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Exploring Antibiotic Susceptibility and Plasmid Characteristics in Sun-Dried Meat Foodborne Pathogens

Opara, C.N.
Department of Microbiology, Faculty of Sciences, Federal University Otuoke, Yenagoa, Bayelsa State, Nigeria

Corresponding author email: oparacn@fuotuoke.edu.ng

Abstract
For this study, eight bacterial isolates from samples of sun-dried meat were employed. The isolates were identified as *Enterococcus sp.*, *Micrococcus sp.*, *Bacillus sp.*, *Staphylococcus auereus*, *Salmonella sp.*, *Escherichia coli*, *Pseudomonas sp.*, and *Klebsiella pneumonia* through morphological and physiological characterisation. Acridine orange (0.75 mg/ml) was used for the plasmid curing process. Using agarose gel electrophoresis, the effectiveness of the plasmid was examined, and antibiotic susceptibility discs were used to measure the effect on antibiotic susceptibility. Pefloxacin (PEF), an antibiotic with a 75% sensitivity, was the most successful in suppressing the isolates. Conversely, all isolates were resistant to ampicillin (APX) and amoxicillin (AM), which were the least effective antibiotics. *Klebsiella sp.* is the most resistant isolate; it exhibited resistance to 88.8% (8 of 9) of the drugs tested. However, *Staphylococcus aureus i*s the most responsive organism, showing sensitivity to 55.5% (5 out of 9) of the antibiotics tested. Of the eight isolates tested, five had plasmids detected in them, while three (*Bacillus sp*., *Salmonella species*, and *Klebsiella pneumonia*) lacked plasmids as evidenced by the absence of a visible band. Following the curing process, *Micrococcus sp* lost its resistance to 3 of the 9 antibiotics (33%) while *Staphylococcus aureus* developed its resistance to 5 of the 9 (56%) drugs. According to this study, foodborne bacteria can carry and potentially spread plasmids that confer persistent antibiotic resistance. Furthermore, it demonstrates that, depending on plasmid curing, some resistance is chromosomally mediated and some are plasmid-mediated.

Keywords: Food-Borne Bacteria, Antibiotics Resistance, Public Health, Plasmid Profile and Curing

Introduction

For many years, contaminated food and food items have been linked to foodborne illness, which poses a serious threat to public health. Some of the main factors promoting food-borne diseases in developing countries are inadequate food handling practices, such as weak or inadequate safety laws, inadequate sanitation exercises and regulatory system enforcement, and lack of enlightenment and infection awareness (Gille et al., 2018). Over the past ten years, there has been an increase in the problem of food contamination with antibiotic-resistant bacteria, which is linked to a variety of anthropogenic and environmental causes (Jeong et al., 2013; Iraporda et al., 2015). It is more likely that food-borne illnesses may develop antibiotic resistance since the elements that determine antibiotic resistance can spread to other bacteria that are dangerous to the public's health. Antimicrobial resistance is becoming more common among food-borne pathogens due to the transferability of antibiotic resistance determinants to other bacteria that are important to public health (Cassani et al., 2019). Separate, circular, self-replicating extrachromosomal DNA fragments with distinct copy counts within the host are called plasmids. Plasmids encode a variety of characteristics, such as resistance to heavy metals and antibiotics, hydrocarbon degradation, bacteriocin and antibiotic production, and more (Ranadheera et al., 2017; Bell et al., 2018). They frequently carry extra genetic information, such as genes that confer antibiotic resistance, which might be useful to bacteria in particular settings. Plasmids, however, can also aid in the spread of undesired features like antibiotic resistance (Bell et al., 2018). One method that aids in determining the possibility of resistant gene dissemination is called primer profiling. It must be investigated as a plasmid as a result. Because plasmids are a key mechanism for the dissemination of antibiotic resistance genes in the bacterial population, this must be investigated (Cassani et al., 2019).

