Bacteriospermia, extended spectrum beta lactamase producing Gram-negative bacteria and other factors associated with male infertility in Mwanza, Tanzania: a need of diagnostic bacteriology for management of male infertility

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Abstract

Background: Infections caused by Extended spectrum beta lactamase (ESBL) producing bacterial are global challenge. There is limited information on the magnitude of bacteriospermia, ESBL producing Gram-negative bacteria (GNB) causing bacteriospermia and factors associated with male infertility. This study determined magnitude of bacteriospermia, ESBL-GNB and other factors association with infertility among presumptive infertile men in Mwanza, Tanzania.

Methods: A cross-sectional hospital-based study was conducted between May 2017 and July 2018 among 137 presumptive infertile men. Semen specimens were self-collected by masturbation into clean, sterile and none-spermicidal containers and processed following laboratory standard operating procedures (SOPs). Data analysis was done using STATA 13.0.

Results: Gram-negative bacteria were predominantly isolated (86.4%), of which 31.6% were ESBL producers. In a total 44 bacteria were isolated from semen culture. The bla_{CTX-M} gene was detected in 75% of phenotypically confirmed ESBL producers. Infertility was independently found to be associated with abnormal spermatozoa morphology (OR (95%CI): 14.48(3.17-66.05)) and abnormal spermatozoa motility (OR (95%CI): 0.05(0.01-0.24)). However, neither bacteriospermia (OR (95%CI): 0.86(0.29-2.59)) nor ESBL bacteriospermia (OR (95%CI): 0.13(0.01-1.22)) was found to be associated with infertility.

Conclusion: One third of bacteriospermia is due to ESBL-producers with history of antibiotic use being protective factor for infertility. Abnormal spermatozoa morphology and poor spermatozoa forward motility independently predicted infertility.

Keywords: bacteriospermia; blaCTX-M; male infertility; extended spectrum beta lactamase; Mwanza; Tanzania.
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Introduction

Bacteria, fungi, protozoa and viruses are agents implicated in male urogenital tract and accessory sex gland infections and account for about 15% of male infertility[1,2]. Bacteria like; *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are the most common pathogens reported to be isolated from semen culture

and causing bacteriospermia[3,4]. Bacteriospermia has been found to cause infertility due to various factors such as: deterioration of spermatogenesis, alteration of acrosome and sperm morphology, auto-immune processes induced by inflammation, increased sperm DNA fragmentation due to formation of reactive oxygen species and obstruction of genital tracts due to fibrosis and inflammation[3,5].

Production of Extended spectrum beta lactamase (ESBL) among multi-drug resistant (MDR) Gram-negative bacteria is the common antibiotic resistance mechanism[6]. *E. coli*, *K. pneumoniae*, *Citrobacter* spp., *Enterobacter* spp., *Acinetobacter* spp. and *Pseudomonas aeruginosa* are common ESBL producing Gram-negative bacteria (GNB)[6,7]. The blaCTX-M gene is documented to account over 75% of ESBL producing gram negative bacteria clinically isolated however little is known on its prevalence among ESBL producing Gram-negative bacteria causing bacteriospermia[8]. Here in, we report the magnitude of bacteriospermia, blaCTX-M among ESBL producing Gram-negative bacteria and other factors associated with male infertility in Mwanza, Tanzania. This is the first study to report magnitude of bacteriospermia, ESBL producing GNB and other factors associated with male infertility from Tanzania. These data are important in the management of bacteriospermia in our setting where there is high prevalence of ESBL producing Gram-negative bacteria[9-11].

Methods

Study design, duration, population and setting

This cross-sectional hospital-based study was conducted between May 2017 and July 2018 involving 137 presumptive infertile men (whose female couples were medically confirmed fertile) attending reproductive health/infertility clinics in Mwanza, Tanzania. Standardized data collection tools used to collect socio-demographic and clinical characteristics of the study participants. Semen specimens were self-collected by masturbation into wide mouth, clean, sterile and spermatozoa non-toxic specimen containers (Hunter Scientific Limited, UK) after consented voluntarily and sexual abstinence of a minimum of 3 days. Patients were instructed to pass urine and then thoroughly cleaned their hands and penis with clean water and non-antiseptic soap[12]. Specimens were brought to Central Pathology Laboratory, department of Histopathology at the Bugando Medical Centre (BMC) for semen analysis at room temperature within 30 minutes after collection and Catholic University of Health and Allied Sciences (CUHAS) multipurpose laboratory in cold box (2-8^{0}C) for semen culture within one hour of collection. PCR to detect blaCTX-M gene was done at National Institute of Medical Research (NIMR), Mwanza.

