



Molecular Characterization and Identification of *Proteus mirabilis* (*Cucumis sativus*) Isolated from Cucumber (*Cucumis sativus*) Sold in Gusau Metropolis, Zamfara State Northwestern Nigeria

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Cucumber, Antibiotics, Kirby-Bauer, florouquinolones, *Proteus mirabilis*, Zamfara State, Resistance, and Susceptibility.

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ABSTRACT

Proteus mirabilis is a gram-negative bacterium commonly found in the environment and is known for its role in human and animal infections. Occurrence of *Proteus mirabilis* in fresh farm produce, such as cucumbers, tomatoes and cabbage raises food safety concerns due to its potential in contamination and transmission. This study was designed to screen and characterize *Proteus mirabilis* isolated from cucumber sold in Gusau metropolis, Zamfara state, Northwestern, Nigeria. Different techniques such as DNA extraction, Polymerase Chain Reaction (PCR), agarose gel electrophoresis, gene sequencing, and antibiotics susceptibility testing were employed in this study. The molecular characterization of the isolates was performed by using Zymo Research Quick-DNA bacteria/fungi miniprep kit. The bacterium identified was found to be *Proteus mirabilis*. The Antibiotics susceptibility of the isolate was determined using modified Kirby-Bauer disk diffusion method. Amoxicillin and ampicillin exhibited resistance. However, the isolate showed high susceptibility to ciprofloxacin, ofloxacin, sparfloxacin, and pefloxacin but showed a moderate sensitivity to gentamycin. Findings from this study clearly showed significant occurrence of *Proteus mirabilis* in cucumber sold in Gusau metropolis, Zamfara state with florouquinolones being the most active first line drugs tested against the bacterium.

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INTRODUCTION

Proteus mirabilis is a family member of Enterobacteriaceae and is a facultative anaerobe. The genus of *Proteus* is composed of about six important members such as *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri*, *Proteus terrae*, and *Proteus cibarius*. Among these, the most implicated specie in clinical isolates is *Proteus mirabilis*, which with other members of enterobacteriaceae such as *Escherichia*, *Klebsiella*, *Enterobacter*, and *Serratia* species are known to cause serious public health infections (Sun *et al.*, 2019). Recently, concerns due to the danger of foodborne pathogens and the impact they have on public health have necessitated the importance of ensuring the safety of various food products (CDC, 2020). *P. mirabilis* is found in diverse settings, such as soil, water, and sewage, playing an important role in decomposition of organic matter (Armbruster *et al.*, 2018). *P. mirabilis* is also part of the normal human and animal intestinal flora, along with *Escherichia coli*. It can cause disease in humans in the form of urinary tract infections and other opportunistic infections (Schaffer and Pearson, 2015), and approximately 90% of *Proteus* infections are caused by *P. mirabilis* (Jacoby, 2009).

Due to increasing consumption of raw fresh vegetables, including cucumber, these vegetables can serve as potential vehicles through which opportunistic and antibiotics resistant bacteria are transmitted (Al-Kharousi *et al.*, 2019). This is evident in the increasing cases of antimicrobial resistant pathogens seen in fresh produce, raising serious food safety concerns. There is increasing recovery of *Proteus mirabilis* from food, environmental and animal sources (Olanbiwoninu, 2024). *P. mirabilis* was clinically reported to be harboring significant carbapenem resistance genes (e.g., blaNDM) from some fresh vegetables especially cucumber (Li *et al.*, 2023). Some chromosomally integrated carbapenemase genes such as blaNDM1 have also been reported through genomic studies to be carried by *Proteus mirabilis* (Wang *et al.*, 2024). Moreover, multidrug-resistant strains of *Proteus mirabilis* have been reported to be in possession of both blaNDM1 and *cfp* genes (Sun *et al.*, 2024). This genetic mutation highlights the potential public-health risk that comes with contaminated produce such as cucumber. This study was undertaken to screen, characterize and determine the antibiotics susceptibility profile of *Proteus mirabilis* isolated from cucumber sold in Gusau metropolis, with a view to identifying the antibiotics that are most effective against the bacterium.