When bacteria adapt to the use of antibiotics, antibiotic resistance develops. Animals or humans do not develop antibiotic resistance in bacteria. Both humans and animals can contract illnesses from these bacteria, and treating

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those infections is more difficult than treating infections from non-resistant bacteria (Gulbandilar et al., 2017). Plasmid curative and anti-plasmid techniques could decrease the frequency of antimicrobial resistance genes and make bacteria more susceptible to antibiotics, but novel approaches are needed to combat antimicrobial multidrug resistance (Adesulu-Dahunsi et al., 2018). Any meat's microbiological quality is influenced by the physical and general health of the animal at the time of slaughter, as well as handling, storage, and environmental cleanliness. Antibiotic-resistant pathogenic Enterobacteriaceae members contaminating meat and meat products are a major global health problem due to their high mortality rate. There have also been prior reports of increased antibiotic resistance and spread among these foodborne pathogens (Ranadheera et al., 2017). Therefore, the goal of the current study was to examine the plasmid profiles, curing, and antibiogram of harmful bacterial isolates from sun-dried beef.

Materials and Methods

The following bacteria were isolated from samples of dried meat: *Salmonella sp*., *Escherichia coli*, *Pseudomonas sp*., *Bacillus sp*., *Micrococcus sp*., *Salmonella sp*., *Enterococcus sp*., and *Klebsiella pneumonia* and were used for this study.

To inoculate Salmonella Shigella Agar (SSA), Nutrient Agar (NA), a multipurpose culture medium for various organisms, and Mannitol Salt agar (MSA) for Staphylococcus spp., an aliquot (100 µl) of the bacterial isolates were diluted to 10^{-4} and $10^{-6}$of the 10-fold serial dilution. applying the spread plate technique. Subsequently, the plates were incubated at 37°C for 24 hours while inverted. The number of distinct, visible colonies was measured and reported as colony-forming units per gram, or cfu/g. The isolates were subcultured, and morphological description, Gram staining, and biochemical tests (oxidase, methyl red, indole, catalase, coagulase, urease, and citrate) were used to identify the isolates (Bukhari et al., 2004).

The modified Kirby-Bauer multi-disk diffusion method was used to assess the isolates' susceptibility to antibiotics (Andrup et al., 2008). Muller Hinton agar was treated with commercial antibiotic discs (ROSCO) containing the antibiotics. The following antibiotics were found on the discs (Celtech Diagnostic) and were tested for effectiveness against the isolates: PEF: 10µg of perfloxacin, Z: 20µg of zincacef, CPX: 30µg of ciprofloxacin, E: 10µg of erythromycin, GN: 10µg of Gentamycin, AM: 30µg of amoxicillin, SXT: 30µg of streptomycin, APX: 30µg of ampiclox, R: 25µg of rocephin, and S: 30µg of septrin.

Plasmid profile analysis was performed on the isolates using the modified Alkali-lysis method. Each bacterial isolate was cultured for a whole night in 5 millilitres of nutritional broth. After carefully mixing the broth culture with a vortex, 1.5 ml was placed into an Eppendorf tube that had been labelled beforehand. The bacterial cells were subsequently extracted from the tubes by centrifuging them for four minutes at 6500 rpm (revolutions per minute). After carefully decanting the supernatant, around 100µl of broth culture remained. This was vortexed rapidly until the bacterial cell pellet was fully suspended. To lyse the bacterial cells, an alkali-lysis solution (350µl; 25 mM Tris, 10 mM EDTA, 0.1N NaOH, 0.5% SDS) was added. After roughly 50 inversions of mixing, the solution turned slimy. Next, 150µl of 3.0M sodium acetate was added, and it was vortexed once more for ten seconds. For 15 minutes, it was centrifuged again at 6500 rpm to remove chromosomal DNA and cell debris. After that, the supernatant was poured into a second 1.5 ml Eppendorf tube that had been labelled, and 900 µl of cold 100% ethanol was added. For ten minutes, the fluid was centrifuged at 6500 rpm. The white pellet containing the plasmid DNA was washed twice with 1000µl of 70% ethanol after the supernatant was discarded. Next, the pellet was left to air dry. Subsequently, the pellet was once again suspended in 50µl of TE buffer and kept cold for future usage (Ekundayo, 2021).

Using a modified version of Udo et al. (2008), the plasmid was cured to ascertain whether it encodes a characteristic that codes for antibiotic resistance or multiple antibiotic resistance. Acridine orange (0.75 mg/1 ml) was used for the curing process. Plasmid curing was applied to the isolate that displayed resistance to various antibiotics as a result of plasmid bands. Acridine orange (100µl) containing 0.75 mg/ml was introduced into 5ml of LB broth that had been infected with the test isolates. In a shaker incubator, the medium was incubated for 48 hours. Following incubation, cultures with the greatest concentration of acridine orange that showed obvious growth were diluted and placed on nutrient agar plates together with the relevant antibiotics to test for susceptibility.

