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Original article

Antibiotic susceptibility testing of meropenem-resistant bacteria isolated from pigs from selected farms in Ado Ekiti, Nigeria

Olajumoke kemi Ekundayo *[1], Isaac Iseoluwa Ajayi [2].

1- Bamidele Olumilua University of Education Science and Technology, Ikere, Nigeria

2- Bamidele Olumilua University of Education Science and Technology, Ikere Ekiti, Nigeria

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a major global health concern, especially with the emergence of carbapenem-resistant Enterobacteriaceae (CRE). This work investigates the antibiotic susceptibility testing (AST) of meropenem-resistant bacteria isolated from pig farms in Ado Ekiti, Nigeria. **Methods:** A cross-sectional study design was employed, with samples collected from 90 pigs across three farms. The bacterial isolates were cultured, identified using genetic techniques. Those resistant to meropenem10µg were tested for antibiotic susceptibility testing with fosfomycin, nitrofurantoin, tigecycline, gentamicin, and polymyxin B. **Results:** The study identified significant bacterial species such as *Escherichia coli*, *Proteus mirabilis*, *Providencia alcalifaciens*, *Shigella flexneri*, *Enterococcus faecalis*, and *Bacillus cereus* and meropenem-resistant bacterial isolates were susceptible to some considered ancient antibiotics such as fosfomycin, nitrofurantoin, tigecycline, and gentamicin, with sensitivity percentages of 92.86%, 85.71%, 92.86%, and 92.86%, respectively. Polymyxin B had the lowest effectiveness, with a sensitivity of 64.23%. The study focuses on meropenem resistance in pigs, raising concerns about their role as reservoirs for antimicrobial-resistant bacteria. **Conclusion:** The findings highlight the outcome of the research, which suggested that fosfomycin, nitrofurantoin, tigecycline, and gentamicin can be used to treat meropenem-resistant bacteria isolated from pigs. It should be noted that there is the possibility of spreading resistant germs to humans through direct contact, environmental contamination, or the food chain. This study underlines the importance of considering "sold" antibiotics for treatment, complete antimicrobial stewardship programs, the prudent use of antibiotics in veterinary practices, and improved biosecurity measures in animal husbandry.

Introduction

Antimicrobial resistance (AMR) has emerged as a major global health issue that affects human, animal, and environmental health. Meropenem resistant bacteria are among the most serious antimicrobial-resistant infections, posing major concerns due to their capacity to evade carbapenem medication, a class of antibiotics frequently used as a last resort [1,2]. Resistance to meropenem, a crucial carbapenem in Enterobacteriaceae, reveals an alarming trend, particularly in animal populations such as pigs, which are important for food production and zoonotic disease transmission.

The frequency of antimicrobial resistance in

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* *Corresponding author:* Olajumoke kemi Ekundayo
E-mail address: ekundayo.olajumoke@bouesti.edu.ng

livestock has prompted worries about its potential influence on public health, particularly where antibiotic use in animal husbandry is common. Pig farming is a popular agricultural technique in Ado Ekiti, Nigeria. It is frequently characterized by intensive rearing systems and the use of antibiotics to promote growth, prevent sickness, and treat illnesses. These methods can promote the emergence and spread of resistant bacterial species. This study focuses on the antimicrobial susceptibility testing (AST) of meropenem-resistant bacteria isolated from pigs from different farms in Ado Ekiti. By detecting resistance patterns and potential drivers, the study hopes to provide useful insights into the level of resistance, potential risk factors, and consequences for the veterinary and public health sectors.

Methods

The purpose of this study was to assess the antimicrobial susceptibility of meropenem-resistant bacteria isolated from pigs on specific farms in Ado Ekiti. The methodology was developed to collect, culture, isolate, identify, and test bacterial isolates resistant to meropenem for sensitivity/resistance patterns.

Study Design and Farm Selection

A cross-sectional study design was employed. Ado was divided into three, where each location is a representation of the area that covers the entire Ado Ekiti. Farms were selected purposively based on the scale of pig production, location, history of antibiotic use, and accessibility. Three (3) farms in Ado Ekiti were included in the study, ensuring a representative sample of pig populations within the region.

