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# Antibacterial Spectrum of Multi-drugs Resistant Bacteria in Grilled Pork Meat

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## Abstract

Meat is a protein source and also highly prone to contamination. This study evaluated the presence and prevalence of Multi Drug Resistant (MDR) bacterial pathogens in grilled pork meat sold as a ready to eat street food in Owerri West, Nigeria. Samples collected randomly were subjected to standard microbiological analysis. Bacteria population ranged between 1.0 x $10^3$ to 2.33 $\times 10^4$ CFU/g which suggested gross contamination. The presence of pathogenic bacteria such as *Salmonella* sp., *Escherichia coli*, and *Staphylococcus aureus* portends serious health implications to consumers. These organisms have been reported to cause food borne illnesses. *Bacillus* are major contaminants of soil and vegetation and could be opportunistic in pathogenicity. Gram negative *Shigella*, *Pseudomonas*, *Enterobacter* and *Klebsiella* have been frequently isolated in soil, water and feaces. Their presence is significant and worrisome. Multi Drug Resistant bacterial isolates could result from frequent administration of antibiotics to animals. *Bacillus* is highest susceptibility to Amikacin (30 µg) at 25mm followed by *E.coli*. *Salmonella* exhibited the highest sensitivity to levofloxacin at 34mm followed by *Pseudomonas* at 32mm. Hygiene indicator microorganisms such as *E. coli* were found in 50% of the samples. *Staphylococcus aureus* and *B. cereus* exhibited multidrug resistance at 85.8% with *Staphylococcus* sensitive to Amoxycilin. *B. subtilis* and *Enterococcus faecalis* exhibited 71.43% multidrug resistance. This study provides valuable information of the distribution of antimicrobial-resistant pathogens in grilled pork meat sold as ready to eat foods. Proper standard operation procedures (SOP) is recommended to develop a systematic strategy for reducing the current emergence and spread of antimicrobial resistance genes in the different phases of pig farming, production and distribution.

**Keywords:** antibacterial, pork meat, MRD resistant

## Introduction

Foods are essential vehicles in human exposure to antibiotic resistant bacteria which serve as reservoirs for resistance genes and a rising food safety concern. Foods can be contaminated by different means, including exposure to irrigation water, manure, faeces or soil with pathogenic bacteria. Foods can also become contaminated as they are harvested, handled after harvest or during processing if food safety standards are not correctly applied. Food-borne diseases caused by resistant organisms are one of the most important public health problems as they contribute to the risk of development of antibiotic resistance in the food production chain (Hashempour-Baltork *et al*., 2019). Also, raw foods and foods that are not processed following standard procedures can introduce several antibiotic-resistant bacteria (ARB) to consumers (Gekenidis *et al*., 2018).

The imprudent use of antimicrobials in both the human and animal sector has resulted in the selection of pathogens resistant to multiple drugs. There is no doubt that the rate of antimicrobial resistance development and spreading far outweighs the rate at which new antimicrobial drugs are being developed. For instance, resistance to colistin, one of the last resort antibiotics used to treat multidrug-resistant Gram-negative infections, has been reported (Baker *et al*., 2001; Prescott *et al*., 1999). Multidrug-resistant (MDR) bacteria present a critical danger to public health and can survive the selective toxicity of antimicrobial use, enabling them to proliferate in clinical, on-farm, and environmental settings (AU-IBAR, 2016).

The presence of multi-drug resistant bacteria in grilled pork meat raises concerns about potential foodborne infections and the transfer of antibiotic resistance genes to human pathogens. Studies have investigated the survival and heat resistance of these bacteria during the grilling process. For instance (Lee *et al*., 2020).

This study reports on the antibacterial spectrum of multi-drugs resistant (MDR) bacteria isolated from grilled pork meat.

## Materials and Methods

### Experimental Design

The microbiological characteristics of grilled pork as sold to consumers were assessed through samples collected from selling sites. Then, the antibiotic profiles of the isolated bacteria were evaluated.

### Samples Collection

A total of 128 samples of grilled pork meat were randomly collected (Kothara, 2004) from roadside vendor in the four locations; Umuchima, Eziobodo, FUTO back gate, and Ihiagwa in Owerri West LGA. The samples were individually packaged in sterile plastic bags and transported in an ice-pack to the laboratory for microbiological analyses.

Nutrient agar (NA), Eosin Methylene Blue agar (EMBA), Salmonella Shigella agar (SSA) and Mannitol salt agar (MSA) were prepared according to manufacturer's specification. Nutrient agar was used in the isolation of heterotrophic bacteria (Cheesbrough, 2000).