Results

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Table 1: Zone of Inhibition in Mm for Antibiotics sensitivity pattern of bacteria isolated from sun-dried meat

ISOLATES	CN (10ug)	R (30ug)	AM (25ug)	CPX (5ug)	APX (30ug)	PEF (5ug)	**S** (10ug)	E (15ug)	Z (20ug)
Pseudomonas sp	R(10)	R(0)	R(0)	S(42)	R(0)	S(34)	R(0)	R(0)	I(16)
Enterobacter sp	I(16)	I(15)	R(0)	S(38)	R(0)	I(18)	R(0)	R(0)	R(0)
Salmonella sp.	I(17)	R(8)	R(0)	S(40)	R(0)	S(32)	R(0)	R(0)	S(20)
Bacillus sp	I(16)	R(0)	R(0)	S(40)	R(0)	S(36)	I(15)	R(10)	S(22)
Escherichia coli	R(12)	R(12)	R(0)	S(36)	R(0)	S(42)	S(20)	R(10)	R(0)
Klebseilla pneumonia	R(12)	S(22)	R(8)	R(0)	R(0)	R(0)	R(10)	R(0)	R(5)
Staphylococcus aureus	R(0)	R(0)	R(0)	S(40)	R(0)	S(34)	S(36)	S(28)	S(28)
Micrococcus sp	R(12)	R(14)	R(0)	S(38)	R(0)	S(36)	R(0)	R(0)	R(10)

Key: pefloxacin(pef), gentamycin(cn), Ampicillin(apx), Cefuroxime(z), amoxicillin(am), Ceftriaxone(r), ciprofloxacin(cpx), streptomycin(s), erythromycin(e). Resistant (R), Sensitive (S), Intermediate (I).

The isolates that were subcultured in this investigation were determined to be *Enterococcus sp.*, *Micrococcus sp.*, *Bacillus sp.*, *Staphylococcus auereus*, *Salmonella sp.*, *Escherichia coli*, *Pseudomonas sp.*, and *Klebsiella pneumonia*. Table 1 displays the isolates' pattern of antibiotic susceptibility. All of the antibiotics that were tested showed some degree of resistance in the isolates. PEF, an antibiotic with a 75% sensitivity, was the most successful in suppressing the isolates. Conversely, all isolates were resistant to ampicillin (APX) and amoxicillin (AM), which were the least effective antibiotics. Klebsiella sp. is the most resistant isolate; it exhibited resistance to 88.8% (8 of 9) of the drugs tested. However, Staphylococcus aureus is the most responsive organism, showing sensitivity to 55.5% (5 out of 9) of the antibiotics tested.

