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Molecular characterization and detection of multidrug resistant bacteria from bacteriospermia patients at a hospital in the southeastern part of Nigeria

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Abstract

Male infertility is simply the male's inability to achieve pregnancy in a fertile female following at least 12 months of regular, unprotected sexual intercourse. There are several reasons for male infertility to occur, including both reversible and irreversible conditions. Various microorganisms, mainly bacteria, viruses, and parasites, can infect the male reproductive system and induce a series of inflammatory responses that impair male fertility. This study explores the antibiotic resistance patterns of *Acinetobacter radioresistens*, *Burkholderia diffusa* and *Acinetobacter junii,* focusing on plasmid-mediated resistance and the efficacy of plasmid curing agents. A total of 196 semen samples were collected from patients attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka. The specimens were analyzed using the basic semen parameters for semen analysis and culture. Molecular identification, plasmid profiling and plasmid curing were also carried out for identification of isolated bacteria and detection of plasmid resistant genes. From the results, bacteriospermia was seen in 65.32% of the samples. *Burkholderia diffusa* (39.84%) had the highest frequency of occurrence, followed by *Acinetobacter radioresistens* (32.81%) and lastly, *Acinetobacter junii* (27.34%). Antibiotic susceptibility testing revealed high resistance patterns, particularly in *Acinetobacter radioresistens,* which exhibited resistance to seven antibiotics out of ten tested. Most of the isolates were susceptible to ciprofloxacin and ofloxacin. It was also observed that the Multiple Antibiotics Resistance (MAR) index was greatly reduced in all the isolates, following treatment with 3 different curing agents (sodium dodecyl sulphate, ethidium bromide and acridine orange). Acridine orange was the best curing agent for *Acinetobacter radioresistens* (MAR reduced from 0.70 to 0.10). Sodium dodecyl sulphate and ethidium bromide were best curing agents for *Burkholderia diffusa* and *Acinetobacter radioresistens* respectively. Following the treatments, plasmid was implicated as the cause of the resistance among most of the isolates. The study highlights a high prevalence of bacteriospermia, particularly in sexually active age groups, which may contribute to male infertility. The findings also emphasize the role of plasmids in antibiotic resistance and the potential for plasmid-curing agents to restore antibiotic sensitivity. These results call for enhanced screening for reproductive infections, targeted antibiotic therapy, and further research into alternative antimicrobial strategies.

Keywords: Bacteriospermia; Semen Specimens; Multiple Antibiotic resistance index; Curing agents

1. Introduction

Male infertility is defined as the male's inability to achieve pregnancy in a fertile female following at least 12 months of regular, unprotected sexual intercourse [1]. There are several reasons for male infertility to occur, including both reversible and irreversible conditions. Other factors that could influence each of the partners could be their age, medications, surgical history, exposure to environmental toxins, genetic problems, and systemic diseases. Among the large group of factors that may compromise the reproductive potential of males, bacteriospermia has emerged as a link

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between acquired and idiopathic aspects of male infertility and represents an important yet often-overlooked element that may compromise semen quality in humans as well as animals. Bacteriospermia is defined as the presence of bacteria in the seminal fluid and is clinically acknowledged when bacteria in the ejaculate exceeds 1,000 colony-forming units (CFU)/mL [2]. The condition is often a consequence of acute or chronic bacterial infection of the male urogenital tract accounting for up to 15% of male infertility cases. Bacteriospermia may be caused by both G^+ and G^- bacteria and *Chlamydia* spp. or *Mycoplasma* spp. These bacteria lead to all kinds of diseases, such as chlamydiosis, gonorrhea and ureaplasmosis, which can cause male reproductive system infections [3]. Furthermore, even in healthy individuals, semen may be contaminated by microorganisms during its passage through the genital tract, starting from the testes and expanding all the way to the prepuce and penile foreskin [4].