In this study, presumptive male infertility refers to male's inability to make fertile female partner pregnant for a period of ≥ 1 year of active sexual practices without protections while medically confirmed infertility among men refers male infertility[13-15]. Male infertility can be medically confirmed by examining spermatozoa quality and quantity in an ejaculate[13,14]. This includes: spermatozoa concentration, morphology and/or forward motility[13,14].

Semen analysis, culture and identification of significant isolated bacteria

Semen analysis involved the following parameters; colour (grey to opalescent), volume (2-6 ml), viscosity (< 2 cm dropping threads from pipette), pH (7.2-8.2), motility, morphology and spermatozoa count (20-120 million/milliliter) per SOPs and WHO guidelines12. Semen analysis was performed by skilled and experienced laboratory scientist (> 5 working years) and two other laboratory technicians (> 3 working years) were used to confirm for the validity of the results. Semen specimens were inoculated onto blood agar (BA) and MacConkey agar (MCA) plates followed by aerobic incubation at 37^{0}C for 24-48 hours. A pure significant growth (≥ 10^3 CFU/ml growth) of bacteria were further identified to species level by in-house biochemical identification tests; Gram stain, catalase, slide coagulase, novobiocin, bacitracin, bile esculin and optochin for Gram-positive bacteria and Gram stain, triple sugar iron (TSI), sulfur indole motility (SIM), Simmons citrate, urease and oxidase for Gram-negative bacteria[16].

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed on Muller Hinton agar (MHA) plates by Kirby-Bauer disc diffusion method as per CLSI:2010 guidelines[17]. Erythromycin 15µg, clindamycin 2µg, vancomycin 30µg, gentamicin 10µg, cefoxitin 30µg (for *S. aureus* only) and ciprofloxacin 5µg were used for gram positive bacteria while ampicillin 10µg, sulphamethoxazole-trimethoprim 1.25/23.75µg, gentamicin 10µg, ciprofloxacin 5µg, amoxycillin-clavulanic acid 20/10µg, ceftriaxone 30µg, ceftazidime 30µg,

piperacillin-tazobactam 100/10μg and meropenem 10μg were used for gram negative bacteria.

Phenotypic detection of ESBL producing gram negative bacteria

Double disc synergy (DDS) technique was used to detect ESBL producing gram negative bacteria as reported previously17. Briefly, ceftazidime-clavulanic acid and ceftazidime plain discs were seeded on MHA plate with test organisms. The plates were incubated for 24 hours at 37^0C. The difference of zones of inhibitions of ≥ 5mm between ceftazidime-clavulanic acid and ceftazidime plain was interpreted as ESBL producer[18,19].

Molecular characterization of blaCTX-M gene from ESBL producing gram negative bacteria

Heat treatment technique was performed to extract bacterial DNA with minor modification from previous study[20]. Two colonies of fresh grown bacteria were suspended into DNase/RNase free tubes containing 500 μL of sterile de-ionized water, mixed by vortexing and boiled at 100^0C for 10 minutes. Tubes were centrifuged at 12000 rpm for 10 minutes to obtain 5 μL of supernatant of each test bacteria for PCR.