Distilled water used as diluents was prepared by dispensing 90 ml and 9 ml portion into conical flask and bijou bottles respectively. Both diluents and media were sterilized in an autoclave at $121^0$C for 15mins.

### Microbiological Analyses

Ten grams (10 g) of each sample was placed aseptically with a sterile forceps into 90 ml of sterile physiological saline and shaken vigorously. The suspension was decimally diluted by transferring 1 ml. An aliquot of the appropriate dilution was inoculated into freshly prepared and surface dried media in duplicates. Inocula were spread evenly to ensure discrete and countable colonies. Plates were incubated at ambient temperature for 24 - 48 hours for heterotrophic bacteria (Sharma, 2000; Beishir, 1987).

### Enumeration of Bacteria Counts and Characterization

Colony counts obtained on the media were expressed as colony forming units per gram (CFU/g) to obtain total population (Harrigan and McCance, 2000). Bacterial isolates were characterized and identified using standard methods (Buchanan and Gibbon, 2009; Harrigan and McCance, 2000).

### Standardization of Bacterial isolates and Determination of MDR

Twenty four hour old broth cultures of the isolates were standardized by McFarland methods of turbidity equivalent to 1.5 x $10^8$ CFU/ml. One-tenth milliliter (0.1 ml) of the broth was spread evenly with a glass rod spreader on a freshly prepared and surface dried Mueller Hinton Agar and allowed to stand for 20 min. Commercial antibiotic disc (Oxoid, UK) of different concentrations were aseptically placed equidistant from each other on the culture plate. The plates were then incubated for 24h at 37$^o$C and zones of inhibition of each measured with a transparent meter rule(CLSI, 2014).

## Results and Discussion

Table 1 shows the mean bacterial population isolated on four bacteriological media. Bacteria grew luxuriantly on all the media. Counts were more on Nutrient Agar and least on Salmonella Shigella agar (Table 1). The counts on the media showed densities ranging from 1.0 x $10^3$ to 2.33 ×$10^4$ Cfu/g.

Table 1 Mean Bacteria Counts (CFU/g) of Pork Meat

| Sample code | NA count | SSA count | MSA count | EMBA count |
|---|---|---|---|---|
| UM | 2.33×$10^4$ | 1.0 ×$10^3$ | 2.1×$10^3$ | 1.1× $10^3$ |
| IH | 2.18×$10^3$ | - | 1.66 x $10^4$ | 1.6 x $10^3$ |
| FBG | 1.80 x $10^3$ | - | 1.44 x $10^4$ | 1.3 x $10^3$ |
| EZ | 1.21 x $10^3$ | 1.5 x $10^3$ | 2.4 x $10^3$ | 1.6 x $10^3$ |

UM, Umuchima; FBG, FUTO Back Gate; IH, Ihiagwa; EZ, Eziobodo; NA, Nutrient Agar; SSA, Salmonella Shigella Agar; MSA, Mannitol Salt Agar; EMBA, Eosin Methylene Blue Agar

The microbiological analyses of grilled pork samples showed the presence of pathogenic bacteria such as *Salmonella* spp., *E. coli*, and *S.aureus* (Table 2 and 3).*Staphylococcus aureus* was frequently isolated from the samples.Bennett *et al*. (2013)reported the presence of pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica* subsp. enterica in pork meat.

Hong *et al*. (2023) isolated five pathogens from pig carcasses and reported *S. aureus* as the most prevalent (40.0% of slaughter houses and 11.5% of carcasses) followed by *Y. enterocolitica* (7.0%), *C. perfringens* (4.0%). Several studies have reported that the most common foodborne pathogens associated with pigs are species of*Campylobacter*, *Salmonella*, *S. aureus*, *L. monocytogenes* and *Y. enterocolitica* (Bennett *et al*., 2013; Heredia and Garcia, 2018). That this study did not identify *Listeria*, *Yersinia* and *Campylobacter*, does not preclude their occurrence. The heat of cooking and subsequent grilling might have eliminated such bacteria from the samples.

The presence of nitrobacteria in grilled pork could be due to an insufficient heat treatment and/or a lack of hygiene after cooking. The presence of *E. coli* indicates faecal contamination or through inappropriate handling after cooking. This bacterium is indeed widespread in the environment and is commonly found in the intestine of animals (AU-IBAR, 2016). The Health Protection Agency (Health Protection Agency, 2009) set up acceptable limits for microbiological criteria in ready-to-eat food. Using these guidelines, it is safe to say that 21% (enterobacteriaceae and *E. coli*) in grilled pork samples were inappropriate for human consumption. (Kim *et al*., 2018; Kim & Yim, 2016).