Given the increasing threat of antibiotic resistance, there has been an increasing interest in the use of plasmid curing as a powerful tool in the study of bacterial genetics and physiology, and its important applications in the development of new therapeutic and biotechnological strategies. Moreover, plasmid curing in bacteria is a useful tool for studying the roles of plasmid-borne genes in bacterial physiology and pathogenesis, and for understanding the mechanisms of plasmid maintenance and replication in bacterial cells [5,6]. Ethidium bromide, acridine orange, and sodium dodecyl sulfate (SDS) are chemicals that can be used to induce plasmid curing in bacteria. These chemicals can interfere with plasmid replication or partitioning, leading to the loss of the plasmid from the bacterial cell [6,7,8].

Although there is an increasing body of evidence supporting the use of plasmid curing agents in drug discovery, limited research has been conducted primarily due to challenges in determining a non-lethal dosage for humans. By eliminating plasmids, researchers can better understand the roles these genetic elements play in antibiotic resistance, virulence, and bacterial behavior, potentially leading to improved treatment strategies for reproductive and sexually transmitted infections.

2. Materials and methods

2.1. Study area

The research was conducted at the General Microbiology Laboratory at Nnamdi Azikiwe University, Awka. The samples were collected from Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka. Molecular analysis was carried out at the Molecular Research Foundation for Students and Scientists in Nnamdi Azikiwe University, Awka.

2.2. Inclusion and exclusion criteria

Eligible participants were males between the ages of 18 and 40. Individual who came for semen analysis and culture due to unusual discharge, itching and for fertility screening were included. Study subjects who engaged in sexual intercourse, took antibiotics, and consumed kolanuts, alcohol and bitter kola within the space of three to five days were excluded. Written informed consent was obtained from each participant.

2.3. Specimen collection

A total of 196 semen specimens were collected directly at the clinic into sterile containers after 3–5 days of sexual abstinence. To prevent contamination, the study subjects were asked to wash and disinfect hands, as well as the external genitalia.

2.4. Semen analysis

Semen analysis was carried out, according to 2010 WHO guidelines, evaluating spermatozoa morphology, motility, and concentration [9].

2.5. Isolation and identification of bacteria

Semen samples were cultured on Nutrient agar, Blood agar and MacConkey agar and incubated at 37^{o}C for 24 hours. Isolates were identified using standard methods such as Gram staining, citrate utilization, sugar fermentation test, coagulase test, urease test, motility test [10] and molecular characterization [11].

2.6. Antibiotics Sensitivity Test of the Isolates

Antimicrobial susceptibility test was performed for all the isolates using disk diffusion method (Kirby-Bauer, 1966) on Mueller Hinton (Oxoid Basinstoke, UK) according to the direction of the Clinical and Laboratory Standards Institute [12]. Five colonies of the test organism were emulsified in 5ml of Nutrient broth and mixed gently. The mixture was

incubated at 37°C and the turbidity of the inoculums were adjusted to 0.5 Mc Farland standards. With sterile swab stick, the mixture was uniformly spread onto Mueller-Hinton agar. The antibacterial impregnated disks were placed using sterile forceps on the agar surface and the zones of inhibition were determined. The zones of inhibition were measured at 24 hours to the nearest millimeter using a transparent ruler. The results obtained were interpreted as sensitive or resistant according to the direction of Clinical Laboratory Standards Institute [12], (resistance 0-16mm and sensitive >16mm). The antibacterial agents in the disks and their concentrations are as follows: Erythromycin (E, 5µg), Ciprofloxacin (CPX, 10µg), Levofloxacin (LEV, 20µg), Gentamicin (GEN 10µg), Ampicillin (AMP 10µg), Amoxicillin (AMX, 20µg), Augmentin (AUG, 30µg) and Streptomycin (S, 30µg). The inoculated plates were inverted and incubated for 24 hours at 37°C. After incubation, a metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used [13].

2.7. Identification of Multi-Drug Resistant (MDR) Isolates

The multi-drug resistant (MDR) character of the isolates was determined by observing the resistance pattern of the isolates to at least five or six of the antibiotics that were used in this study, taken from at least three of the following classes of antibiotics: Aminoglycosides, Beta-lactam, quinolones [14,10].

