Detection of beta-lactamase production among Gram-negative bacteria isolated from semen of male patients with bacteriospermia

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ABSTRACT

Background: Bacteriospermia, a condition characterized by the presence of bacteria in male ejaculate may influence male infertility. However, presence of bacteria exhibiting resistance to extended-spectrum antibiotics could be a serious challenge to the treatment of bacteriospermia.

Objectives: This study examined semen of patients for bacteriospermia and screened for the presence of common beta-lactamases in Gram-negative bacteria isolated, in two teaching hospitals in Oyo State, Nigeria.

Methods: Semen samples were collected by masturbation after abstinence for 3 days among 182 male patients attending fertility clinic of Ladoke Akintola University of Technology Teaching Hospital and Bowen University Teaching Hospital between February 2015 and February 2016. Gram-negative bacteria were isolated and identified using standard methods. Antibiotic susceptibility test was by disc-diffusion, beta-lactamase detection by double-disc synergy test and Polymerase Chain Reaction.

Results: Bacteriospermia was observed in 26.4% of the patients (age range 21 and 60 years). *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Entetrobacter* spp. and *Proteus* spp. were isolated. Resistance to amoxicillin, amoxicillin-clavulanate, cefepime, ceftriaxone, cefotaxime, ceftazidime and cefuroxime was observed in 87.5%, 62.5%, 45.8%, 41.7%, 37.5%, 37.5% and 27.1% of the isolates respectively. Resistance to imipenem, ciprofloxacin, ofloxacin and gentamicin was 8.3%, 41.7%, 43.8% and 54.2% respectively. MDR was exhibited by 68.8%. ESBL, AmpC and MBL was produced by 31.3%, 22.9% and 14.6% respectively, with 3(6.3%) co-producing all. bla_{TEM} and $bla_{CTX\text{-}M}$ were detected but AmpC and MBL genes were not. Sequencing revealed $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$. Six of the eight $bla_{CTX\text{-}M\text{-}15}$ positive strains lost the gene to mutagenic treatment.

Conclusion: High level of MDR bacteria including those producing beta-lactamases in this study calls for caution in the antibiotic treatment of bacteriospermia.

Keywords: Bacteriospermia, Antibiotic susceptibility, Beta-lactamase, semen

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Détection de la production de bêta-lactamase chez les bactéries gram-négatives isolées du sperme de patients mâles atteints de bactériospermie

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RESUME

Contexte: La bactériospermie, une affection caractérisée par la présence de bactéries dans l'éjaculation des hommes, peut influencer la stérilité des hommes. Cependant, la présence de bactéries présentant une résistance aux antibiotiques à spectre étendu pourrait constituer un défi sérieux pour le traitement de la bactériospermie.

Objectifs: Cette étude a examiné le sperme des patients pour la bactériospermie et a examiné la présence de bêta-lactamase communes dans des bactéries gram-négatives isolées, dans deux centres hospitaliers universitaires de l'Etat d'Oyo, au Nigéria.

Méthodes: Les échantillons de sperme ont été recueillis par masturbation après une abstinence de 3 jours chez 182 patients qui suivaient un traitement dans une clinique de fertilité des centres hospitaliers universitaires de l'Université de Technologie Ladoke Akintola et de l'Université Bowen entre février 2015 et février 2016. Les bactéries gram-négatives ont été isolées et identifiées selon des méthodes standardisées. Le test de susceptibilité aux antibiotiques a été effectué par diffusion de disque, détection de bêta-lactamase par test de synergie à double disque et réaction en chaîne par polymérase.