PCR was performed for phenotypically confirmed ESBL producing gram negative bacteria to determine the presence of blaCTX-M gene as previously reported[21]. CTX-M3G; forward primer: 5'-GTTACAATGTGTGAGAAGCAG-3' and reverse primer: 5'-CCGTTTCCGCTATTACAAAC-3' were used. Briefly, PCR amplification was carried on thermocycler machine (GeneAmp® PCR System 9700, ThermoFishers Scientific, Singapore) as previously explained. Briefly, PCR were conditioned at; initial denaturation at 94^0C for 5 minutes and cycles: 1; denaturation at 94^0C for 60 seconds, 2; annealing at 55^0C for 30 seconds and 3; extension at 72^0C for 60 seconds and final extension at 72^0C for 5 minutes. PCR products were visualized under UV illumination on gel electrophoresis by using 2% agarose gel stained with redsafe (7.5 μL of redafe were added into 150 ml of TBE suspended with 3g of agarose powder). The amplicon with band size of 1000bp was annotated as blaCTX-M gene, (Figure 1). *E. coli* ATCC 25922 was used as negative control organisms.

Statistical analysis

Data analysis was done by using STATA 13.0 version. Continuous data were presented as mean (± SD) and categorical data as percentages. Logistic regression analysis was used to show association between male infertility and independent variables. A p-value of less than 0.05 at 95% confidence interval was considered as statistically significant.

Ethical considerations

Ethical clearance to conduct this study was obtained from a joint BMC/CUHAS ethics and review committee and given ethical numbers: CREC 329/2017 and updated in 2018 by certificate number 719/2018. Written informed consent forms were obtained from study participants before enrollment in this study. Laboratory results; semen analysis, and culture and sensitivity were submitted to respective clinicians for patient management

Results

Socio-demographic and clinical characteristics of study participants

A total of 137 participants were enrolled during this study period. The mean age (±SD) and mean infertility duration (±SD) was 33±6.9 years and 2.7±2 years, respectively. The majorities of participants were living in urban areas (64.2%), 54.0% had tertiary education and 97.1% enrolled from BMC. The following participants reported history of; 2.2% fever, 16.1% antibiotic use within one month prior to enrollment in this study, 18.3% UTI and 3.7% sexually transmitted diseases (STDs). Of the 22 participants with history of antibiotic use, 13 used for ≤ 5 days while of 25 participants with history of UTI, 7 purchased antibiotic without prescription, (Table 1).

Table 1: Socio-demographic and clinical characteristics of study participants

Variables		Frequency (n)	Percentage (%)
Mean (+/- SD) age (years)		33 (+/- 6.9)	-
Mean (+/-SD) infertility duration (years)		2.7 (+/- 2)	-
Residence (N=137)	Urban	88	64.2
	Rural	49	35.8
Level of education (N=137)	Uneducated	3	2.2
	Primary	10	7.3
	Secondary	50	36.5
	Tertiary	74	54.0
Recruitment clinic (N=137)	Bugando Medical Centre	133	97.1
	Kamanga Hospital	2	1.4
	Manjis health care centre	2	1.4
History of fever (N=137)	Yes	3	2.2
	No	134	97.8
Previous antibiotic (N=137)	Yes	22	16.1
	No	115	83.9
Duration of antibiotic use (N=22)	≤ 5 days	13	59.1
	1 week	2	9.1
	2 weeks	6	27.3
	1 month	1	4.5
Type of antibiotic used (N=22)	Ceftriaxone	9	40.9
	Ciprofloxacin	8	36.4
	Azythromycin	3	13.6
	Cotrimoxazole	1	4.5
	Amoxycillin	1	4.5
History of UTI (N=137)	Yes	25	18.3
	No	112	81.7
UTI treatment facility (N=25)	Healthcare facility	18	72
	Pharmacy or drug shops	7	28
History of STD (N=137)	Yes	5	3.7
	No	132	96.3
STD treatment (N=137)	Complete	4	80
	Incomplete	1	20
Catheterization (N=137)	Yes	1	0.7
	No	136	99.3
Other co-morbid (N=137)	Yes (Hypertension)	1	0.7
	No	136	99.3

Semen analysis, bacteriospermia and ESBL bacteriospermia

All participants (100%) had normal semen appearance (color) during semen analysis. Of 137 studied participants, majority of participants had abnormal semen volume (63.5%): 92.0% hypospermia and 8.0% hyperspermia, and poor forward motility of spermatozoa 63.5%. Infertility was observed among 35.0% of participants of which 64.6% had oligospermia (Table 2).