MATERIALS AND METHODS

Sample Collection and Preparation

Cucumber (*Cucumis sativus*) samples were obtained from Tudun wada, Yar-hanya, Damba, Sabuwar kasuwa, Unguwar gwaza, and Sabon gari markets, Gusau, Zamfara state, Nigeria. They were properly washed and mashed up using mortar and pestle at the Microbiology Laboratory of Federal University Gusau, and were stored aseptically for further analysis.

Sample Inoculation

The petri-dishes were first labeled with a marker, and the wire loop was then flamed red hot. A 10-fold serial dilution of the cucumber sample was done and a 0.1 mL of the suspension was inoculated into freshly prepared nutrient agar and incubated aerobically at 37°C overnight for 24 hours as described by Cheesbrough (2002).

Isolation and Sub-Culturing of Bacteria

After incubation, the bacterial isolates were sub-cultured into fresh culture medium using slant bottles. The sub-cultured isolates were incubated at 37°C for 24 hours allowing active growth and multiplication (Madigan *et al.*, 2021).

Identification of isolates

The isolate was identified based on gram's staining, colony morphology and standard conventional biochemical tests such as IMVIC for proper identification as described by Cheesbrough, (2010).

Molecular identification of bacterial isolate

DNA Extraction

DNA extraction was carried out using DNA extraction kit (Qiagen, USA), following manufacturer's instructions.

Polymerase chain reaction of 16S rRNA Gene

PCR targeting 16S rRNA gene was performed using some designed primers. The 25 µL PCR mix was prepared containing the following: Master Mix 12.5 µL (Qiagen, USA), 2.5 µL of coral load, 1 µL of 10 mM each of 27-F and 1942-R, 3 µL of nuclease free water and 5 µL of DNA template. The tubes were carefully mixed and transferred to applied bio-system 9700 programmed with the following cycling conditions. Initial denaturation at (94°C for 5 minutes) followed by 40 cycles of denaturation at (94°C for 30 seconds), annealing specific to 16S rRNA gene at (60°C for 1 minute) and the extension at (72°C for 1 minute 30 seconds), followed by final extension at 72°C for 10 minutes. The PCR products thus obtained were kept at 4°C until required.

Agarose Gel Electrophoresis

The PCR amplicons (products) were loaded on 1.5% agarose gel and run at 100 volts for 40 minutes. To determine the size of the amplified PCR product, 1 kb DNA ladder from New England Biolabs, UK was loaded in the first well. Electrophoresis was performed at 70V for 1 hour and the mobility was monitored by the migration of the dye in the gel. After appropriate migration, the Agarose gel was visualized under UV trans-illuminator in a Bio-Rad gel documentation device and the results were documented. The gel was allowed to solidify after 30 minutes and then the comb was gently removed. The gel slab along with the running tray was submerged carefully into the electrophoresis tank containing 1X TAE buffer (Sambrook & Russell, 2001; Green & Sambrook, 2012).

Sequencing of 16S rRNA

From the amplified products, the amplicons with single band were chosen, and they were purified according to the manufacturer's instructions (QIAquick PCR Purification Kit, cat. No.28106). Big Dye terminator sequencing kit (Applied Bio-Systems) was used for the sequencing process. Ethanol EDTA solution was used to purify and precipitate the unincorporated dye terminators. Next, the pellets were again dissolved in Applied Biosystems Cat. No.4311320 HiDiformamide buffer. This sequencing was done with a 3130xl Genetic Analyzer (Yang *et al.*, 2022).

Antibiotics Susceptibility Testing

Proteus mirabilis was inoculated into sterile nutrient broth in test tubes and left at room temperature for 24 hours. The broth was observed for growth by checking its turbidity and comparing it to the McFarland standard. An applicator stick was used to swab from the standardized inoculum and inoculated on sterile Mueller Hilton agar plates. Gram negative bacteria antibiotics disc was placed on the inoculated plates with the aid of a sterile forceps, and incubated at 37°C for 24 hours. The zones of inhibition were

measured in millimeters with a meter rule and recorded. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020).