Résultats: La bactériospermie a été observée chez 26,4% des patients (tranche d'âge de 21 à 60 ans). L'*Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Entetrobacter* spp. et *Proteus* spp. étaient isolés. La résistance à l'amoxicilline, à l'amoxicilline-clavulanate, au céfépime, à la ceftriaxone, au céfotaxime, au ceftazidime et à la cefuroxime a été observée respectivement chez 87,5%, 62,5%, 45,8%, 41,7%, 37,5%, 37,5% et 27,1% des isolats. La résistance à l'imipenème, à la ciprofloxacine, à l'ofloxacine et à la gentamicine était respectivement de 8,3%, 41,7%, 43,8% et 54,2%. 68,8% ont fait preuve de multiple résistance. ESBL (Bêta-lactamase à Spectre étendu), AmpC (Céphalosporines de classe moléculaire C) et MBL (lymphocytose à cellules B monoclonales) ont été produits respectivement de 31,3%, 22,9% et 14,6%, avec 3 (6,3%) coproducteurs de tout. bla_{TEM} and $bla_{CTX\text{-}M}$ ont été détectés mais les gènes AmpC et MBL ne l'étaient pas. Le séquençage a révélé $bla_{CTX\text{-}M\text{-}15}$ et $bla_{TEM\text{-}1}$. Six des huit souches positives $bla_{CTX\text{-}M\text{-}15}$ ont perdu le gène en traitement mutagène

Conclusion: Le niveau élevé de bactéries MDR, y compris celles produisant les bêta-lactamase dans cette étude exige de la précaution dans le traitement antibiotique de la bactériospermie.

Mots-clés: bactériospermie, susceptibilité au Antibiotiques, bêta-lectamase, sperme

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INTRODUCTION

Healthy couples who are not on contraceptive may achieve pregnancy usually within few months of continuous unprotected sexual intercourse. While some may achieve pregnancy within one month of regular sexual intercourse, some may take three to twelve months before pregnancy can be established.[1] Normally, if no contraceptive measures are adapted, conception often times is achieved in 80 – 85% of healthy couples within twelve months of regular unprotected sexual intercourse.[1,2,3] However, in some cases, couples who are not under the influence of contraceptive could not still establish a successful pregnancy that will lead to a live-birth even after twelve months of continuous unprotected sexual intercourse. This condition is what is termed infertility[2,4] and is a problem affecting about 15% of couples worldwide.[5]

Clinicians referred to a medical condition in which the couples for the first time in their life time are unable to get pregnant after regular unprotected sexual intercourse as primary infertility, while a condition in which the couples are unable to get pregnant after an earlier pregnancy that lead to a live-birth is referred to as secondary infertility.[4,6]

Infertility could be due to the male partner where it is principally due to poor semen parameters or due to the female partner where factors such as occlusions of fallopian tubes, uterine or endometrial abnormalities, abnormal cervix and anovulation are indicated.[7] Other factors responsible for infertility are congenital and hormonal disorders, lifestyle, environmental hazards, psychological state and infection of the organ of reproduction. All these can lead to impairment in the function of genital organs, abnormal production of reproductive cells and semen quality, difficulty in sperm cell transport to the oocyte, impairment in fertilization and embryo implantation steps.[8,9,10,11]

Infertility is often misconstrued to be as a result of the female factor only while the male factor is completely neglected or undermined.[12] However, recent findings have shown that factors affecting male partners account for more than 40% of the causes of infertility among couples.[12] Male factors affecting fertility are usually detected through semen quality analysis which is used as a surrogate measure of male fecundity.[12,13] A normal seminal fluid should contain fluids from the vas deferens, seminal vesicles, prostate gland and mucus glands.[14,15] Infertility may result from aberration in the normal sperm morphology (teratozoospermia), reduced sperm motility (asthenozoospermia), lack of sperm (azoospermia) or too little sperm (oligozoospermia).[16,17] Sperm cells on the other hand is the essential part contained in semen and are the male reproductive cells of which the genetic material must combine with the genetic material from an egg of female partner, in a process called fertilization to have a child. A man's fertility and sexual characteristics depend on the normal functioning of the male reproductive system.[18]

Among many factors such as environmental toxins, drugs (like cimetidine, nitrofurantoin, cannabis), smoking and alcohol use that can decrease sperm count and reduces fertility in males,[19] infections of the urogenital tract are directly or indirectly a major cause of infertility in some couples. The agents of infection may impair the reproductive organs both in men and women, and may cause the agglutination of motile sperm, impairment of acrosome reaction and alteration in cell morphology.[20,21,22] A condition involving the presence of bacteria in male ejaculate or semen which could be symptomatic or asymptomatic is called bacteriospermia.[18] Apart from certain microorganisms such as *Neisseria gonorrhoeae, C. trachomatis, Ureaplasma urealyticum* that are usually associated with urinary tract and male accessory gland *infections*, other common microorganisms that have been implicated are *Chlamydia trachomatis*, *Staphylococcus* spp., *Pseudomonas* spp. and bacteria of the Enterobacteriaceae family.[19] These microorganisms have been reported to influence semen parameters including the sperm cell characteristics.[19] It is imperative to note that infection of the genitourinary tract of men may contribute to infertility by adversely affecting sperm function, morphology and their motility, altering the chemical composition of seminal fluid and or cause anatomical obstruction of some parts of the male reproductive organs.[23]