Among 137 semen culture, 32.1% had positive bacteriospermia of which Gram-negative bacteria were predominantly isolated (86.4%). *K. pneumoniae* (27.3%) was the most frequently isolated bacteria followed by E. coli (20.5%) and *Acinetobacter* spp. (15.9%). Out of 38 Gram-negative bacteria, 31.6% were phenotypically ESBL producers. *K. pneumoniae* was predominant ESBL producer detected (58.3%). ESBL blaCTX-M gene was found among 75% of phenotypically confirmed ESBL producers (Table 2).

Table 2: Semen analysis, bacteriospermia and ESBL bacteriospermia results

Variables		Frequency (n)	Percentage (%)
Quality of spermatozoa and semen			
Semen appearance (N=137)	Normal	137	100
	Poor	0	0
Semen PH (N=137)	Normal (7.2 – 8.2)	131	95.6
	Increased alkaline (≥ 9)	6	4.4
Semen viscosity (N=137)	Normal	55	40.1
	Abnormal	82	59.9
Semen volume (N=137)	Normal	50	36.5
	Abnormal	87	63.5
Abnormal semen volume (N=87)	Hypospermia	80	92.0
	Hyperspermia	7	8.0
Spermatozoa morphology (N=137)	Normal	115	83.9
	Abnormal	22	16.1
Spermatozoa motility (N=137)	Good forward motility	50	36.5
	Poor forward motility	87	63.5
Quantity of spermatozoa in semen			
Male infertility (N=137)	Normalspermia	89	65.0
	Infertility	48	35.0
Infertility types (N=48)	Oligospermia(<20 mil/ml)	31	64.6
	Azoospermia (no sperms)	16	33.3
	Necrospermia (dead)	1	2.1
Bacteriospermia			
Bacteriospermia (N=137)	Positive	44	32.1
	Negative	93	67.9
Isolated bacteria spp (N=44)	*K. pneumoniae*	12	27.3
	E. coli	9	20.5
	Acinetobacter spp	7	15.9
	Enterobacter aerogenes	3	6.8
	Enterococcus faecalis	3	6.8
	K. oxytoca	3	6.8
	P. aeruginosa	3	6.8
	Others*	3	6.8
ESBL Bacteriospermia			
ESBL producing GNB (N=38)	Producers	12	31.6
	None producers	26	64.8
ESBL-GNB species	*K. pneumoniae*	7	58.3
	Acinetobacter spp	3	25
	Enterobacter aerogenes	1	8.3
	Pseudomonas aeruginosa	1	8.3
ESBL *bla*$_{CTX-M3G}$gene (N=12)	Positive	9	75
	Negative	3	25

The mean age (±SD) and mean infertility duration from seeking medical intervention (±SD) of the 15 infertile participants with bacteriospermia was 31.5 (±8.1) years and 2.5 (±1.5) years, respectively. The majority of infertile participants with bacteriospermia had semen hyper-viscosity (73.3%, n=11), hypospermia (66.7%, n=10) and poor forward spermatozoa motility (66.7%, n=10) while 4 (26.7%) participants had no spermatozoa in their semen (Table 3).

