Prevalence and Antimicrobial Resistance in Bacteria from Seminal Microbiota of Males Undergoing Evaluation for Infertility: A Cross-sectional Study in Port Harcourt, Nigeria

Abstract

The role of bacterial and other microbial infections on seminal quality and male fertility has been attracting the attention of researchers and public health experts in recent times. A number of studies have reported negative impacts of microorganisms, including those of the seminal microbiota, though there have been few reports on the antimicrobial resistance status of the organisms. This study is thus focused on determining the prevalence and antimicrobial resistance in bacteria associated with seminal microbiota of males undergoing evaluation for infertility.

Methodology

This retrospective study involved the review of laboratory records of 243 males undergoing evaluation for infertility from January 2020 to December 2023. The statistical analysis involved the use descriptive statistics to present the results and use of Pearson's chi square and Fisher's exact test to test for association between the variables.

Results

The prevalence of positive cultures was 43.2%. The dominant species were *Escherichia coli* (29.2%), followed by *Enterococcus faecalis* (23.6%), *Staphylococcus aureus* (20.8%), *Streptococcus agalactiae* (17.0%), *Acinetobacter Baumannii* (2.8)%, *Klebsiella pneumoniae* (1.9%), *Pseudomonas aeruginosa* (1.9%), *Enterobacter cloacae* (0.9%) and *Proteus mirabilis* (0.9%). A cumulative of 79.2% of the bacteria were resistant to Amoxicillin/clavulanate and Norfloxacin They were followed by Chloramphenicol (69.5%), Rifampicin (58.1%), Azithromycin (37.1%), Streptomycin (37.1%) Ceftriaxone (34.3%) Ciprofloxacin (32.4%) Gentamicin (30.5%) Levofloxacin (12.4%)

Conclusion

This study has helped to fill the gap of inadequacy of data on the subject matter, especially in Port Harcourt. It is hoped that more scholars will build on the outcome of this study in the area of seminal quality, infertility, microbial infections and antimicrobial resistance.

Keywords: Antimicrobial resistance, Bacteriospermia, Infertility, Semen quality, Seminal microbiota

Introduction

The human body exists in a mutually beneficial and dynamic relationship with well-coordinated and highly evolved microbial communities, constituting the various microbiota of the human microbiome. The microbial communities are populated by various microorganisms such as bacteria, viruses, and fungi and protozoa which constitute the microbiota.[1] In recent times the interest of researchers are drawn towards the microbial communities found in the male genitourinary systems, and the beneficial and harmful roles they play in human health, fertility and sustenance of future of generations.[2,3] Several studies have reported on the adverse effects of the bacteria found in the semen on the quality and wellbeing of the spermatozoa, yet it remains contentious in the face of studies linking decrease in sperm quality to asymptomatic bacteriospermia[4,5]

There are several organisms, particularly bacteria, involved in the male genitourinary infections and inflammations, these include sexually transmitted pathogens, pathogens causing lower urinary tract infections, and male accessory glands infections. Some of the microorganisms involved in these conditions include chlamydia, mycoplasma, gonococcus, trichomonas and viruses such as HIV, hepatitis viruses, Human Papilloma Virus and others having consequent effect on the semen and the genitourinary system.[6] Bacteria in the genitourinary tracts are recognized to infect many organs causing such conditions as epididymitis, orchitis, urethritis, prostatitis, and others. The most frequently isolated bacteria from semen culture include *Escherichia coli*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Streptococci*, *Staphylococcus saprophyticus, Pseudomonas aeruginosa* and *Enterococcus faecalis*.[7] Some of these are part of the seminal fluid microbiota.

It is not all bacteria isolated from semen culture that cause harm to the human body. It has been reported that semen is not always sterile but may harbour a number of commensal bacteria such as *Staphylococcus epidermidis* or *Streptococcus viridans* and contaminants from the anterior urethra which are free-living as part of the male genitourinary microbiota. On the other side are bacteria like *E. coli* and *E. faecalis* which are known pathogens implicated in close to 90% of the cases of chronic bacterial prostatitis.[8] Furthermore, *Staphylococcus aureus*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis*, and *Escherichia coli* are reported to impede sperm function in vitro; and *S. aureus* as a pathogen is linked with the aetiology of seminal fluid infections.[9]

Besides the harmful impacts of bacteriospermia on semen quality, the genitourinary and fertility on a man, there is the concern about antimicrobial resistance in bacteria associated with the semen microbiota whether as pathogens or commensals. While antimicrobial resistance in a wide spectrum of clinical specimens have been studied, it appears that only very little attention has been spared for studies of drug resistance in semen specimens.[6] The use of antimicrobial drugs in the treatment of bacteriospermia and related conditions, as well as in the control of bacteria during processing and storage of semen preparatory for artificial reproductive techniques are likely to pose the public health challenge through proliferation of antimicrobial drugs resistance.[10]