This study therefore investigated the occurrence of bacteriospermia and beta-lactamase producing Gram-negative bacteria in seminal fluids of male patients visiting fertility clinics in two tertiary hospitals in Ogbomoso, Oyo state.

MATERIALS AND METHODS

Study area

Ogbomoso is a town in Oyo state, south-western Nigeria founded in the mid 17th century and with a population of 299.535 as at the 2006 census.[24] The town is divided into two local government councils namely Ogbomoso north and south. Most inhabitants were from the Yoruba ethnic group but other ethnic groups are also present in their thousands. The population is mostly

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Christians and Muslims, and some are traditional worshipers. Their main occupation is farming, teaching, artisan and trading. Ogbomosho has two tertiary hospitals namely Ladoke Akintola University of Technology Teaching Hospital (LTH) and Bowen University Teaching Hospital (BUTH).[25]

Study population and sample size

Male partner of couples with cases of infertility visiting the out-patients unit of the infertility clinics of the LTH and BUTH were recruited for the study between February, 2015 and February, 2016. The sample size was calculated using the online Raosoft, Inc sample size calculator[26] at 5% error margin and 95% confidence level, a total of 182 samples including 105 samples from LTH (based on monthly average sample of 12) and 77 samples from BUTH (based on monthly average sample of 8) were collected.

Study design

Ethical clearance for the study was obtained from Ladoke Akintola University of Technology Teaching Hospital (LTH) Ethical committee. Participation in the study was voluntary with inform consent obtained from each participant and all information obtained kept confidential.

Collection and handling of samples

Semen samples were collected using the method previously described by Oyeyipo *et al*.[27] Only those patients who gave their consent, not on antibiotic two weeks prior to the study and not on any form of illness were included in the study.

The participants were instructed on how to collect the sample as described by Oyeyipo *et al*.[27] Briefly, aseptic collection of samples was achieved by washing their hands and genital parts before expressing the semen into sterile wide-mouth plastic containers through masturbation after 3 days of abstinence from sexual intercourse. Upon collection, samples were transferred within one hour of collection to the laboratory for analysis and further processing.[28]

Isolation and Identification of bacteria from semen samples

The seminal fluids were cultured on *cetrimide agar, eosin methylene blue agar and MacConkey agar media* and were incubated aerobically in an inverted position at 37°C for 24 hours. The culture plates were examined for growth of colonies of bacterial isolates present in the samples. Pure bacteria colonies were isolated and identified by Gram's reactions and standard biochemical tests such as production of oxidase, catalase, indole and urease, utilization of citrate and fermentation of sugars and methyl red test.[29]

Antimicrobial susceptibility testing of isolates

Antibiotic susceptibility test was carried out using the disc-diffusion method as recommended by Clinical and Laboratory Standards Institute, CLSI.[30] Standard bacterial inoculums were prepared from 24 hours bacteria cultures by emulsifying three colonies of each isolate in 5 mL of sterile normal saline and the suspension adjusted to 0.5 McFarland. The standardized bacteria suspensions were inoculated on freshly prepared Mueller-Hinton agar plate using sterile swab sticks. With the aid of sterile forceps, the following antibiotics (Oxoid Ltd, England) were placed on the surface of the inoculated agar plates- amoxicillin (30µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (10 µg) and ofloxacin (10 µg)**.** The plates were allowed to stand for one hour on the working bench for pre-diffusion of the antibiotic before incubation at 37°C for 24 hours. Zones of growth inhibition was recorded and interpreted as resistant, intermediate and sensitive using the breakpoints for each antibiotic as stated in the Clinical and Laboratory Standards Institute guideline.[30] Isolates resistant to more than three classes among the five classes of antibiotics used in this study were designated as multidrug resistance strains as described by Magiorakos *et al*.[31]