Table 3: Description of 15 infertile participants with bacteriospermia

Age (years)	Infertility duration	History of UTI	Semen analysis					Bacteria spp	ESBL
			Viscosity	Volume	Morphology	Motility	Remarks		
25	1	Yes	Abnormal	Reduced	Normal	Normal	Oligospermia	*E. faecalis*	N/A
20	2	No	Abnormal	Reduced	N/A	N/A	Azoospermia	*S. pyogenes*	N/A
33	2	No	Abnormal	Increased	Normal	Poor	Oligospermia	*E. faecalis*	N/A
26	1	No	Abnormal	Normal	Normal	Poor	Oligospermia	*E. coli*	NEG
32	3	No	Abnormal	Increased	Normal	Poor	Oligospermia	*K. pneumoniae*	NEG
26	1	No	Abnormal	Normal	Normal	Poor	Oligospermia	*E. coli*	NEG
45	5	No	Normal	Reduced	Normal	Poor	Oligospermia	*K. pneumoniae*	POS
36	4	No	Normal	Reduced	N/A	N/A	Azoospermia	*K. pneumoniae*	POS
35	5	No	Abnormal	Reduced	Abnormal	Poor	Oligospermia	*E. coli*	NEG
32	2	Yes	Abnormal	Reduced	Normal	Poor	Oligospermia	*K. pneumoniae*	NEG
35	3	No	Normal	Increased	Abnormal	Poor	Oligospermia	*Acinetobacter* spp	NEG
29	1	No	Abnormal	Reduced	Normal	Poor	Oligospermia	*E. coli*	NEG
49	5	No	Normal	Reduced	Normal	Poor	Oligospermia	*K. oxytoca*	NEG
19	1	No	Abnormal	Reduced	N/A	N/A	Azoospermia	*P. aeruginosa*	NEG
31	2	No	Abnormal	Reduced	N/A	N/A	Azoospermia	*Acinetobacter* spp	NEG

KEY: ID=Identification number, N/A=Not Applicable, NEG=Negative and POS=Positive

Antibiotics resistance pattern

Percentage resistance of Gram-negative bacteria to antibiotics ampicillin, trimethoprim-sulphamethoxazole and amoxycillin-clavulanic acid was 100%, 100% and 92.1% respectively while ESBL-GNB resistance to ampicillin, trimethoprim-sulphamethoxazole, amoxycillin-clavulanic acid and gentamicin was 100%, 100%, 91.7% and 66.7%. Percentage resistances of Gram-positive bacteria to erythromycin were 66.7% (Table 4).

Table 4: Antibiotics susceptibility patterns of isolated bacteria causing bacteriospermia and ESBL producing GNB

ISOLATES	INT	ANTIBIOTIC SUSCEPTIBILITY PROFILES												
		AMP n(%)	SXT n(%)	AK n(%)	CIP n(%)	GEN n(%)	AMC n(%)	CRO n(%)	CAZ n(%)	TZP n(%)	MEM n(%)	E n(%)	VA n(%)	CD n(%)
GNB (N=38)	R	38(100%)	38 (100%)	2(5.3%)	5(13.2%)	9(23.7%)	35(92.1%)	15(39.5%)	11(28.9%)	3(7.9%)	-	N/A	N/A	N/A
	I	-	-	4(10.5%)	1(2.6%)	1(2.6%)	2(5.3%)	3(7.9%)	5(13.2%)	3(7.9%)	-			
	S	-	-	32(84.2%)	32(84.2%)	28(73.7%)	1(2.6%)	20(52.6%)	22(57.9%)	32(84.2%)	38(100%)			
ESBL-GNB (N=12)	R	12(100%)	12(100%)	2(16.7%)	2(16.7%)	8(66.7)	11(91.7%)	10(83.3%)	10(83.3%)	2(16.7%)	-	N/A		
	I	-	-	1(8.3%)	-	-	1(8.3%)	2(16.7%)	2(16.7%)	1(8.3%)	-			
	S	-	-	9(75%)	10(83.3%)	4(33.3%)	-	-	-	9(75%)	12(100%)			
GPB (N=6)	R	N/A	N/A	N/A	1(16.7%)	2(33.3%)	N/A	N/A	N/A	N/A	N/A	4(66.7%)		
	I				-	1(16.7%)						1(16.7%)		- -
	S				5(83.3%)	3(50%)						1(16.7%)	6(100%)	6(100%)

AMP=ampicillin, SXT=cotrimoxazole, AK=amikacin, CIP=ciprofloxacin, GEN=gentamicin, AMC=amoxycillin-clavulanic acid, CRO=ceftriaxone, CAZ=ceftazidime, TZP=piperacillin-tazobactam, MEM=meropenem, E=erythromycin, VA=vancomycin, CD=clindamycin, INT=interpretation, R=resistance, I=intermediate, S=sensitive and NA=not applicable

Factors associated with male infertility

On Chi square analysis, male infertility was significantly associated with: semen hyper-viscosity (p=0.022), abnormal semen volume (p=0.015), abnormal spermatozoa morphology (p<0.001) and poor spermatozoa motility (p<0.001).On multivariate regression analysis, male infertility was significantly associated with: antibiotic use (OR (95%CI): 0.14(0.02-0.85),p=0.033), abnormal spermatozoa morphology (OR (95%CI): 14.48(3.17-66.05),p=0.001) and abnormal spermatozoa motility (OR (95%CI): 0.05(0.01-0.24),p<0.001). Bacteriospermia and ESBL bacteriospermia did not have significant association with infertility on both; univariate and multivariate regression analysis (Table 5).