Ethical consideration

Ethical approval was obtained from Ekiti State Veterinary Clinic, and informed consent was secured from farm owners before sample collection.

Sample Collection

Pig samples were collected using sterile swabs dipped inside the rectum and rotated to obtain adequate samples. To prevent contamination, each sample was collected aseptically and maintained in a cooler with ice packs before being promptly transported to the laboratory for analysis. Samples of apparently healthy pigs were collected and labeled accordingly. In total, 90 samples were obtained from the three locations.

Isolation of Enterobacteriaceae

The samples were processed in the laboratory within 24 hours of their collection. They were inoculated on MacConkey agar and incubated at 37°C for 24-48 hours. Distinct colonies were sub-cultured to obtain pure isolates.

Identification of bacteria isolates

Bacterial isolates were identified by PCR. Genomic DNA was extracted as previously reported (PLEASE add reference). For each amplification, the PCR mixture included 10 µl of 5X GoTaq colorless buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of a 10 mM dNTPs mix, 1 µl of 10 pmol of each primer (27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and 1525R 5'-AAGGAGGTGATCCAGCC-3′), 0.3 units of Taq DNA polymerase (Promega, USA), 8 µl DNA template, and 42 µl of sterile distilled water. The PCR was carried out using a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystems Inc., USA), with an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 50°C for 60 seconds, and 72°C for 90 seconds, concluding with a final extension at 72°C for 10 minutes, and then cooled to 4°C [3]. The amplification of the approximately 1.5 kb gene fragment was confirmed by passing the result through a 1.5% agarose gel. The gel was electrophoresed at 120 V for 45 minutes before being visualized and photographed using UV transillumination. The PCR product sizes were determined by comparing them to the mobility of a 100 bp molecular weight ladder that was run alongside the experimental samples on the gel [3]. The amplified fragments were sequenced using an Applied Biosystems Genetic Analyzer 3130xl sequencer according to the manufacturer's instructions, with the Big Dye Terminator v3.1 cycle sequencing kit. Genetic studies were conducted using Bio-Edit software and MEGA X [4,5]

Antibiotics susceptibility testing

Antibiotics susceptibility testing The Kirby-Bauer Disc diffusion method was performed to examine the antibiotic sensitivity of all bacterial isolates using a Mueller-Hinton agar plate, and Zones of inhibition were measured to determine whether they were resistant or sensitive to the selected antibiotics [6,7]. A sterile loop was used to inoculate the pure isolate in 3ml of sterile peptone water, and a sterile swab stick was used to pick out the sample, which was then inoculated on sterile Mueller-Hinton agar. The selected antibiotic discs

were aseptically placed on sterile Mueller-Hinton agar at a distance from one another. The antibiotic sensitivity testing was conducted using a commercially available single Oxoid disc and antibiotics including gentamicin (30µg), fosfomycin (100µg), nitrofurantoin (300µg), tigecycline (15µg), polymyxin B (200µg) and meropenem(10µg). Used antibiotics were (Oxoid): gentamicin (30µg), fosfomycin (100µg), nitrofurantoin (300µg), tigecycline (15µg), polymyxin B (200µg) and meropenem(10µg). Bacteria isolates resistant to meropenem were selected for the sensitivity testing. The antimicrobial susceptibility test used Escherichia coli ATCC 25922 as the control strain [CLSI 2023].