Phenotypic detection of selected beta-lactamase production

Presumptive detection of the production of beta-lactams hydrolyzing enzymes among the Gram negative bacterial isolates was carried out as previously described by CLSI,[30] Tan *et al*.[32] and Franklin et al.[33] to detect the production of extended-spectrum beta-lactamase (ESBL), AmpC beta-lactamase and Metallo beta-lactamase (MBL) enzymes respectively. ESBL and AmpC production was determined among the isolates that showed resistance to the cephalosporins using the double-disc synergy test (DDST) and cefoxitin/cloxacillin double-disc methods respectively,[30,32] while MBL production was determined among the isolates resistance to carbapenems.[33,34] Briefly, bacterial suspension equivalent to 0.5 McFarland standard was prepared and spread over the surface of Mueller Hinton agar plates using sterile

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cotton swab. For ESBL detection, separate discs of ceftazidime (30 µg) and cefotaxime (30 µg) were placed 20 mm centre-to-centre around amoxicillin-clavulanic acid (20/10 µg) disc on the agar plates with the aid of sterile forceps. For AmpC detection, two cefoxitin discs one inoculated with 200 µg of cloxacillin and the other without, were placed on the inoculated agar surface, while for the MBL detection, two imipenem discs, one inoculated with 10 µL of 750 µg of EDTA solution and the other without, were placed on the inoculated agar surface using sterile forceps. All the plates were left for an hour on the bench for pre-incubation diffusion before incubation at 37°C for 24 hours. ESBL production was inferred from those having the zone of inhibition around any of the two discs (cefotaxime and ceftazidime) expanded towards the clavulanic acid disc. AmpC production was inferred from those having the zone of inhibition around the cloxacillin inoculated cefoxitin discs expanded ≥4 mm by the presence of the cloxacillin,[32] while MBL production was inferred from those having the zone of inhibition around the EDTA inoculated imipenem discs expanded >4 mm by the presence of the EDTA.

PCR detection of common beta-lactamases

The DNA of bacterial isolates positive for ESBL, AmpC and MBL were extracted using the boiling method.[35] PCR technique was used to screen for the presence of TEM, SHV and CTX-M genes using specific primers as described by Maynard et al.[36] and Mendonca et al.[35] bla_{OXA}, $bla_{DHA\text{-}1}$ and bla_{CMY} were amplified as described by Caroline et al.[37] and $bla_{NDM\text{-}1}$, bla_{IMP}, bla_{VIM} and bla_{KPC} were amplified as described by Nordmann et al.,[34] Yong *et al.*,[38] Toleman et al.[39] and Marschall et al.[40] respectively using specific primers as presented in table 1 below.

Briefly, a reaction volume of 20 µL containing 10 µL of pre-mixed Master Mix, 7 µL of sterile distilled water, 0.5 µL of each of the forward and reverse primers and 2 µL of the DNA of the isolate to be screened was prepared in PCR tubes for each of the isolates and mounted on the PCR machine. The PCR conditions for detecting TEM and SHV genes are 5 min at 94°C, followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1.5 min. For CTX-M gene, 5 minutes at 94°C followed by 31 cycles of 1minute at 94°C, 1minute at 56°C, 1minute at 72°C and concluded by 10minutes at 72°C. The PCR conditions for the AmpC genes were 7 minutes at 94°C followed by 30 cycles of 1minute at 94°C, 1minute at 52°C, 1minute at 72°C and concluded by 10minutes at 72°C. The PCR conditions for NDM-1 amplification are 94°C for 10min followed by 36 cycles of amplification consisting of 30 sec at 94°C, 40 sec at 52°C, and 50 sec at 72°C; and 5 min at 72°C for the final extension. For IMP and VIM, 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, annealing at 45°C for 1 min, an extension at 68°C for 1 min, ending with incubation for 5 min at 68°C. For KPC, 95°C for 5min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 30sec, and extension at 72°C for 1 min 30 sec and a final extension cycle at 72°C for 10 min.