The acquisition and dissemination of antimicrobial resistance are primarily fallouts of human activities within and outside of healthcare facilities, including management of baceriospermia and related conditions and also due to several other interdependent environmental factors related to misuse and abuse of antimicrobials healthcare, agriculture and elsewhere such as unmitigated use as animal growth promoters. These have worsened the problem of antimicrobial-resistance bacteria beyond imagination and put the world on the tenterhooks of a public health disaster. It has severely constricted the options in therapeutic intervention in infectious diseases, with a resultant surge in morbidity, mortality and costs in human and financial resources.[11]

This study was thus conceived to ascertain the prevalence of aerobic bacteria in the seminal fluid microbiota of males undergoing evaluation for infertility, and evaluate the antimicrobial resistance patterns of the isolates.

Methods and materials

Study period and area

The study was conducted from January 2020 to December 2023, at Diagnostix Medical Laboratories in Port Harcourt, South-South of Nigeria. The facility is a private diagnostic laboratory, rendering services to persons attending public and private healthcare facilities within and outside the Port Harcourt metropolis. The laboratory services are managed by a team of medical laboratory scientists led by an experienced medical laboratory scientist. who is skilled in the analysis of semen and other body fluid.

Study design

The study is a facility-based retrospective cross-sectional study involving the review of the laboratory records of men who conducted semen analysis, culture and sensitivity testing within the study period. The records of all persons with complete documentation for semen analysis and culture were included while those without complete documentation were excluded. On the whole records of 261 laboratory results were reviewed while 243 where included.

Semen Culture

Semen specimens were inoculated onto blood agar (BA) and MacConkey agar (MCA) plates and aerobically incubated at 37°C for 24 hours, negative cultures were left in the incubator for another 24 hours. A pure significant growth ($\geq 10^3$ CFU/ml growth) of bacteria were further identified to species level by inhouse 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[12]

Antibiotic susceptibility testing

Antimicrobial Susceptibility Testing, as stated in the Standard Operating Procedure was carried out by the Kirby Bauer disk diffusion method using Mueller-Hinton agar (Oxoid, Hampshire, England). The following antimicrobial agents tested: Amoxicillin/cloxacillin(20µg), Azithromycin (30 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Levofloxacin(20µg), Gentamicin (10 µg), Norfloxacin (10 µg), Rifampicin (20 µg), Streptomycin (30 µg) (Oxoid, England).[13] Resistance data were read and interpreted in accordance with the standards of the Clinical Laboratory Standards Institute (CLSI).[13,14]

Results

The laboratory of records of 243 males undergoing evaluation for infertility were reviewed between January 2020 and December 2023. The mean age of the participants was 39.09 years, the median age was 38 years, the mode, 35 years, standard deviation (7.264), the minimum and maximum ages were 27 and 62 years respectively. (Figure 1)