Results

Bacteria isolated were identified as *Proteus mirabilis, Providencia alcalifaciens* species, *E. coli, Shigella flexneri*, *Enterococcus faecalis,* and *Bacillus cereus* using the molecular identification by DNA extraction, amplification, and sequencing of 16S rRNA using 27F and 1625R universal primer pair [8]

Table 1 shows the 14 bacterial isolates identified, and the sequences have been submitted to the NCBI database with their accession numbers

Table 3 is the percentage susceptibility/resistant pattern of bacterial isolates to antibiotics and it shows that 64.23% of bacterial isolates were sensitive to polymyxin b and 35.7% were resistant. 92.86% of bacterial isolates were sensitive to gentamicin and 7.14% resistant. 85.71% of bacterial isolates were sensitive to nitrofurantoin and 14.29% resistant, 92.86% of bacterial isolates were sensitive to fosfomycin and 7.14% resistant and 92.86% of bacterial isolates were sensitive to Tigecycline and 7.14% resistant

Table 1. Identified bacteria isolates

S/N	Scientific Name		Percentage Identity	Accession no
.1	fr4	*Proteus mirabilis*	99.66%	ON715740
.2	F11	*Providencia alcalifaciens*	99.77%	ON715731
.3	F14	*Bacillus cereus*	99.66%	ON715732
.4	Fr1	*Bacillus cereus*	99.55%	ON715737
.5	Fr2	*Enterococcus faecalis*	99.89%	ON715739
.6	M11	*Escherichia coli*	100.00%	ON715741
.7	M12	*Escherichia coli*	100.00%	ON715742
.8	M12p	*Escherichia coli*	100.00%	ON715743
.9	M13	*Escherichia coli*	99.88%	ON715744
.10	M16	*Escherichia fergusonii*	99.88%	ON715745
.11	M24	*Shigella flexneri*	99.76%	ON715746
.12	M25	*Shigella flexneri*	100.00%	ON715749
.13	Mr11	*Bacillus cereus*	99.55%	ON715748
.14	Mr5	*Bacillus cereus*	99.55%	ON715747

Table 2. represents the antibiotic susceptibility testing of the bacterial isolates and this shows that the bacterial isolates resistant to Meropenem are still susceptible to old antibiotics fosfomycin, nitrofurantoin, tigercycine, and gentamicin even though some of the isolates like *E.coli* and *Providencia alcalifaciens* were still resistant to gentamicin, polymyxin B.

S/N	Isolates Code	Organisms	PB(200) µg Polymyxin B	CN(30) µg Gentamicin	F(300) µg Nitrofuratoin	Fos(100 µg Fosfomycin	Tgc(15 µg) Tigercycline
1	M11	*Escherichia coli*	R	S	R	S	S
2	M12	*Escherichia coli*	S	S	S	S	S
3	M12p	*Escherichia coli*	S	S	S	S	S
4	M13	*Escherichia coli*	S	S	S	S	S
5	M16	*Escherichia fergusonii*	S	S	S	S	S
6	M24	*Shigella flexneri*	S	S	S	S	S
7	M25	*Shigella flexneri*	S	S	S	S	S
8	Mr11	*Bacillus cereus*	S	S	S	S	S
9	M11	*Escherichia coli*	R	S	R	S	S
10	F11	*Providencia alcalifaciens*	R	R	S	R	S
11	F14	*Bacillus cereus*	R	S	S	S	S
12	Fr1	*Bacillus cereus*	R	S	S	S	S
13	Fr2	*Enterococcus faecalis*	S	S	S	S	S
14	Fr4	*Proteus mirabilis*	S	S	S	S	S

Table 3. Percentage susceptibility/resistant of bacterial isolates to antibiotics.

Antibiotics	**Sensitive**	**Resistant**
Polymyxin B	64.23%	35.7%
Gentamicin	92.86%	7.14%
Nitrofuratoin	85.71%	14.29%
Fosfomysin	92.86%	7.14%
Tigecycline	92.86%	7.14%

Discussion

Animals, like humans, contain bacteria in their stomachs. Antibiotic-resistant bacteria found in animals' stomachs can enter food in a variety of ways, specifically when animals are slaughtered and processed; resistant bacteria can contaminate meat or other animal products, and resistant bacteria can enter the environment through animal waste. People can get antibiotic-resistant intestinal illnesses by handling or consuming contaminated food or coming into contact with animal waste (poop), whether through direct connection with animals and animal environments or via contaminated drinking or swimming water [9,10].