The PCR amplicons were run on 0.8 - 2% Agarose gel, visualized under UV-trans-illuminator and then sequenced to determine the variant of each gene type.

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Table 1: Primers sequences for the amplification of the beta-lactamase genes

Target	Primer	Sequence 5' – 3'	Fragment size (bp)	Reference
TEM	TEM-for	GAGTATTCAACATTTTCGT	857	36
	TEM-rev	ACCAATGCTTAATCAGTGA		
SHV	SHV-for	TCGCCTGTGTATTATCTCCC	768	
	SHV-rev	CGCAGATAAATCACCACAATG		
CTX-M	CTX-M-f	TTT GCG ATG TGC AGT ACC AGT AA	543	35
	CTX-M-r	CGA TAT CGT TGG TGG TGC CAT A		
OXA-1	OXA-1-F	GGCACCAGATTCAACTTTCAAG	564	37
	OXA-1-R	GACCCCAAGTTTCCTGTAAGTG		
DHA-1	DHA-1-F	TGATGGCACAGCAGGATATTC	997	37
	DHA-1-R	TGATGGCACAGCAGGATATTC		
CMY (2-7,12-18,21-23)	CMY-F	CGAAGAGGCAATGACCAGAC	538	37
	CMY-R	ACGGACAGGGTTAGGATAGY		
NDM-1	NDM-1-F	GGTTTGGCGATCTGGTTTTC	621	34
	NDM-1-R	CGGAATGGCTCATCACGATC		
IMP	IMP-F	TTTCATATGGCAGAGTCTTTGCCAGATT	741	38
	IMP-R	ATCCTAGAAATTTAGTTGCTTGGTT		
VIM	VIM-F	ATGTTCAAACTTTTGAGTAAG	801	39
	VIM-R	CTACTCAACGACTGAGCG		
KPC	KPC-F	ATGTCACTGTATCGCCGTC	872	40
	KPC-R	CTCAGTGCTCTACAGAAAACC		

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Treatment of CTX-M-15-positive strains with mutagen
All the bacterial isolates that were positive for the CTX-M-15 gene were subjected to R-plasmid curing experiment using modified method described by Adeleke *et al.*[41] Briefly, each of the isolates were sub-cultured into sterile nutrient broth and incubated for 24 hours at 37°C. They were then inoculated into two sets of tubes containing Mueller Hinton broth to which 200 and 100 µg/mL ethidium bromide has been added and then incubated for another 24 hours at 37°C. Pure colonies of the ethidium bromide treated isolates were then selected on MacConkey agar and Nutrient agar plates, and were suspended in tubes and diluted to 0.5 MacFarland standard. The ethidium bromide treated isolates were then screened for the presence of the CTX-M gene as described previously above.[36]

Data Analysis

Analysis of data was in SPSS version 16 and outcomes were presented as proportions and percentages.

RESULTS

Subjects and isolates characteristics

From the 182 male patients that participated in this study, 48 (26.4%) of them had bacteriospermia. Age distribution of the volunteers ranged between 21 and 60 years with age-group 31-40 years (45.8%) recording the highest case of bacteriospermia, followed by age-group 21-30 years (33.3%) while age-groups 41-50 years and 51-60 years recorded low bacteriospermia of 16.7% and 4.2% respectively (Table 2). The Gram-negative bacteria isolated included *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Entetrobacter* spp. and *Proteus* spp. *E. coli* was the most predominant (41.7%) while *Proteus* spp. (4.7%) was the least bacteria isolated (Table 3).

Table 2: Distribution of bacteriospermia among the patients according to age-group

Age-group (Years)	Patient with bacteriospermia N = 48(26.4%)		Patient without bacteriospermia N = 134 (73.6%)		Total screened N = 182(100%)	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)
21-30	16	33.3	35	26.1	51	28.0
31-40	22	45.8	50	37.3	72	39.6
41-50	8	16.7	38	28.4	46	25.3
51-60	2	4.2	11	8.2	13	7.1

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Table 3: Number and Percentage bacteria isolated

Isolates	N	%
Escherichia coli	20	41.7
Klebsiella spp.	11	22.9
Proteus spp.	2	4.2
Pseudomonas spp.	10	20.8
Enterobacter spp.	5	10.4
Total	48	100

Antibiotic Susceptibility test

The bacteria showed resistance to amoxicillin (87.5%), amoxicillin-clavulanic acid (62.5%) and gentamicin (54.2%). Less than 50% of the isolates exhibited resistance to the fluoroquinolones and cephalosporins while 8.3% showed resistance to imipenem (Figure 1). Multidrug resistance phenotype was observed in 33 (68.8%) of the isolates (Figure 2).