2.8. Determination of Multiple Antibiotic Resistant (Mar) Index

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula **MAR = a/b**, where **a** represents the number of antibiotics to which the test isolate depicted resistance and **b** represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility [15].

2.9. Plasmid extraction

Plasmid was extracted using ZymoPURE™ Plasmid Miniprep Kit (Zymo Research) [11].

2.10. Plasmid curing

The methods of Uba et al*.* [16], Zaman et al*.* [8] and Olawale et al*.* [17] as modified in this study were adopted in the plasmid curing analysis**.** Acridine orange (AO) (0.0075 % w/v), ethidium bromide (EB) (0.00125 % w/v) as well as sodium dodecyl sulphate (SDS) (0.100 %) solutions were prepared by adding 0.0075g, 0.00125g and 0.100g of the acridine orange powder into 1 mL of sterile distilled water. These solutions are equivalent to 750 µg/mL, 1250 µg/mL and 100 mg/mL of acridine orange, ethidium bromide and sodium dodecyl sulphate. One millilitre of the acridine orange, ethidium bromide and sodium dodecyl sulphate solutions were then dispensed aseptically to each test tubes containing 9 mL of sterile nutrient broth. The concentration of acridine orange, ethidium bromide and sodium dodecyl sulphate in each 10 ml broth-acridine, ethidium bromide and sodium dodecyl sulphate solutions are 75µg/mL, 125 µg/mL, and 10 mg/mL. The bacterial strains labelled 1, 2 and 3 were inoculated into the acridine orange, ethidium bromide and sodium dodecyl sulphate broths and rotated on a shaker at 120 rpm. After 24-48 h of incubation at 37°C, the organisms were freed from the chemicals (acridine orange, ethidium bromide and sodium dodecyl sulphate) by subculturing on sterile nutrient agar slants and incubated at 37°C. Antibiotic susceptibility test was done again for the bacterial strains that showed resistance to antibiotics prior to the curing and the changes in resistance pattern was noted. The bacteria that displayed clear changes in resistance pattern after curing were regarded as bearing their resistance factor in the plasmid.

3. Results and discussion

3.1. Morphological characteristic, microscopic and biochemical test results of isolates

There are many causes of male infertility, of which 8-35% are related to genital tract infections [18]. Based on this research, three major bacterial species were isolated, and they are *Acinetobacter radioresistens, Burkholderia diffusa* and *Acinetobacter junii* (Table 1). The role of these bacteria as the important causes of male infertility is still a controversial issue. Most women and men who are infected with these bacteria are not aware of their infection because they are asymptomatic. Therefore, detection of these agents is important.

The microscopic analysis showed that the bacterial isolates are Gram negative. They consist of rods. The results of the microscopic and biochemical tests are displayed in the table 2.

Table 1 Morphological Characteristics of Bacteria Isolates

S/N	Form	Colour	Elevation	Margin	Texture	Transparency	Bacterial Isolate
1	Circular	Pink	Convex	Entire	Smooth	Opaque	*Acinetobacter radioresistens*
2	Circular	Pale-yellow	Convex	Entire	Smooth	Opaque	*Burkholderia diffusa*
3	Circular	Creamy	Convex	Entire	Smooth	Opaque	*Acinetobacter junii*

Table 2 Microscopic and biochemical test of the isolates

S/N	Gram stain	Rod/ Cocci	Catalase test	Citrate test	Hemolysis	Coagulase test	Indole test	Urease test	Motility	Sugar Fermentation				Bacterial Isolate
										Glucose	Fructose	Sucrose	Dextrose	
1	-	Rod	+	+	Gamma	-	-	-	-	-	-	-	-	*Acinetobacter radioresistens*
2	-	Rod	-	+	Beta	+	-	+	+	-	-	-	-	*Burkholderia diffusa*
3	-	Rod	+	+	Gamma	_	-	-	-	-	-	-	-	*Acinetobacter junii*

Key:- = Negative Reaction; + = Positive Reaction

3.2. Distribution of bacterial isolates from semen samples

A total of 196 semen samples were collected from male patients attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra state. The frequency (%) distributions are shown in figure1 below. The figure identifies and quantifies the bacterial isolates found in semen samples. The distribution shows that *Burkholderia diffusa* (38.84%) was the most prevalent, followed by *Acinetobacter radioresistens* (32.81%) and *Acinetobacter junii* (27.34%).