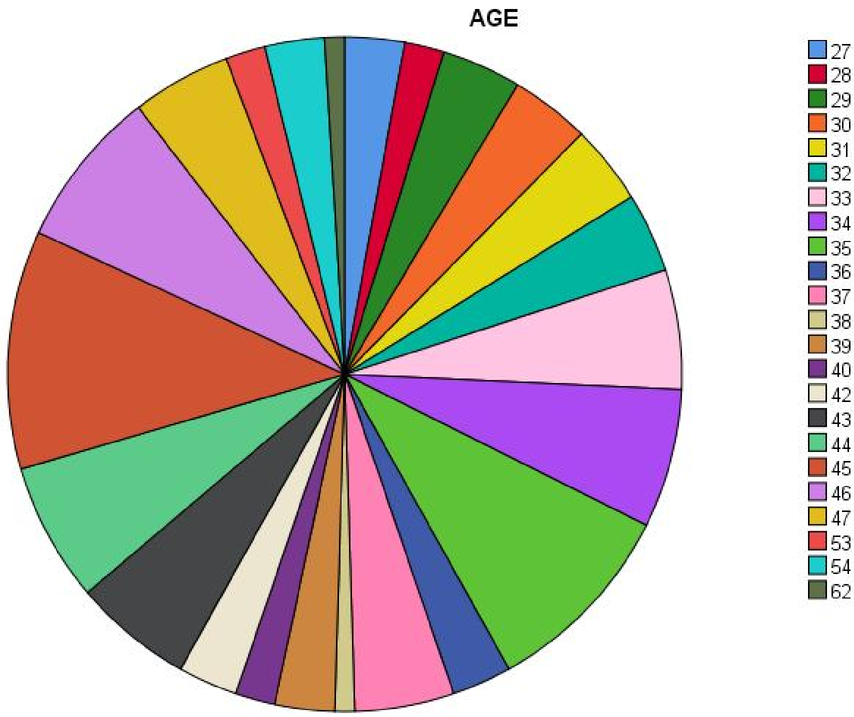

Figure 1: The Age Distribution of the Participants

Occurrence of Bacterial Strains obtained from Semen Specimens

Out of the 243 semen specimen cultures, 105 (43.2%) were positive, producing nine bacterial species; 138 (56.8%) were negative cultures. (Figure2) The most dominant species were *Escherichia coli* (29.2%), followed by *Enterococcus faecalis* (23.6%), *Staphylococcus aureus* (20.8%), *Streptococcus agalactiae* (17.0%), *Acinetobacter Baumannii* (2.8), *Klebsiella pneumoniae* (1.9), *Pseudomonas aeruginosa* (1.9), *Enterobacter cloacae* (0.9%) and *Proteus mirabilis* (0.9%). (Table 1)

