton agar using the CLSI protocols. The antibiotics by which the bacteria were tested on were Nitrofurantoin (NIT) 300 µg, Ceftriaxone (CRO) 45 µg, Cefuroxime (CXM) 30 µg, Gentamicin (GN) 10 µg, Cefixime (ZEM) 5 µg, Ofloxacin (OFX) 5 µg, Amoxicillin/Clavulanic acid (AUG) 30 µg, Imipenem (IMP) 10 µg, Ampiclox (ACX) 10 µg, Levofloxacin (LBC) 5 µg, Cefotaxime (CTX) 25 µg and Nalidixic acid (NA) 10 µg (Abtek, Ahmedabad, Gujarat India). *Escherichia coli* ATCC 25922 was used as negative control throughout the study. Interpretation of antimicrobial susceptibility test was carried out by the guidelines of the Clinical Laboratory Science Institute (CLSI, 2017; The European Committee on Antimicrobial Susceptibility Testing [EUCAST], 2021; Founou *et al.,* 2018; Ajuga *et al.,* 2021; Aworh *et al.,* 2022).

Phenotypic Virulence Testing

Three virulence traits (Hemolysis, Protease production and biofilm forming potential) were then assayed for as previously described (Otokunefor et al., 2020). In brief, hemolytic ability was determined by inoculating to blood agar and observing for zones of clearance around the growth (Lad *et al*., 2022). Protease production was similarly detected but using skim milk agar. Biofilm forming potential was detected by culturing on Congo Red Agar (CRA), with isolates producing a black pigment indicative of a positive result.

Results

An assessment of the antimicrobial susceptibility of the isolates showed resistance rates ranging from 82.7% to 100% in uncured isolates (Figure 1). This was similar to results obtained following plasmid curing. In general, plasmid curing had a minimal effect on resistance rates with changes of 1% to 2% observed in majority of the antibiotics tested (91.7%). A greater than 5% decrease (6.2%) was observed only with respect to a single antibiotic (ofloxacin). Looking at the MAR Index calculated as the percentage of number of observed resistances to antibiotics divided by total number of antibiotics tested, where a MAR index of 1 indicates resistance to all tested antibiotics, there was a slight shift in decrease of MAR index (Figure 2).

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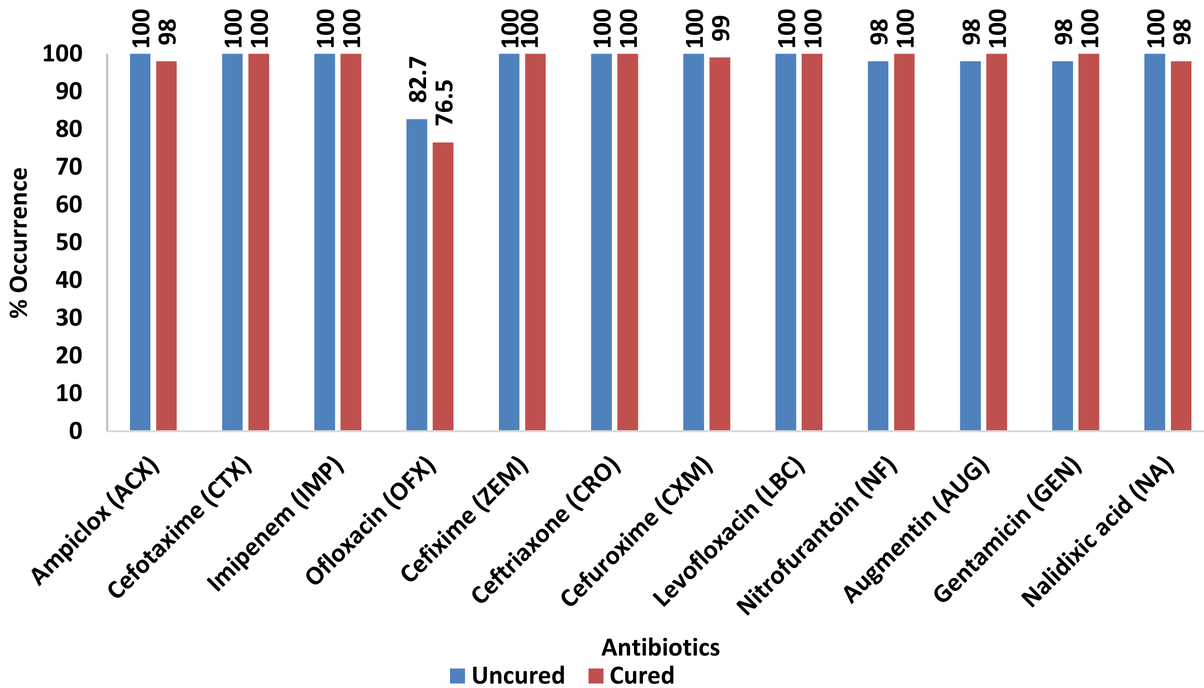