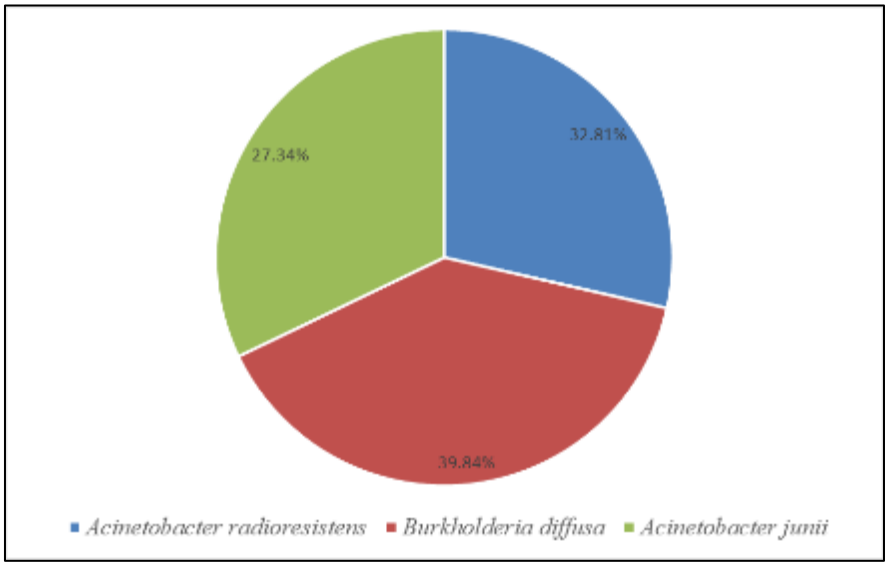

Figure 1 Frequency (%) distribution of bacterial isolates from semen

3.3. Plasmid profiling

The Plasmid Profiling before and after curing were presented in figures 2 and 3, respectively. Figure 2 shows the gel electrophoresis image containing four lanes. The M (marker/ladder) contains the DNA ladder, which serves as a molecular weight reference. The distinct bands allow estimation of the plasmid sizes in the other lanes labelled 1, 2 and 3 (sample lanes). These sample lanes contain plasmid DNA samples showing different migration patterns. The multiple bands visible in some lanes suggest different plasmid conformation (multiple plasmids). Lanes 1, 2 and 3 show multiple bands, likely representing different plasmid conformations; supercoiled, nicked, and linearized forms. Lane 2 shows more distinct bands which could indicate multiple plasmids. Lanes 1 and 2 have visible plasmid bands but lower intensity, possibly due to lower DNA yield or degradation. Lane 3 appears to have a higher DNA concentration due to brighter band intensity.