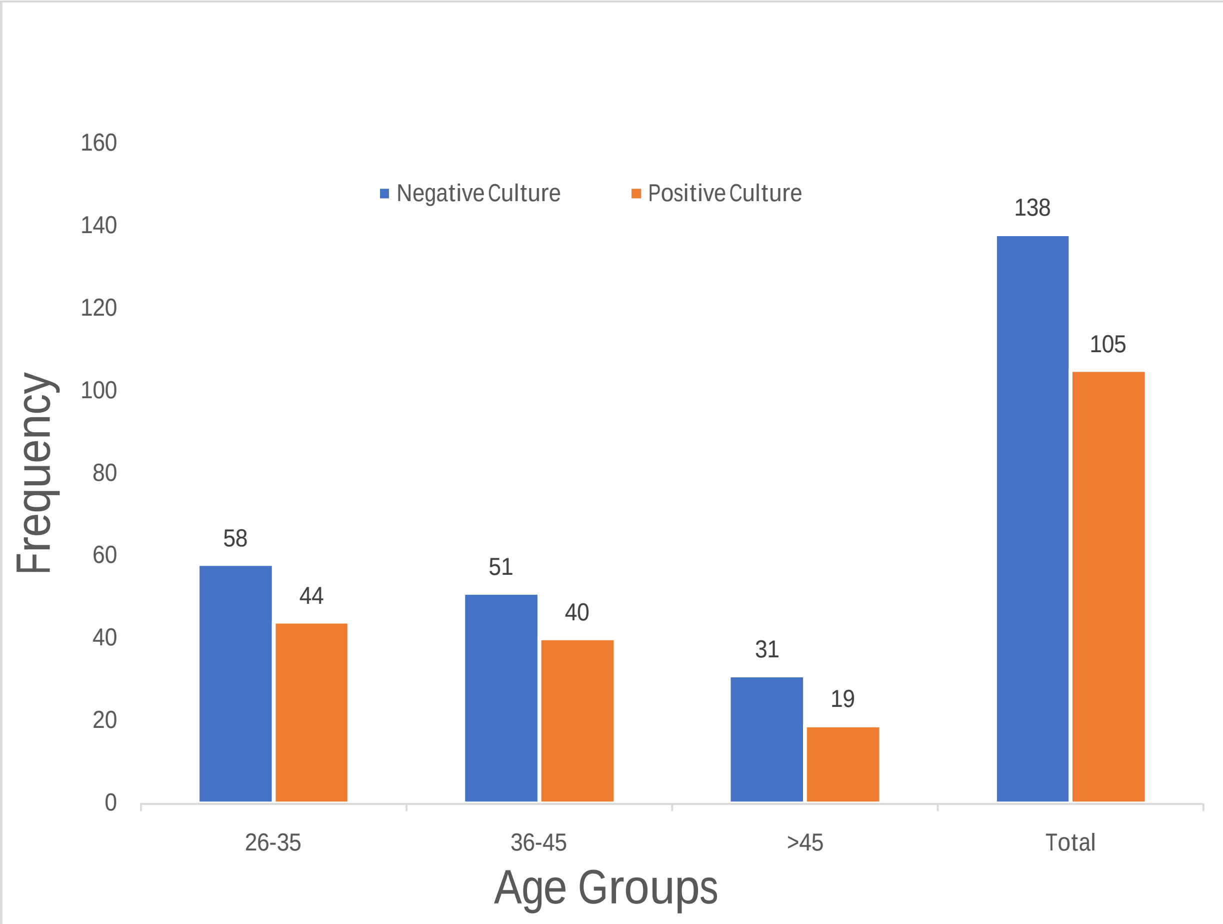

Figure 2: The Distribution of Positive and Negative Cultures

Table 1: Frequency and Occurrence of Bacterial Isolates obtained from Semen

Bacterial Isolates	**26-35 years** n	%	**36-45 years** n	%	**>45 years** n	%	**Total** n	%
Acinetobacter baumannii	1	33.3	2	66.7	0	0.0	3	2.9
Escherichia coli	2	6.5	21	67.7	8	35.8	31	29.5
Enterobacter cloacae	0	0.0	0	0.0	1	100	1	1.0
Enterococcus faecalis	9	36	9	36	7	28	25	23.8
Klebsiella pneumoniae	2	100	0	0.0	0	0.0	2	1.9
Proteus mirabilis	1	100	0	0.0	0	0.0	1	1.0
Pseudomonas aeruginosa	2	100	0	0.0	0	0.0	2	1.9
Streptococcus agalactiae	14	77.8	4	22.2	0	0.0	18	17.1
Staphylococcus aureus	13	59.1	6	27.3	6	27.7	22	21.0
Total	**44**	**41.9**	**42**	**40**	**19**	**18.1**	**105**	**100.0**

n: Number of strains

Antimicrobial Resistance/Susceptibility Patterns of the Antimicrobial Agents

Over all, the bacterial isolates were observed to be resistant to 53.8% of the test antimicrobial agents. Amoxicillin/clavulanate and Norfloxacin were the most ineffective agents as the bacteria strains exhibited resistance against 79.2% of each of the two agents. They were followed by Chloramphenicol (69.5%), Rifampicin (58.1%), Azithromycin (37.1%), Streptomycin (37.1%) Ceftriaxone (34.3%) Ciprofloxacin32.4 Gentamicin (30.5%) Levofloxacin (12.4%)

Table 2: Antimicrobial Resistance/Susceptibility Patterns of the Antimicrobial Agents

Antimicrobials	Resistant	Percent	Susceptible	Percent
Amoxicillin/cloxacillin	79	75.2	26	24.8
Norfloxacin	79	75.2	26	24.8
Chloramphenicol	73	69.5	32	30.5
Rifampicin	61	58.1	44	41.9
Azithromycin	39	37.1	66	62.9
Streptomycin	39	37.1	66	62.9
Ceftriaxone	36	34.3	69	65.7
Ciprofloxacin	34	32.4	71	67.6
Gentamicin	32	30.5	73	69.5
Levofloxacin	13	12.4	92	87.6
Cumulative	**485**	**46.2**	**565**	**53.8**

Antimicrobial Resistance Profiles of Bacterial Isolates obtained from Semen specimens

Overall, 46.3%. of the test isolates were found to be resistant to as least one antimicrobial agent. The highest prevalence of 70% was recorded among *Enterobacter cloacae* and *Pseudomonas aeruginosa* which had only one and two strains respectively. This was followed by *Acinetobacter baumannii* (63.3%) having three stains. The three dominant strains also recorded high prevalence of AMR as follows: *Escherichia coli* (50%), *Staphylococcus aureus* (49.6%), *Enterococcus faecalis* (42.4 %) and *Streptococcus agalactiae* (37.3%); the prevalences for the other strains are: *Klebsiella pneumoniae* (40%) and *Proteus mirabilis* (10%). (Table 3)

Table 3: Antimicrobial Resistance Profiles of Bacterial Isolates obtained from Semen specimens

Bacterial Isolates	n	nx10	APX	AZT	CTX	CHL	CPX	LV	GN	NB	RD	STR	R (%)
Acinetobacter baumannii	3	30	3	1	1	2	1	1	2	2	3	3	19(63.3)
Escherichia coli	31	310	25	10	1	23	14	5	9	25	20	13	155(50.0)
Enterobacter cloacae	1	10	1	1	1	1	0	0	0	1	1	0	7(70.0)
Enterococcus faecalis	25	250	19	7	8	18	6	2	7	19	11	9	106(42.4)
Klebsiella pneumoniae	2	20	1	2	1	1	15	0	0	1	1	0	8(40.0)
Proteus mirabilis	1	10	0	0	0	0	0	0	0	0	1	0	1(10.0)
Pseudomonas aeruginosa	2	20	2	2	1	1	1	1	2	2	2	0	14(70.0)
Streptococci agalactiae	18	180	10	7	4	11	4	2	6	10	8	5	67(37.3)
Staphylococcus aureus	22	220	19	9	9	15)	7	2	6)	19	14	9	109(49.6)
Total (%)	105	1050	79	39	36	73	34	13	32	79	61	3	486(46.3)