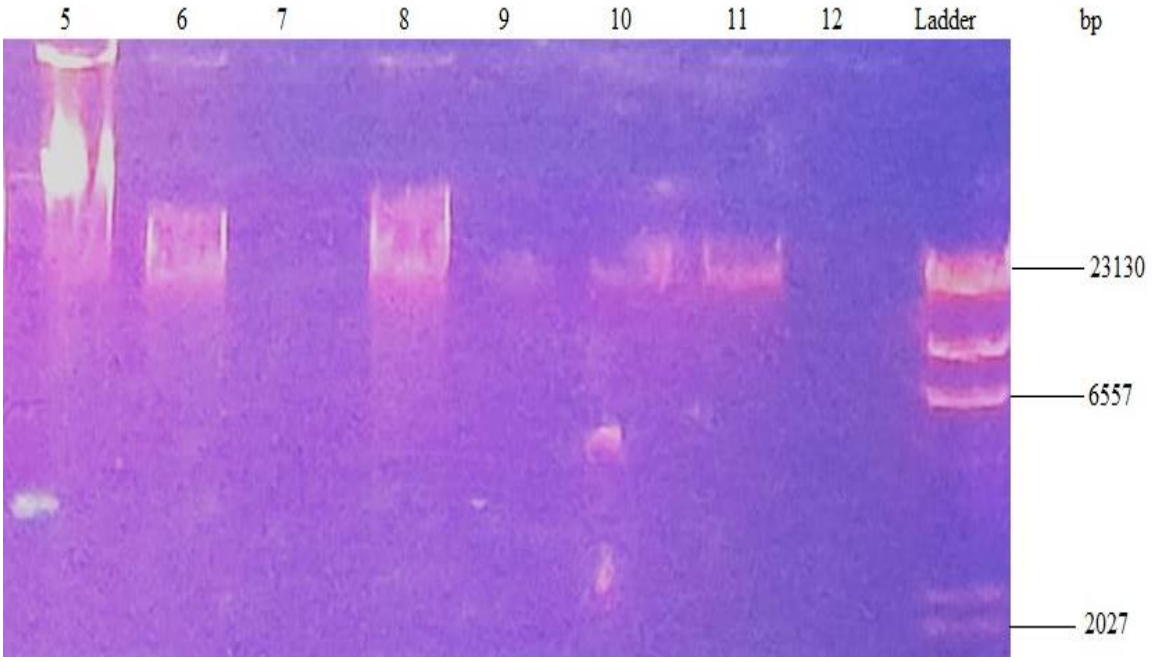

Fig. 1: Plasmid profile pattern of bacterial isolates in 0.5% agarose gel.

Key: Lane 5**;** *Enterobacter sp***,** lane 6**;** *Micrococcus sp*, lane 7; *Bacillus* sp, lane 8; *Staphylococcus aureus*, lane 9; *Salmonella sp*, lane 10; *Escherichia coli*, lane 11; *Pseudomonas sp* lane 12; *Klebsiella pneumonia*.

As shown in Figure 1, plasmid profiles of the bacteria isolates were performed and displayed in a gel electrophoresis field. Out of the eight isolates examined, five demonstrated the presence of plasmids, and three isolates had no discernible band, which suggests the lack of plasmids.

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Table 2: Diameter of Zone of Inhibition (Mm) of the Antibiotics after plasmid curing

ISOLATES	CN (10ug)	R (30ug)	AM (25ug)	CPX (5ug)	APX (30ug)	PEF (5ug)	S (10ug)	E (15ug)	Z (20ug)
Pseudomonas	18	20	30	22	7	34	0	10	15
Enterobacter spp	23	10	18	0	12	9	0	34	23
Salmonella spp.	20	20	13	26	30	0	10	30	20
Bacillus spp	20	25	20	22	30	0	12	28	25
Escherichia coli	25	21	23	20	27	0	10	25	20
Klebseilla spp.	0	26	18	10	30	41	9	12	15
Staphylococcus spp.	15	20	0	0	0	0	0	24	15
Micrococcus spp	22	25	0	22	24	0	0	30	22

Key: pefloxacin(pef), gentamycin(cn), Ampicillin(apx), Cefuroxime(z), amoxicillin(am), Ceftriaxone(r), ciprofloxacin(cpx), streptomycin(s), erythromycin(e).>20mm =Sensitive (S); 15-19mm =Intermediate (I); <14mm =Resistant (R).

As indicated by Table 2, after curing, there was a little decrease in resistance to some of the antibiotics, but the bacterial isolates' susceptibility to other antibiotics did not change. After the cure, the bacterial isolates developed resistance to some antibiotics, which could be linked to the overuse and abuse of these drugs, which led to the creation of strains resistant to the drugs.

Discussion

All gram-negative bacteria, except *Klebsiella pneumonia* were shown to be susceptible to both pefloxacin (pef) and ciprofloxacin (cpx). A recent analysis found a comparable report of *Klebsiella pneumonia* resistance to antibiotics in the quinolone group (Salmanov et al., 2021). The bacteria that were most susceptible to Ciprofloxacin (cpx) were gram-positive strains of Micrococcus and *Staphylococcus sp.* They also showed sensitivity to cefuroxime (z) and pefloxacin (pef). Comparable reports of Ciprofloxacin sensitivity in *Staphylococcus* and *Micrococcus* have been published by (Dajcs et al., 2004; Ajiboye, 2021). 100% of the bacterium isolates were resistant to ampicillin and amoxicillin, or penicillin. The bacteria found in this study have penicillin resistance, which is in line with findings from earlier research on this expanding trend (Breijyeh et al., 2020). *Serratia marcescen*t resistance to the variety of antibiotics used in this investigation is in line with earlier experimental findings published by Guerra et al. (2003), which showed that *Serratia marcescens* was only susceptible to Ciprofloxacin (cpx) and Pefloxacin (pef), and that it was resistant to other widely used antimicrobial agents. This assay's result demonstrates a significant frequency of antimicrobial resistance since all isolated organisms exhibit multi-antibiotic resistance. This is in line with the Ministry of Health of Nigeria's report on the burden of disease and antibiotic resistance, which discovered a significant prevalence of bacteria resistant to many drugs in Nigeria, raising the country's potential death rate (Egwuenu et al., 2018).