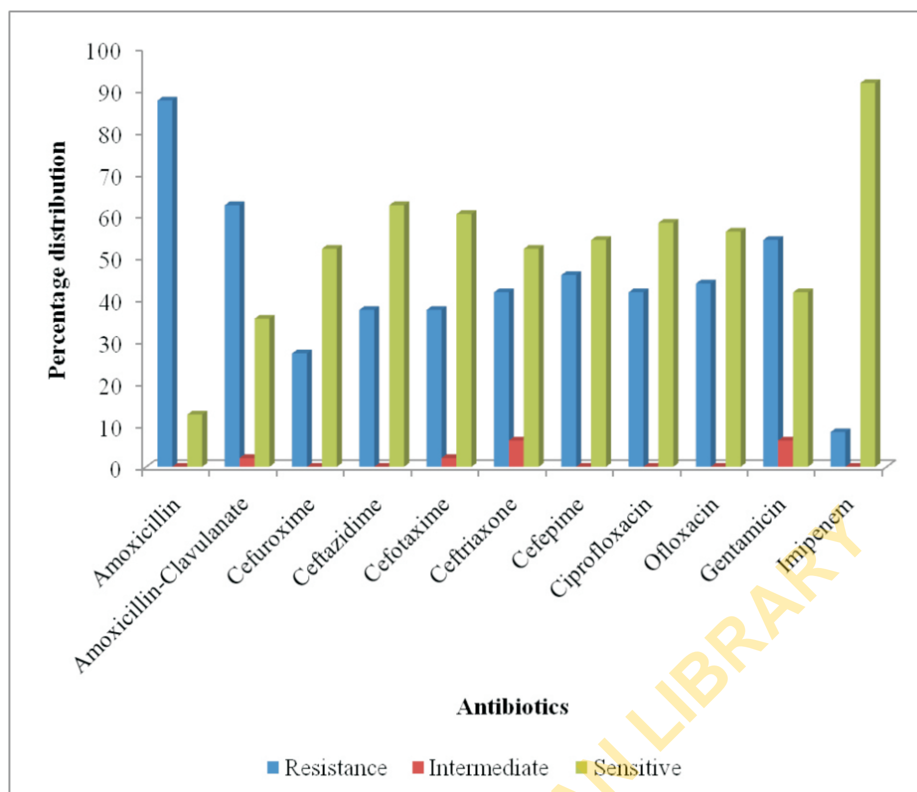

Figure 1: Percentage Antibiotic Susceptibility

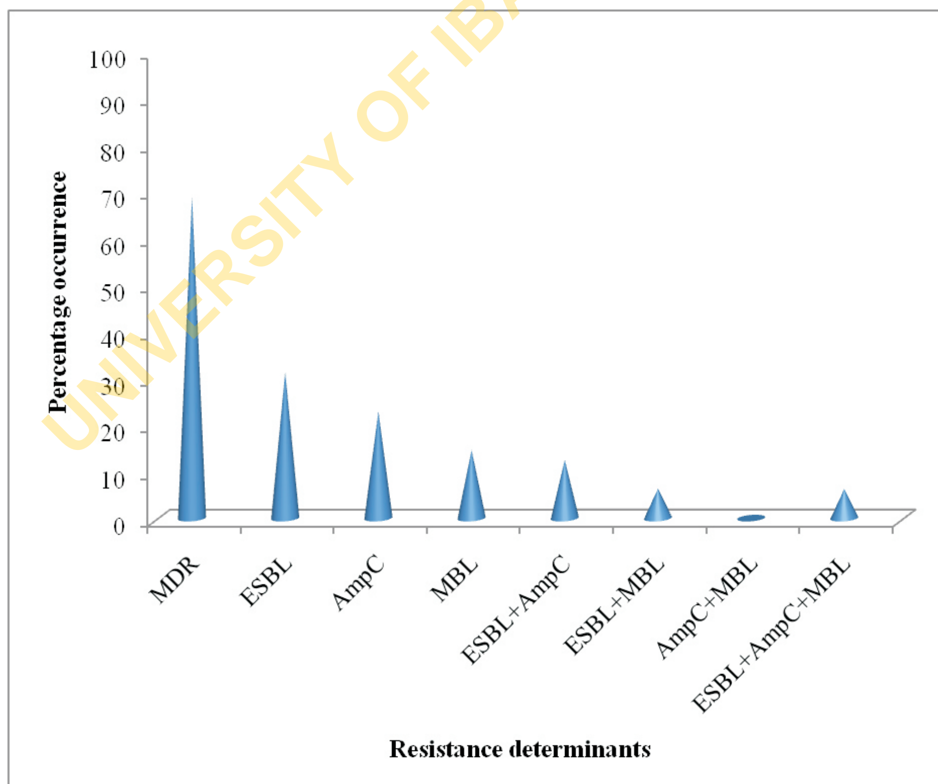

Figure 2: Multidrug resistance and occurrence of resistance determinants among isolates

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Detection of beta-lactamases and mutagen treatment of $bla_{CTX-M-15}$ positive strains

Of the 48 bacterial isolates, 15 (31.3%), 11 (22.9%) and 7 (14.6%) phenotypically produced ESBL, AmpC and MBL respectively. Six (12.5%) of the isolates was found to co-produced ESBL and AmpC, 3 (6.3%) co-produced ESBL and MBL, none co-produced AmpC and MBL while 3 (6.3%) co-produced all the three enzymes (Figure 2).

Polymerase chain reaction was able to amplify TEM and CTX-M genes but not SHV, *Amp*C and MBL genes. Sequencing analysis revealed the presence of $bla_{CTX-M-15}$ in 8 (53.3%) of the ESBL-producers (Table 4).

All the $bla_{CTX-M-15}$ positive *E. coli* and *Pseudomonas* spp. strains as well as one of the three $bla_{CTX-M-15}$ positive *Klebsiella* strains lost their CTX-M-15 genes to the effect of the mutagen (Table 4).

Table 4: Mutagenic treatment of $bla_{CTX-M-15}$ positive strains