Table 5: Factors associated with male infertility among presumptive infertile men

Variables		Infertility N=48, %	Chi square	P value	Multivariate	
					OR(95%CI)	P value
Antibiotics use	Yes (22)	2 (9.1)	7.7514	0.005	0.14(0.02-0.85)	0.033
	No (115)	46 (40)				
History of UTI	Yes (25)	3 (6.3)	7.1299	0.008	*	*
	No (112)	45 (93.7)				
History of STDs	Yes (5)	2 (40)	0.0562	0.811	3.39(0.33-34.82)	0.304
	No (132)	46 (34.8)				
Semen viscosity	Hyper (82)	35 (42.6)	5.2469	0.022	1.18(0.39-3.53)	0.762
	Normal (55)	13 (23.6)				
Semen volume	Abnormal (87)	37 (42.5)	5.8790	0.015	2.85(0.97-8.39)	0.057
	Normal (50)	11 (22)				
Spermatozoa morphology	Abnormal (22)	19 (39.6)	30.3354	0.000	14.48(3.17-66.05)	0.001
	Normal (115)	29 (60.4)				
Spermatozoa motility	Poor (87)	45 (51.7)	29.1655	0.000	0.05(0.01-0.24)	0.000
	Normal (50)	3 (6.0)				
Bacteriospermia	Positive (44)	15 (30.1)	0.0255	0.533	0.86(0.29-2.59)	0.797
	Negative (93)	33 (35.5)				
ESBL Bacteriospermia	Positive (12)	2 (16.7)	1.9499	0.163	0.13(0.01-1.22)	0.073
	Negative (125)	46 (36.8)				

*had collinearity with previous history of antibiotic use

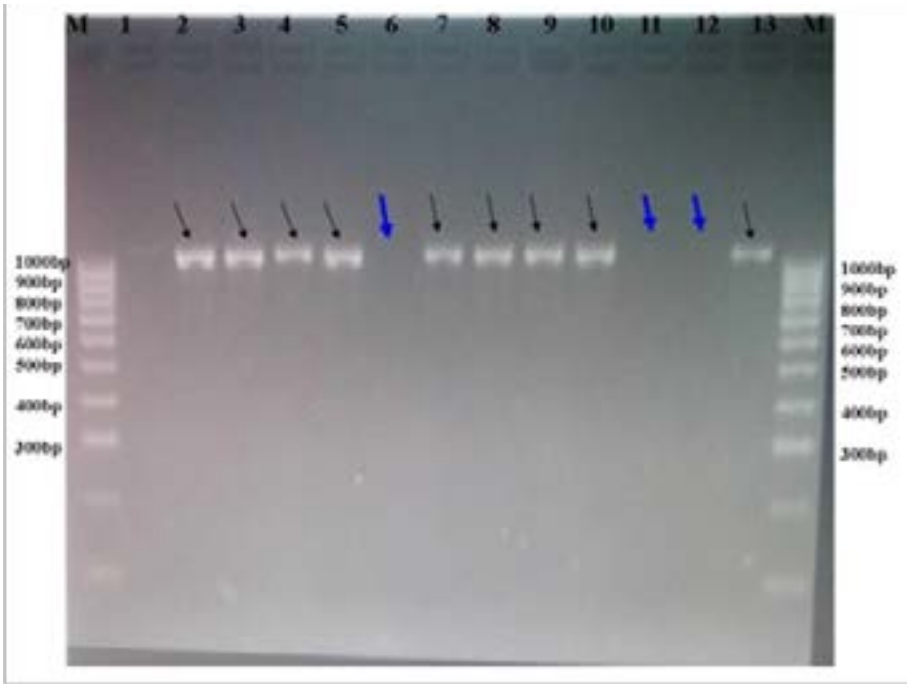

Figure 1: Visualization of PCR products on 2% agarose gel stained with redsafe. Lane M; ladder marker and lanes 1-13; phenotypic confirmed bacterial isolates. Bacteria with positive *bla*$_{CTX-M}$ genes are shown by black arrows while negative are shown with with thick blue arrows.