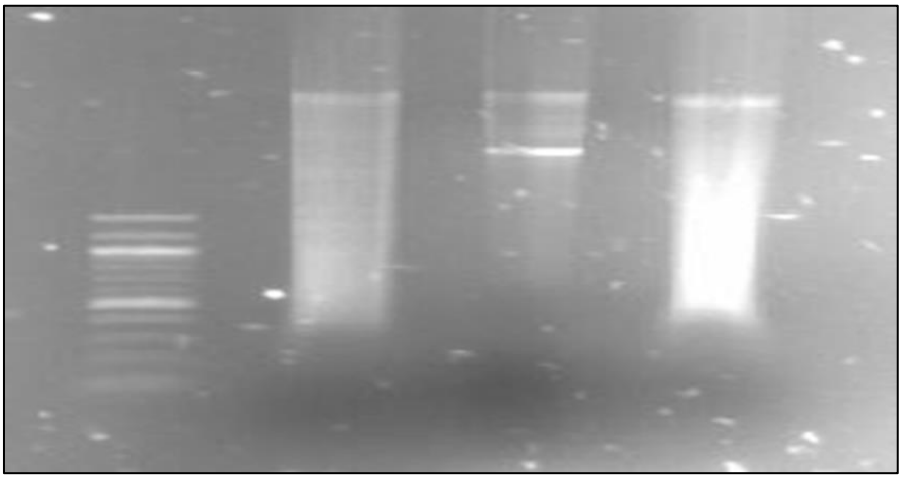

Figure 2 Plasmid Profiling Before Curing

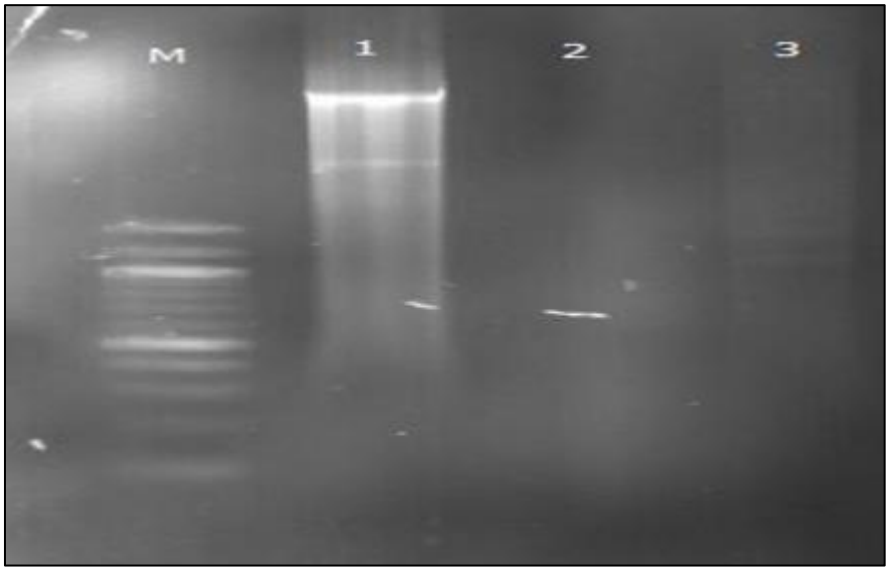

Figure 3 Plasmid Profiling After Curing using Sodium dodecyl sulphate

Before curing, lanes 1,2 and 3 had strong plasmid bands, indicating the presence of plasmid DNA. Lane 2 showed multiple bands, suggesting different plasmid conformations. After curing, lane 1 showed a visible band possibly genomic DNA or residual plasmid. Lanes 2 and 3 have significantly reduced plasmid bands, indicating successful plasmid curing in these samples. Only faint bands are visible in lanes 2 and 3, which might be degraded plasmid or chromosomal DNA. Observations of plasmid presence in *Acinetobacter radioresistens*, Burholderia diffusa and *Acinetobacter junii* align with the findings of El-shazly et al. [19] and Hayatsu et al. [20] suggesting that plasmids play a crucial role in the phenotypic traits of these bacteria.

3.4. Multiple Resistance of Bacterial Isolates to Different Antibiotics

Table 3 Antibiotic sensitivity patterns (mm) against the bacterial pathogens before and after curing