RESULTS

Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates

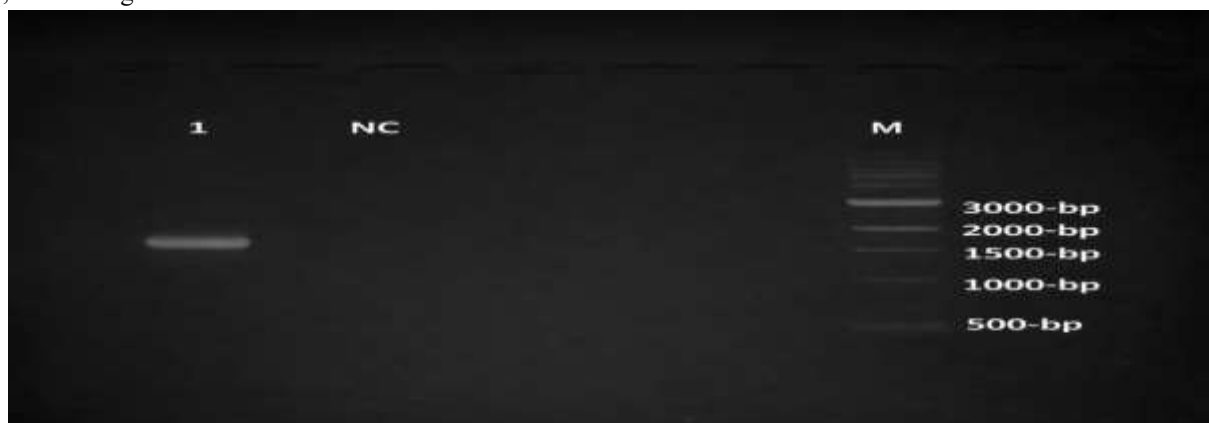
The table below summarizes the colony appearance, Gram's reaction, cell morphology, and biochemical test results of bacterial isolates. The uniform Gram-negative rod morphology, positive urease and motility tests, non-lactose fermentation, and positive methyl red and indole reactions were consistent with the characteristics of *Proteus* spp.

Sample codes	Gram	Shape	Cit	Urea	Gluc	Suc	Lac	H ₂ S	Gas	Mot	MR	Indole	Probable orgs
DMB I	-ve	Rod	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>
DMB II	-ve	Rod	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>
NM I	-ve	Rod	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	<i>Prot. spp.</i>
NM II	-ve	Rod	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>
YHNY I	-ve	Rod	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	<i>Prot. spp.</i>
YHNY II	-ve	Rod	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>
U/GWZ	-ve	Rod	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>
S/GARI	-ve	Rod	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	<i>Prot. spp.</i>

Key: DMB I: Damba I; DMB II: Damba II; NM I: New market I; NM II: New market II; YHNY I: Yarhanya I; YHNY II: Yarhanya II; U/GWZ: Unguwar Gwaza; S/GARI: Sabongari. Cult.Xtics: Cultural characteristics, Gram: Gram staining, Cit: Citrate, Urea: Urease, Gluc: Glucose, Lac: Lactose, H₂S: Hydrogen sulfide, Gas: Gas, Mot: Motility, MR: Methyl red

Figure 1: Amplified Gene Profiles of Bacterial Isolates Obtained through Gel electrophoresis

Image below shows the result of PCR amplification visualized on an agarose gel. Each lane represents a DNA sample, and the bright bands show a successful amplification. The marker on the right was used to estimate DNA size. The negative template control shows no band, confirming that there was no contamination in the reaction.



Agarose Gel Electrophoresis of 16S rRNA Amplicons (1500 bp) of *Proteus mirabilis*

Key: M: 1 kb Molecular ladder

Lane 1: DMB I

Lane NC: Negative template control

Table 2: Antibiotics Susceptibility Profile of *Proteus* species isolated from cucumber sold in Gusau Metropolis

Table 2 presents the antibiotics susceptibility profile of *Proteus mirabilis* isolated from cucumber samples obtained from different locations in Gusau metropolis. The isolates showed high susceptibility to ciprofloxacin, ofloxacin, and sparfloxacin. Amoxicillin, ampicillin and streptomycin demonstrated resistance to *Proteus mirabilis*.