To determine the molecular weight of the plasmid DNA, use the ladder, a 1 kilobase marker with standard molecular weight (Roberts and Crawford, 2000). It is known that bacteria can become resistant to drugs in response to external environmental pressure. This can happen when medicines or resistant strains are introduced directly into the environment or when plasmids are used to transfer resistance gene sequences. (Carattoli, 2013). Thus, the gel electrophoresis data suggest that plasmids may be a mediating factor in the resistance to the antibiotics under investigation. Plasmids are how bacteria develop resistance, according to several studies (Bennett, 2008); nevertheless, some studies indicate that plasmid presence positively correlates with bacterial resistance (Talukder et al., 2021). Numerous bacterial genera have been shown to exhibit horizontal gene transfer, including Enterobacter isolates carrying plasmids that give other bacteria antibiotic resistance (Vaidya, 2011). Research on Serratia sp. has been done in a manner comparable to this (Ugochukwu et al., 2022). Furthermore, bacterial DNA can incorporate the DNA carried by plasmids, giving the bacteria the capacity to withstand antibiotics and making the resistance genes fully inherited (Talukder et al., 2021). Large plasmids and their capacity for conjugation are occasionally linked to patterns of bacterial antibiotic resistance (Alitheen et al., 2009). On the other hand, isolates lacking a plasmid exhibited a high number of antibiotic resistance patterns, suggesting that resistance to most of these antibiotics stems from chromosomal origins or mobile genetic elements. This could facilitate the spread of resistant genes to other human clinically significant bacteria (Atuanya & Ogunleye, 2015). Carattoli (2003) and Yah et al. (2007) state that rather than being plasmid-mediated, the antibiotic resistance in isolates that don't appear to have plasmids was linked to chromosomes and/or transposons.

From Table 2 after curing, S. aureus was demonstrated to have a broad spectrum of antibiotic resistance, suggesting that its resistance genes may be chromosomal rather than plasmid-carried (Nguyen et al., 2014). The isolates' plasmid-mediated antibiotic resistance was discovered using the plasmid curing test, which is consistent with findings from earlier research (Zaman et al., 2010; Vinay et al., 2013; Ojo et al., 2014). While all isolates remained resistant to streptomycin, a 10 mm diameter zone of inhibition was seen in Salmonella sp. isolates compared to none before curing, which could suggest that both the bacterial chromosome and the plasmid were mediating the resistance to streptomycin (Jesús et al., 2014).

Conclusion

This is an early study that demonstrates the food-borne pathogens' susceptibility, resistance, plasmid profile, and ability to cure when exposed to different tested antibiotics. Resistance was noted to common antibiotics such as ampicillin, amoxycillin, and erythromycin. The cure experiment revealed that the majority of the antibiotic resistance was plasmid-mediated, and this type of resistance is easily transmissible across strains or between organisms living in the same environment. However, since antibiotic abuse and overuse contribute to the development of antibiotic resistance and the majority of antibiotic resistance is plasmid mediated, easily transmissible to non-resistant microbial species, Policies that limit the indiscriminate use of antibiotics are advised, particularly for those that have fewer reports of resistance emerging. The investigation also showed that, although resistance was still seen, plasmid curing enhanced susceptibility (sensitivity) to the test antibiotics.

Recommendation

Antibiotic overuse and sales over the counter should be discouraged to reduce the threat of antibiotic resistance. Furthermore, since there aren't many studies on this topic, care should be taken while using antibiotics in chicken farms. Additionally, a study on Staphylococcus sp.'s chromosomal-mediated resistance to antibiotics should be done.

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