Key: n=Number of strains, R= Number of resistant strains

Discussion

This study has contributed in ascertaining the prevalence of aerobic bacteria in the seminal fluid microbiota of males undergoing evaluation for infertility, and evaluate the antimicrobial resistance patterns of the isolates. The prevalence of positive sperm culture of 43.2% as observed in this study aligned closely with the prevalence of 43.5%; reported for male partners of infertile couples in an Italian Study.[6] It was however higher than the 21.0% among asymptomatic in men with primary infertility in another Italian study[15] and 35.3% foe infertile males in an Indian study.[16] The outcomes of this and the other studies is a restatement of the fact that the male genitourinary tract is not sterile. The differences in prevalence may be ascribed to environmental and related factors.

The bacterial isolates obtained here are largely environmental organisms and part of the bacterial communities of organ systems contiguous to the genitourinary system particularly the digestive system.

The bacterial species recovered in this study were in agreement with the outcome of several studies elsewhere.[6,9,15,16,17] The most prevalent isolates recovered in this study, namely *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococci*; were some of the commonest species obtained in some other places.

Some of the organisms have been reported to impact adversely on seminal quality; *Staphylococcus aureus*, and *Escherichia coli* have been known to impede sperm function in vitro and also cause infections in the semen.[9] *E.coli*, *K. pneumoniae*, and *E. cloacae* are reported have significant reduction on sperm motility.[18] The presence of *Streptococcus viridians* or *Haemophilus parainfluenzae* has been reported to correlate with impaired sperm morphology' while *coagulase-negative Staphylococci* or *Enterococcus faecalis* has been linked with pathological low counts of live spermatozoa.[17] Thus, there appear to be ample research evidence to support the adverse impacts of bacteriospermia on the quality of sperm. This will lead to more beneficial outcomes in the diagnostic evaluation and management of male factor infertility.

The results for antimicrobial resistance show a high resistance against most of the antimicrobial agents. More than 50% of the bacteria were resistant to penicillin (Amoxicillin/cloxacillin), the fluoroquinolone (Norfloxacin), Chloramphenicol and Rifampicin, while only Levofloxacin had less than 15% resistance. There was high levels of resistance among the four predominant isolates *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococci*; as well as the less dominant ones. This level of resistance was however less than the resistance of 100%, 100%, 91.7% and 66.7%. reported for ampicillin, trimethoprim-sulphamethoxazole, amoxycillin-clavulanic acid and gentamicin and erythromycin respectively in Tanzanian study.[12] Slightly lower levels of resistance than the ones observed in this study was reported in an Italian study.[6] A study in America reported much lower prevalence for resistance ranging from 0-17% for males undergoing evaluation for infertility.[19]

Though there were not much reported studies of antimicrobial resistance in bacteria recovered from semen samples, it can be seen from the few studies that bacteria in semen microbiota is not an exception to the high prevalence of antimicrobial resistance which has become a looming global threat to public health. The differences in the outcome of the studies may attributed in jurisdictional attitude to drug use, abuse and misuse as it pertains to rules on regulation of drugs, enforcement and compliance to the rules and the awareness of the threats posed by increasing antimicrobial resistance.

It is noteworthy that five of the isolates belong to the ESKAPE pathogens, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter cloacae. Klebsiella pneumoniae* and *Acinetobacter baumannii;* (which also include *Enterococcus faecium*) a group of bacteria of very difficult to treat bacteria high propensity for the acquisition of resistance to antibiotics and major causes of nosocomial infections worldwide, making them a threat to global public health.[20,21]

The limitations of the study are mainly those faced in retrospective studies, given that the samples have been analyzed before the commencement of the study. The sociodemographic data are limited to those contained in the laboratory records, and there is no personal interaction with the participants. Some information that may enrich the study are therefore not captured. Future studies may be designed to overcome these limitations

Conclusion

This study has, by the determination of the prevalence and antimicrobial resistance patterns in bacteria associated with seminal microbiota of males undergoing evaluation for infertility in Port Harcourt helped in filling the gap of inadequacy of data on the subject matter, especially in Port Harcourt. It is hoped that more scholars will build on the outcome of this study in the area of seminal quality, infertility, microbial infections and antimicrobial resistance.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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