Organisms	Antibiotics	Zone of inhibition(mm)	Interpretation
<i>Proteus mirabilis</i>	CN	24 ± 1.41	Susceptible
	PEF	24 ± 1.36	Susceptible
	OFX	23 ± 1.33	Susceptible
	S	11 ± 0.59	Resistant
	CFX	24 ± 1.8	Susceptible
	SP	24 ± 0.83	Susceptible

AMX	15 ± 1.23	Resistant
AMP	13 ± 0.91	Resistant

Key: OFX-Ofloxacin (10mcg); PEF- Pefloxacin (10mcg); CPX- Ciprofloxacin (10mcg); AMP- Ampicillin (30mcg); CN-Gentamycin (10mcg); S- Streptomycin (30mcg); SP- Sparfloxacin (30mcg); AMX- Amoxicillin (30mcg).

Table 3: Blast Outputs of Total Score, Percentage Identity and Accession Number Obtained from the Isolates' Sequence

Isolates code	Top hit from the NCBI Data base	Accession No.	% Identity	Total hit scores
DMB I	<i>Proteus mirabilis strain IS10 16S rRNA</i>	OP164711.1	98.28	1223

Key: NCBI= National Centre for Biotechnology Institute

DISCUSSION

Concerns posed by the danger of foodborne pathogens and their impact on public health have caused researchers into finding an important way of ensuring the safety of various food products (CDC, 2020). *P. mirabilis* is a bacterium found in soil, water, and sewage, where it plays a role in the decomposition of organic matter (Armbruster *et al.*, 2018). This study confirmed the occurrence of *Proteus mirabilis* in cucumber samples using both phenotypic and genotypic methods. *Proteus mirabilis* in cucumber has been reported to be detected in fresh produce when consumed raw where it acts as a vehicle for opportunistic bacteria (Islam *et al.*, 2023). The contamination may result from irrigation with contaminated water, soil exposure, or unhygienic handling at market places. Although *Proteus mirabilis* is more frequently associated with clinical infections, their presence in food raises concerns for food safety and potential transmission to consumers, particularly those with weakened immunity.

Antibiotics susceptibility patterns of *Proteus mirabilis* in this study showed high sensitivity to fluoroquinolones (pefloxacin, ofloxacin, and sparfloxacin), and aminoglycosides (gentamicin and streptomycin). This finding is consistent with other reports that these antibiotics remain effective against many *Proteus* species (Zhang *et al.*, 2023). However, the bacteria exhibited significant resistance to ampicillin and amoxicillin, which mirrors emerging trends of resistance of *Proteus* species especially *P. mirabilis* to these antibiotics as reported by Srinivasan *et al.* (2024). Resistance to β -lactam antibiotics (amoxicillin and ampicillin) is often mediated by β -lactamase production and other genetic mechanisms that reduce drug efficacy. Recent studies have documented carbapenem-resistant *P. mirabilis* in vegetables containing bla_{NDM} and other carbapenemase genes (Li *et al.*, 2023; Wang *et al.*, 2024). Although carbapenem resistance was not specifically examined in this study, the presence of resistant genes to β -lactam antibiotics indicates potential pathways for emerging AMR threats. However, moderate resistance of this bacterium to some antibiotics suggests selective pressure possibly linked to overuse of antibiotics in agriculture and clinical contexts. The resistance patterns identified in this study are consistent with multidrug resistance (MDR) profiles previously reported among foodborne *Proteus* isolates (Odoki *et al.*, 2019; Olanbiwoninu, 2024), underscoring the public-health implications of consuming contaminated cucumber especially within the study area.

CONCLUSION

Proteus mirabilis was successfully isolated and characterized molecularly using both phenotypic and genotypic methods from cucumber samples obtained across multiple markets in Gusau metropolis, Zamfara state, Northwestern Nigeria. The antibiotics susceptibility profile of the bacterium characterized, revealed that fluoroquinolones were the most active antibiotics against the bacterium while beta-lactam antibiotics were virtually ineffective against the bacteria.

RECOMMENDATION

Molecular detection and antibiotics susceptibility profile of *Proteus mirabilis* from cucumber sold in Gusau metropolis, Zamfara state, as part of food safety, stewardship and surveillance should be encouraged by government and other regulatory agencies.

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