Organism	Diameter of Zone of Inhibition (mm)										
	OFX	**AU**	**PEF**	**CTZ**	**CN**	**CPX**	**CEP**	**TRX**	**S**	**CEF**	**MAR index**
Acinetobacter radioresistens	0.00	0.00	0.00	0.00	1.60±0.07	2.00±0.10	0.00	0.00	2.40±0.15	0.00	0.70
Cured AR strain SDS	2.50±0.17	0.00	2.00±0.12	0.00	1.80±0.07	2.30±0.10	0.00	0.00	1.70±0.05	1.20±0.02	0.40
Cured AR strain EB	1.70±0.05	1.40±0.01	0.00	0.00	1.30±0.02	1.30±0.01	1.20±0.04	0.00	1.40±0.09	1.30±0.05	0.30
Cured AR strain AO	1.70±0.06	0.00	1.90±0.05	1.80±0.07	2.50±0.10	2.00±0.10	1.90±0.05	1.50±0.04	1.90±0.08	2.50±0.08	0.10
Burkholderia diffusa	1.70±0.05	0.00	1.50±0.04	0.00	1.50±0.02	1.60±0.03	0.00	0.00	0.00	0.00	0.60
Cured BD strain SDS	2.20±0.16	0.00	2.20±0.10	1.20±0.03	1.40±0.10	2.20±0.13	2.30±0.16	2.40±0.10	2.30±0.10	1.30±0.10	0.10
Cured BD strain EB	1.30±0.06	0.00	1.50±0.08	0.00	2.00±0.05	1.30±0.09	1.40±0.07	1.10±0.07	1.20±0.08	1.30±0.10	0.20
Cured BD strain AO	1.30±0.10	0.00	1.40±0.11	1.10±0.02	2.00±0.10	1.50±0.11	1.10±0.04	0.00	2.40±0.20	2.00±0.08	0.20
Acinetobacter junii	1.30±0.05	0.00	0.00	0.00	2.50±0.13	2.20±0.15	0.00	0.00	0.00	2.00±0.11	0.60
Cured AJ strain SDS	2.20±0.10	0.00	1.80±0.10	1.20±0.03	1.30±0.04	1.70±0.11	1.30±0.04	1.20±0.03	1.40±0.05	1.10±0.04	0.10
Cured AJ strain EB	1.30±0.06	1.40±0.05	1.50±0.06	2.30±0.10	2.00±0.10	1.20±0.05	1.20±0.01	0.00	1.20±0.03	1.30±0.04	0.10
Cured AJ strain AO	1.40±0.03	0.00	1.90±0.04	2.00±0.10	1.70±0.03	1.80±0.02	1.30±0.05	0.00	2.40±0.10	1.20±0.06	0.20

Key: MAR = Multiple antibiotic resistance; SDS = Sodium dodecyl sulphate; EB = Ethidium bromide; AO = Acridine orange; mm = millimeter; 0.00 = No zone of inhibition; OFX = Oflaxacin; AU = Augmentin; PEF = Peflacine; CTZ = Ceftazidime; CN = Gentamycin; CPX = Ciprofloxacin; CEP = Ceporex; TRX = Ceftriaxone; S = Streptomycin; CEF = Cefuroxime