Discussion

In the current study, male infertility was found among one third of the participants, of which oligospermia was prevalent encountered type followed with azoospermia while one participant had necrospermia as observed previous[22]. Significantly, male infertility was associated with poor forward spermatozoa motility and abnormal spermatozoa morphology. Poor forward spermatozoa motility means that spermatozoa cannot swim properly hence unable to reach the egg for fertilization and abnormal morphology of the spermatozoa means that spermatozoa may be unable to penetrate an egg for fertilization[23,24]. Therefore, the two factors reduce spermatozoa quality and ability of fetilization[25]. These factors may be used as surrogate markers for diagnosis of male infertility[14].

Neither bacteriospermia nor ESBL bacteriospermia was associated with male infertility as observed elsewhere[3,4]. This might be due to small sample size of this study resulting to wide 95%CI and imprecise estimate of the effect therefore results didn't have statistical significance[26]. However, about one third of infertile men had bacteriospermia, suggesting that bacteria might have adverse impact on spermatozoa quantity and/or quality. This is further supported by the fact that history of antibiotic use was protective factor of male infertility. Therefore, there is a need of infectology to be part of infertility diagnosis and management among presumptive infertile men.

Gram-negative bacteria, specifically *K. pneumoniae*, *E. coli* and *Acinetobacter* spp. were prevalently isolated in this study as previously reported from other studies[22,27]. This is contrary to a study3 which reported that, Gram-positive bacteria, specifically *Enterococcus faecalis* and *Staphylococcus aureus* were predominantly isolates causing bacteriospermia. This difference may be due to overall increase trend of multi resistant gram negative infections as the commonest cause of bacterial infections, in the current study's setting Gram-negative bacteria are the most leading causative agents of bacterial infections[10].

About one third of the Gram-negative bacteria were found to be ESBL producers with three quarters of phenotypic ESBL producers carrying blaCTX-M gene. The other quarter might be carrying other CTX-M groups and/or other ESBL families (SHV and TEM) as previously observed[28]. It should be noted previously studies[21,29,30] have found blaCTX-M-15 which is a member of group 1 to be the commonest allele (>75%) in the majority of ESBL producers in Tanzania. The observation of about 30% of Gram-negative bacteria from semen culture to carry ESBL genes is significantly higher than what has been observed in other studies[31,32]. This could be due to high ESBL carriage in our setting due to overuse of antibiotics[29,33]. In this study it was observed that 16.1% of participants used antibiotics mainly cephalosporins (40.9%) without prescriptions. As previously observed, in the current study, resistance to non –beta lactam antibiotics was very high among ESBL- producers[33]. The observation is worrisome as treatment options for ESBL producing bacteria are expensive and most of the time not available in most health facilities in developing countries.

Conclusion

The magnitude of bacteriospermia and ESBL bacteriospermia is high among presumptive infertile men. We recommend that, infectology should be part of diagnosis and management of male infertility.

Limitations of the study

Due to limited funds, this study neither characterized other ESBL families (SHV and TEM) nor other CTX-M groups. Furthermore, we did not investigate other pathogens such as fungi and viruses which could have adverse impact on the quality and/or quantity of spermatozoa in semen of infertile men. Another limitation is, we lack data schistosomiasis screening which is endemic in this study setting and reported elsewhere to be associated with male infertility.

Competing interests

None declared.

Authors' contributions

VS, JI, FC, MFM and SEM conceived and designed this study; YM and ALH collected study data; VS, YM and ALH participated in laboratory procedures; VS, JI, FC, MFM and SEM participated in data analysis; VS wrote the first draft of manuscript; all authors critically revised and approved the final draft of manuscript.

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