Bacterial Isolates	Number of isolates with $bla_{CTX-M-15}$ treated	Number/Percentage not cured	Number/Percentage cured
Escherichia coli	4	0 (0%)	4 (100%)
Klebsiella spp.	3	2 (66.7%)	1 (33.3%)
Proteus spp.	0	0 (0%)	0 (0%)
Pseudomonas spp.	1	0 (0%)	1 (100%)
Enterobacter spp.	0	0 (0%)	0 (0%)
Total	8	2 (25%)	6 (75%)

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DISCUSSION

In this study, the percentage occurrence of bacteriospermia was found to be low (26.4%) compared to the study of Ibadin and Ibeh;[42] Isaiah *et al.*[43] that reported percentage occurrences of bacteriospermia among infertile male as 41.4% and 65.7% respectively in University of Benin Teaching Hospital, Benin city. Similarly, Omosigho *et al.*[44] reported a percentage occurrence of 39.9% bacteriospermia among infertile male in Bida, Niger state. Oyeyipo *et al.*[27] reported a percentage occurrence of 75% bacteriospermia among selected HIV/AIDS patients in Port Harcourt, Nigeria. This higher percentage occurrence reported by Oyeyipo *et al.*[27] may be due to the depressed immunity of the AIDS patients which could have resulted in high colonization of bacteria that are usually not found in healthy person.