Table 3 shows the multiple resistance of bacterial isolates to different antibiotics. The antibiotics susceptibility results, reveal that *Acinetobacter radioresistens*, *Burkholderia diffusa* and *Acinetobacter junii* exhibit significant multidrug resistance (MDR), with resistance to six or more antibiotics. However, most isolates remained susceptible to ciprofloxacin and ofloxacin, likely due to fluoroquinolones mechanism of action, which targets bacterial DNA gyrase and topoisomerase IV, and their ability to penetrate bacterial cells efficiently due to their lipid solubility and high pka values [20,21]. *Acinetobacter radioresistens* showed resistance to seven of ten antibiotics used in the sensitivity testing, *Burkholderia diffusa* and *Acinetobacter junii* were resistant to six antibiotics each. Table 3 also shows the antibiotic sensitivity patterns (mm) against the bacterial pathogens before and after curing. Before curing, *Acinetobacter radioresistens* showed the highest resistance (0.00) to Oflaxacin (OFX), Augmentine (AUG), Peflacine(PEF), Ceftazidime(CTZ), Ceftriaxone(TRX) and Cefuroxime(CEF) indicating strong β-lactam and fluoroquinolone resistance. The lowest resistance was seen in Streptomycin (2.40 ± 0.15) as opined by Wang *et al.* [23] suggesting partial susceptibility to aminoglycosides. *Burkholderia diffusa* exhibited complete resistance (0.00) to Ceporex (CEP), Ceftriaxone (TRX), Streptomycin(S), Augumentin (AUG), Ceftazidine (CTZ) and Cefuroxime (CEF) while the lowest resistance (1.70±0.05) was seen in Ofloxacin (OFX) reinforcing the general trend of fluoroquinolone susceptibility. *Acinetobacter junii* also showed the highest resistance (0.00) to Augmentin (AUG), Peflacine (PEF), Ceftaxidime (CTZ), Ceporex (CEP), Ceftriaxone (TRX) and Streptomycin (S) while the lowest resistance (2.50 ± 0.13) was to Gentamycin (CN) suggesting some degree of aminoglycoside effectiveness. After treatment using curing agents (SDS, ethidium bromide and acridine orange), noticeable improvements in antibiotic susceptibility were observed including the MAR index reduction where *Acinetobacter radioresistens* reduced from 0.70 to 0.10 showing a major loss of resistance and the zones of inhibition increased for peflacine (2.00mm), ceftazidime (1.80mm) and gentamycin (2.50mm). *Burkholderia diffusa* decreased from 0.60 to 0.10, confirming the involvement of plasmids in resistance and susceptibility increased for ceftazidime (1.20mm), ceftriaxone (2.40mm) and peflacine (2.20mm). *Acinetobacter junii* curing resulted in increased sensitivity to ceftazidime (2.00mm), ceftriaxone (2.40mm), and gentamycin (2.40mm). Also, MAR index dropped from 0.60 to 0.20 after curing.

3.5. Antibiotics Plasmid Curing Profile of Bacterial Pathogens Before and After Curing

Table 4 shows Antibiotics plasmid curing profile of bacterial pathogens before and after curing. After curing using Sodium dodecyl sulphate (SDS); *Acinetobacter radioresistens* which was resistant to seven out of the ten antibiotics Oflaxacin (OFX), Augmentine (AUG), Peflacine(PEF), Ceftazidime (CTZ), Ceporex (CEP), Ceftriaxone (TRX) and Cefuroxime (CEF) became sensitive to three more antibiotics Oflaxacin(OFX), Ceftazidime(CTZ) , Ceporex(CEP), Ceftriaxone(TRX) and still resistant to four antibiotics Oflaxacin(OFX), Ceftriaxone(TRX), Ceporex(CEP) and Cefuroxime(CEF). This showed that resistance is plasmid mediated and that Sodium dodecyl sulphate (SDS) might not be the best curing agent for *Acinetobacter radioresistens*. *Burkholderia diffusa* was resistant to six antibiotics Ceporex (CEP), Ceftriaxone (TRX), Streptomycin (S), Augumentin (AUG), Ceftazidine (CTZ) and Cefuroxime (CEF) pre-curing and became sensitive to five (Ceporex (CEP), Ceftriaxone (TRX), Streptomycin (S), Ceftazidine (CTZ) and Cefuroxime (CEF)) out of the six antibiotics it was previously resistant to and only resistant to Augumentin (AUG). This shows that SDS could be the best curing agent for *Burkholderia diffusa*. *Acinetobacter junii* which was resistant to Augmentin (AUG), Peflacine (PEF), Ceftaxidime (CTZ), Ceporex (CEP), Ceftriaxone (TRX) and Streptomycin (S) became sensitive to all the antibiotics tested against it and maintaining resistance to just Augmentin (AUG) showing that SDS is a good curing agent for *Acinetobacter junii* and that resistance is plasmid mediated. After curing using Ethidium bromide (EB), *Acinetobacter radioresistens* remained resistant to three of the seven antibiotics it was previously resistant to, showing that EB is not a good curing agent for this pathogen. *Burkholderia diffusa* remained resistant to two out of 6 antibiotics it was previously resistant to showing that for this species, EB is a fair curing agent. *Acinetobacter junii* remained resistant to one of the six antibiotics it was initially resistant to showing that for this species, EB is a good plasmid curing agent. After curing using Acridine orange (AO), *Acinetobacter radioresistens* remained resistant to Augmentin and sensitive to other antibiotics indicating that AO is a good curing agent for this species and that resistance is plasmid mediated. *Burkholderia diffusa* remained resistant to ceftriaxone and sensitive to all the other antibiotics also showing that AO is a good curing agent. *Acinetobacter junii* remained resistant to two out of the six antibiotics it was previously resistant to, showing that for this species, AO is a fair curing agent.