*S. aureus* was one of the most common causes and was particularly dangerous at slaughterhouses because of its potential for transmission from animals to slaughter operators and vice-versa (Peton and Loir, 2014). In Germany, Greece, and South Africa, the prevalence of *S. aureus* in pig carcasses were reported to be 6.0%, 15.5%, and 32.5%, respectively (Beneke *et al.,* 2011; Komodromos *et al.,* 2022; Tanih *et al.,* 2015).

Table 2 Colonial and Microscopic Characteristics of Bacteria isolated from Samples

| Colonial characteristics | Spore Formation | Motility | Gram Morphology | Most Probable Identity |
|---|---|---|---|---|
| Small circular shiny black fish eye colonies on SSA | - | + | Gram negative rods in short chains | *Salmonella* sp |
| Moist and shiny purple metallic sheen colonies on EMBA | - | + | Short gram negative rods predominantly in singles, few in chains | *Escherichia coli* |
| Smooth moist and shiny low convex golden yellow colonies on NA | - | - | Gram positive cocci predominantly in clusters, few in pairs | *Staphylococcus* sp |
| Dull and dry serrated flat cream colonies on NA | + | + | Large gram positive rods in short chains with central spores | *Bacillus cereus* |
| Mucoid and slimy cream colonies on NA | + | + | Gram positive rods in chains | *Bacillus* sp |
| Small circular moist and shiny low convex cream colonies on NA | - | - | Gram positive cocci in long chains, few in pairs and clusters | *Enterococcus* sp |
| Smooth moist and shiny pink colonies on EMBA | - | - | Gram negative rods in short chains | *Enterobacter* sp |
| Circular moist and shiny light pink colonies | - | - | Gram negative rods in short chains some in singles | *Shigella* sp |
| Mucoid and slimy domed shaped pink colonies on MCA | - | + | Small short Gram negative rods in singles and short chains | *Klebsiella* sp |

Table 3 Microscopic and Biochemical Characteristics of Bacteria isolated from Samples

| Spo | Mot | Gram stain | Oxi | Cat | Coag | In | MR | VP | S | L | G | M | Identity of isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| - | + | -R | - | + | - | + | - | + | + | + | + | - | *Escherichia coli* |
| - | - | -R | - | + | - | - | + | - | - | + | + | - | *Enterobacter* sp |
| - | + | -R | - | + | - | - | + | - | - | - | + | + | *Salmonella* sp |
| - | - | +S | - | + | + | - | - | + | + | + | + | + | *Staphylococcus* sp |
| - | - | +S | - | - | - | - | + | - | + | + | + | + | *Enterococcus faecalis* |
| + | + | +R | - | + | - | - | - | + | - | - | - | - | *Bacillus cereus* |
| + | + | +R | - | + | - | - | - | + | - | - | - | + | *Bacillus subtilis* |
| - | + | -R | - | + | - | - | - | + | + | + | + | + | *Klebsiella* sp |

*B. cereus* showed the highest susceptibility to Amikacin (30 µg) at 25mm followed by *E. coli*. *Salmonella* exhibited the highest sensitivity to levofloxacin at 34mm followed by *Pseudomonas* at 32 mm (Table 4).

Dongryeoul *et al.* (2022) reported pathogens and their antimicrobial resistance isolated from pig production to pork meat distribution phases. All the foodborne pathogens: *L. monocytogenes*, *S.aureus*, and *Y. enterocolitica* isolated from the samples were sensitive to amoxicillin/clavulanate, ciprofloxacin, and gentamicin, whereas some, *L. monocytogenes*, and *S. aureus* isolates were resistant to various antibiotics, including ampicillin, erythromycin, tetracycline, and vancomycin. The most common antimicrobial resistance pattern in the pathogenic isolates was AMP-KAN-STR-SXT-TET (CLSI, 2014). The *Staphylococcus* species and *B. cereus*, isolated from the samples in this present study exhibited the most multidrug resistance at 85.8% with *Staphylococcus* sensitive to Amoxycilin and *B. cereus* to amikacin. This was followed by *B. subtilis* and *Enterococcus* which exhibited 71.43% multidrug resistance (Table 5).

Antibiotic resistance, though harbored in non-pathogenic bacteria, can potentially be spread through horizontal gene transfer to other species including opportunistic pathogens that are present in the environment or after consumption of ARB-contaminated foods. When ARB-contaminated foods are consumed, the spread of antibiotic resistant genes may affect the gut microbiome thereby contributing to the pool of antibiotic-resistance genes (ARG) in the human gut (Gekenidis *et al*, 2018). The use of antimicrobials in animal production (especially in poultry and pigs) remains a key contributor to AMR (Van Boeckel *et al*., 2019). Its use is expected to increase exponentially due to the expansion of intensive production systems and the surge in disease burdens. Over the next 20–40 years, meat consumption in Africa is forecast to grow by 30% by 2030) due to growth in the human population (from the current 1.2 billion to over 2.5 billion by 2050), increasing purchasing power and urbanization (FAO, 2018). Across Africa, the current per capita annual consumption of meat and milk is about 14 kg and 30 L, respectively, and is projected to more than double to 26 kg and 64 L, respectively, by 2050 (AU-IBAR, 2016).