In this study, bacteriospermia was found to be higher among male patients within the age range of 31 and 40 years, this is in agreement with the reports of Ibadin and Ibeh;[42] Oyeyipo *et al.*[27] that reported high bacteriospermia among patients in the age ranged 36 to 40 and 38 to 47 years respectively. The high cases of bacteriospermia in both age range 21 – 30 and 31 – 40 year may be attributed to the sexual activeness common to these age groups. However, the reduced cases among the age groups 41 – 50 years and 51 – 60 years may be due to reduced frequency in sexual activity usually attributed to medical ailments, partners' failing health and anxiety about sexual performance in men of that age.[45]

Bacteria isolated in this study includes *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Proteus* spp. which have been similarly reported by some authors.[27,42,44,46,47] The close proximity of the female anus to their vagina and inappropriate cleaning of the anus among some female partners may contribute to the colonization of the vagina with coliform bacteria.[48] Poor wiping of the anus after defecating by some female partners have been reported to result in the contamination of their vagina with faecal microorganisms.[48] The contaminating microorganisms may eventually colonize and become established in that region leading to the development of asymptomatic urinary tract infections which may be transferred to the male partner during sexual intercourse. This may be a good explanation for the presence of high number of coliforms recorded in the semen of the male partners involved in this study.

The results of the antibiotics susceptibility test against the isolates in this study revealed that neither amoxicillin nor amoxicillin-clavulanic acid is suitable in the treatment of bacteriospermia as higher percentage (87.5% and 62.5% respectively) of the bacteria exhibited resistance against them. This result was similar to that of Al-Dahmoshi *et al.*[49] where 75 to 100%

and 47.4 to 75% resistance was reported among the isolates against amoxicillin and amoxicillin-clavulanic acid respectively. Although third and fourth generation cephalosporins, aminoglycoside and fluoroquinolones exhibited good antibacterial activity against some (> 50%) of the isolates, imipenem gave better activity particularly against the multidrug resistant (MDR) strains and thus could be considered by physicians as the drug of choice in cases of bacteriospermia caused by MDR bacteria. The high percentage (68.8%) of MDR isolates reported in this study is alarming and pointed to the fact that antibiotic treatment of bacteriospermia requires serious caution. It is therefore important that antibiotic susceptibility test be carried out on semen of patients suspected to have infection before the commencement of antibiotic treatment. The common practices of empirical or blind treatment of infections with extended spectrum antibiotics without adequate culture and sensitivity test should be discourage so as to prevent increased selective pressure and further spread of MDR strains in cases of antibiotic treatment failure.

Extended-spectrum beta-lactamases are well known enzymes mediating resistance against beta-lactam antibiotics and have been reported to spread among so many Gram-negative bacteria particularly the Enterobacteriaceae worldwide.[50,51] In this study, a sizable percentage of the isolates were phenotypically detected to harbour ESBL, AmpC and MBL either singly or co-existing together in an organism (Figure 2). The co-existing beta-lactamase enzymes in addition to other resistance determinants may be responsible for the multiple antibiotic resistance cases observed among the bacterial isolates in this study. Although some of the phenotypically detected beta lactam resistance determinants in this study could not be amplified molecularly, this does not nullify their existence in these isolates. The non amplification of these genes could be due to certain factors such as the PCR conditions and the set of primers used also the variants of the resistance determinants present in the isolates may be different from those targeted in this study. Hence screening for more variants of the classes of resistance genes or carrying out whole genomic sequencing for these set of isolates would give a clearer picture of the variants harboured by the isolates.

The effect of the ethidium bromide on the plasmid of some of the $bla_{CTX\text{-}M\text{-}15}$ positive isolates as observed in this study suggested that the $bla_{CTX\text{-}M\text{-}15}$ gene harboured by these isolates may be plasmid mediated. Those still retaining their $bla_{CTX\text{-}M\text{-}15}$ gene after mutagenic treatment may have their gene located on the chromosomes.

The main limitation to this study was that the semen samples were collected only in two tertiary hospitals in Oyo state. The sampling was not extended to other parts of the country which could have given a different overall result from what was obtained from this study.

CONCLUSION

This study has shown low percentage occurrence of bacteriospermia caused by Gram-negative bacteria mostly with MDR phenotype among the understudied subjects within the time frame of the study. Accumulation of resistance determinants mediating resistance against the beta-lactam antibiotics as seen among some of the isolates in this study could be responsible for the observed resistance and hence, antibiotics, especially the beta-lactams, must be used with caution in the treatment of bacteriospermia in Ogbomosho town of Oyo state to avoid further increase in the accumulation and spread of MDR bacteria in the future.

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