Figure 1: Antibiotic resistance of test isolates before and after plasmid curing

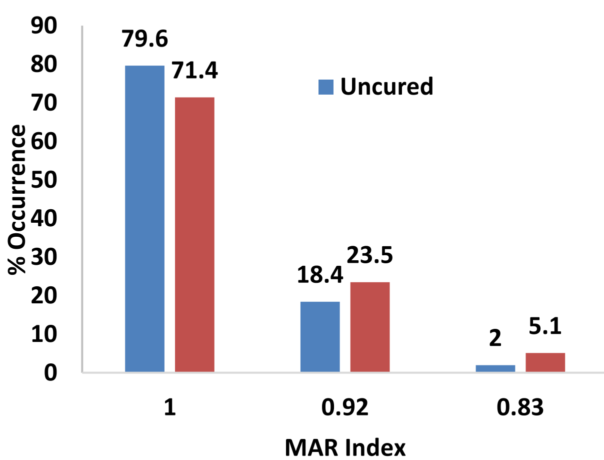

Fig. 2: Effect of plasmid curing on MAR index values of *E. coli* isolates

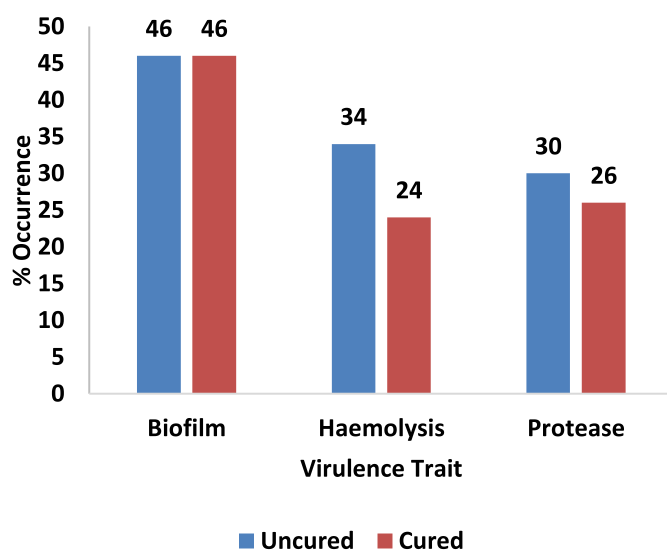

Fig. 3: Occurrence of virulence traits in test isolates before and after plasmid curing

A similar result was observed with respect to the virulence traits studied (Figure 3). Before curing, occurrence of virulent traits ranged from 30% to 46%, while after curing it ranged from 24% to 34%.

For majority of the tested traits, a less than 5% reduction in occurrence was noted following curing. The greatest level of reduction was observed with respect to hemolysis with a 10% decrease observed.

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Discussion

Carriage of the genes encoding antimicrobial resistance and/or virulence on mobile genetic elements is a major public health risk because this can result in a rapid dissemination of such traits between bacterial populations. Despite the fact that several previous studies had reported an effect of plasmid curing on resistance rates, results from this present study showed that this was not a universal occurrence with a greater than 5% decrease only observed against ofloxacin.

This lack of effect of plasmid curing on resistance rates to specific antibiotics was also reported by Olanrewaju and colleagues in 2020. The study exploring effect of plasmid curing in isolates from a River noted no effect on resistance against gentamycin, ciprofloxacin and amoxicillin.

In contrast to results of this current study however, wide decreases in resistance rates of up to 45% was noted with respect to six antibiotics, ofloxacin included. Sule and colleagues (Sule *et al.,* 2023), also reported a decrease in resistance of some bacterial isolates to a number of antibiotics including nitrofurantoin, ceftazidime, gentamicin, ciprofloxacin and ofloxacin after plasmid curing with acridine orange.

Similar reports were made with respect to bacterial isolates from various aquatic/water sources, clinical sources, food sources and chicken faeces (Onifade *et al*., 2019, Churchill and Romanus, 2019, Asiton-a *et al*., 2015, Ekundayo 2021, Rasool *et al.,* 2023). Majority of these studies however failed to quantify the level of the decrease. An assessment of the raw data presented in these papers indicated varying levels of decrease. For the Sule study out of the 8 organisms subjected to plasmid curing, for most antibiotics a decrease was only noted in 1 out of 8 cases.

The Asiton-a *et al*., 2015 study focused on water sources in the Niger Delta region of Nigeria and reported remarkable decreases in MAR indices of isolates following plasmid curing with the highest decrease of 0.875 to 0.125. Churchill and Romanus (2019) studying the effect of plasmid curing in two isolates noted a reduction in resistance from 7 (0.7) to 2 (0.2) antibiotics in one and 3 (0.3) to 1 (0.1) in another.

But once again, these studies failed to quantify the levels of decrease in total. Sulaiman et al. (2020) did a comparative study on clinical *Escherichia coli* and clearly reported effects of curing on resistance to several antibiotics with rates of decrease ranging for 14.3% to 100%. All these studies highlight that the genes for resistance for a specific antibiotic are not necessarily chromosomally borne or plasmid borne. Assessing the situation in a specific locale is important because science could evolve to the point whereby an antibiotic can be simultaneously administered with a plasmid curing agent ensuring efficacy of the antibiotic. These basic research works would therefore be essential to contributing to empirical studies in a particular locale.

With respect to the association of virulence with plasmids, there are fewer current reporting studies. Most studies focus not on effect of plasmid curing on virulence characteristics but on extraction and characterization of potential virulence plasmids often via whole genome sequencing (Quan *et al.*, 2025, Ribeiro-Almeida et al., 2025). A 2009 Egyptian study (El-Baghdady *et al*., 2009) noted a total loss of invasive and adherence capacities of their test isolates following plasmid curing, minimal differences were however noted for the other tested virulence traits (cytotoxicity, elastase activity, urease activity and swarming migration). A more recent study carried out in Nigeria (Mahmod and Husen, 2020), noted an effect on plasmid curing on their test isolate with respect to protease and hemolysin production. The Mahmod study however involved only a single isolate. The variable effect of plasmid curing on both hemolysis and protease production in this current study highlight the variable pathway, bacteria take to achieving the end goal.

Conclusion

This study reports the low effect of curing the bacterial plasmid on their antibiotic resistant traits on the tested antibiotics except a slight shift to ofloxacin. Although same were observed in the virulence traits however there was a remarkable effect on hemolysin production by the organisms. This study provides very useful data on the role of plasmid curing on virulence traits in *Escherichia coli* isolated from abattoir.

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