Plasmid-mediated resistance was confirmed as certain resistances were lost post curing. Chromosomal resistance is likely present for antibiotics that remained unaffected after curing. Different curing agents have varying efficiency, with some removing more resistance markers than others. Comparing the three curing agents, it can be said that Acridine orange is the best curing agent, followed by Ethidium bromide and lastly, Sodium dodecyl sulphate. Table 4 also shows that Ethidium bromide was the only curing agent capable of removing resistance to Augmentin.

While specific studies on *Acinetobacter radioresistens*, *Burkholderia diffusa* and *Acinetobacter junii* are limited, the documented presence of plasmid-mediated resistance in related *Acinetobacter* species suggests a similar mechanism

may be at play in these bacteria. The loss of certain antibiotic resistances following plasmid curing in these isolates support the hypothesis that plasmids are instrumental in mediating these resistances.

Table 4 Antibiotics plasmid curing profile of bacterial pathogens before and after curing

Bacterial strain	Antibiotics gene marker before curing	Cured Bacterial strains	Antibiotics gene marker after curing
Acinetobacter radioresistens	Oflaxacin, Augmentin, Peflacine, Ceftazidime, Ceporex, Ceftriaxone, Cefuroxime	Cured AR strain SDS	Augmentin, Ceftazidime, Ceporex, Ceftriaxone
		Cured AR strain EB	Peflacine, Ceftazidime, Ceftriaxone
		Cured AR strain AO	Augmentin
Burkholderia diffusa	Augmentin, Ceftazidime, Ceporex, Ceftriaxone, Streptomycin, Cefuroxime	Cured BD strain SDS	Augmentin
		Cured BD strain EB	Augmentin, Ceftazidime
		Cured BD strain AO	Augmentin, Ceftriaxone
Acinetobacter junii	Augmentin, Peflacine, Ceftazidime, Ceporex, Ceftriaxone, Streptomycin	Cured AJ strain SDS	Augmentin
		Cured AJ strain EB	Ceftriaxone
		Cured AJ strain AO	Augmentin, Ceftriaxone

Key: SDS = Sodium dodecyl sulphate; EB = Ethidium bromide; AO = Acridine orange;AR = Acinetobacter radioresistens; BD = Burkholderia diffusa; AJ = Acinetobacter junii.

Plasmid has been documented to have encoded gene that provides resistance to naturally occurring antibiotics in competitive environmental niche [24,25]. Findings from this study indicated that resistance shown by the bacterial isolates is mainly plasmid mediated. Although, most of the bacterial isolates carried plasmid bands but not all are responsible for the antibiotic resistance. This is supported by Thomas et al. [26] who reported that plasmid mediated mechanism may increase the likelihood of horizontal spread. Resistance of bacterial organism not due to plasmid or chromosome might be due to efflux pump mechanism [27] or other factors like mutation of genes encoding ribosomal protein which decrease permeability of the cell envelope in enteric bacteria. These findings underscore the importance of monitoring plasmid-mediated resistance to inform effective treatment strategies and curb the spread of multidrug-resistant bacteria.

4. Conclusion

This study has the potential to significantly impact both the clinical management of bacteriospermia and the broader field of antimicrobial resistance. By focusing on the molecular characterization and plasmid curing of multidrug resistant bacteria and evaluating their impact on treatment strategies, we can contribute to improving patient care and shaping public health policies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical clearance was gotten from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra state, Nigeria.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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