The increasing number of meropenem-resistant bacteria poses a substantial threat to public health, particularly in areas where livestock antibiotic usage is widespread. This study examines the incidence of meropenem-resistant bacteria isolate in pig farms in Ado Ekiti, Nigeria, identifying key bacterial species such as *Escherichia coli, Proteus mirabilis, Providencia alcalifaciens, Shigella flexneri, Enterococcus faecalis*, and *Bacillus cereus.* These findings highlight the pigs' crucial role as reservoirs for antimicrobial-resistant bacteria, raising concerns about zoonotic transmission via direct contact, polluted surroundings, or the food chain.

The study's findings indicate that meropenem-resistant isolates demonstrated high sensitivity to several older antibiotics, including fosfomycin (92.86%), nitrofurantoin (85.71%), tigecycline (92.86%), and gentamicin (92.86%). However, polymyxin B was less effective, with only 64.23% sensitivity. This suggests the potential utility of certain "ancient" antibiotics in managing infections caused by CRE in resource-limited

settings, where newer antimicrobials may not be readily available which corroborates the finding of [6,7,11] who provided updates on therapeutic options for carbapenemase-producing bacteria and recommended the use of polymyxin b, gentamicin, nitrofurantoin, fosfomycin, and tigecycline but polymyxin was not as effective in this study as other antibiotics were more effective as stated in the previous study

According to this study, antimicrobial resistance has also been recorded in enteropathogens such as *Proteus mirabilis, Providencia alcalifaciens, E.coli, Shigella flexneri, Enterococcus faecalis, and Bacillus cereus,* and this corroborates the report of [11] that antimicrobial resistance has been observed in commensal bacteria (e.g., *Escherichia coli, enterococci*), bacterial pathogens of animals (e.g., *Pasteurella, Actinobacillus* spp., and zoonotic. However, the prevalence of resistance varies, especially when found in food animals such as pigs, poultry, birds, and farm animals, which can enhance the transfer of resistant genes between animals, humans, animal products, and the environment [12].

The use of antibiotics without proper prescription may have contributed to the issues of antibiotics because in the course of this research, farm animal owners confessed to self-treat animals without the help of veterinary doctors, which can be one of the reasons for the increase in antibiotic resistance globally, and this was by the report of (13) that several antimicrobials (e.g., ceftiofur, sulfonamides, tetracyclines, tiamulin) are used to treat and prevent. Although some antibiotics are used in feed for growth, mostly in pigs after weaning, because they are most vulnerable.

Environmental contamination from animal feces exacerbates the proliferation of resistant bacteria. The One Health strategy, which considers human, animal, and environmental health, is crucial for combating AMR. Mitigating the public health hazards associated with AMR requires measures such as careful antibiotic use, increased biosecurity, and constant monitoring of resistance patterns. [11].

This work emphasizes the need to revisit the considered old antibiotics to reconsider trying to use them to treat infections rather than developing new ones and nitrofurantoin, gentamicin, fosfomycin, and tigecycline can be used to treat meropenem-resistant bacteria.

Conclusion

The research study highlights the crucial issue of antimicrobial resistance (AMR) in pig farming, specifically meropenem-resistant bacteria in Ado Ekiti, Nigeria. The findings emphasize the persistence of resistance in major bacterial species, such as *Escherichia coli, Shigella flexneri,* and *Providencia alcalifaciens*, while also indicating the ongoing efficacy of older antibiotics like fosfomycin, nitrofurantoin, tigecycline, and gentamicin. Therefore, old antibiotics should be revisited and re-tested against bacteria to ensure they are not obsolete. These findings highlight the necessity of using antibiotics wisely in veterinary clinics and implementing thorough antimicrobial stewardship programs. Furthermore, the report advocates for stronger biosecurity measures and continued molecular research to track resistance patterns and discover genetic drivers of resistance.

List of abbreviations

AST- Antibiotic resistance testing

AMR-Antimicrobial resistant

Conflict-of-Interest Disclosure

There is no conflict of interest among the authors.

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None

Data availability

All data generated or analyzed during this study are included in this puplished article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

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