Table 4 Susceptibility of Bacterial Isolates to Commercial Antibiotics

| . Bacterial Isolates | Amikacin 30µg | Ciprofloxacin 5µg | Amoxycilin 30µg | Erythromycin 15µg | Penicillin 10µg | Ampicillin 10µg | Levofloxacin 5µg |
|---|---|---|---|---|---|---|---|
| *B. cereus* | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. subtilis* | 25 | 0 | 0 | 34 | 0 | 0 | 0 |
| Klebsiella sp | 19 | 18 | 0 | 0 | 0 | 0 | 20 |
| *E. coli* | 20 | 34 | 0 | 0 | 0 | 0 | 20 |
| Staphylococcus sp | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| Pseudomonas. sp | 18 | 34 | 0 | 0 | 0 | 0 | 32 |
| Enterococcus. sp | 19 | 0 | 0 | 15 | 0 | 0 | 0 |
| Salmonella sp | 14 | 30 | 0 | 0 | 0 | 0 | 34 |

Table 5 Level of Multi-Drug Resistance per Isolate in percentages

| Isolate | Sensitivity (%) | MDR (%) |
|---|---|---|
| *B. cereus* | 14.28 | 85.72 |
| *B. subtilis* | 28.57 | 71.43 |
| *Klebsiella* | 42.85 | 51.15 |
| *E. coli* | 42.85 | 51.15 |
| *Pseudomonas* | 42.85 | 51.15 |
| *Staphylococcus* | 14.28 | 85.72 |
| *Salmonella* | 42.85 | 51.15 |
| *Enterococcus* | 28.57 | 71.43 |

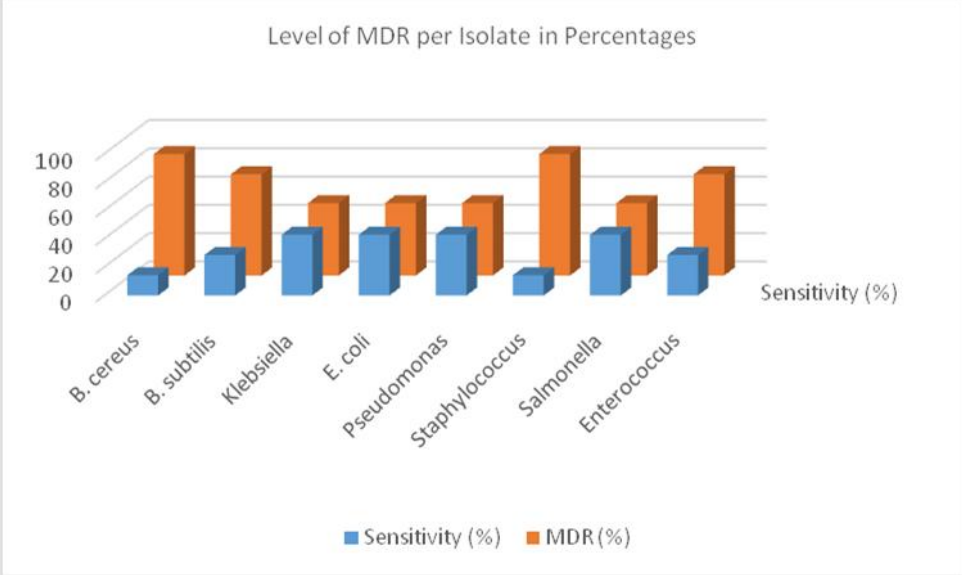


Fig 1 Level of MDR per Isolate in percentages

## Conclusion and Recommendations

The results of this study revealed that grilled pork processed and sold as ready-to-eat street food in Owerri West, Imo State could be a source of foodborne diseases and a great vehicle through which multidrug resistance genes can be transferred to humans. Most of the organisms isolated results from post-cooking operations with a higher probability occurring from handling. This is explainable because they are mostly sold in an open market and unhygienic environment.

This study provides valuable information for the distribution of antimicrobial-resistant pathogens in grilled pork meat sold as ready to eat foods and advised that a systematic strategy for reducing the current emergence and spread of antimicrobial resistance be put in place. It is also imperative that handlers and sellers be trained on matters of food hygiene, good handling practices and standard operation procedures to ensure food safety and security of consumers.

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