Original Article

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Bacteriological Profiles of Semen Culture in Male Patients Having Primary Infertility, Attending Mombasa Assisted Reproduction Centre

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ABSTRACT

Background: Infertility is of global health concern affecting one out of six couples worldwide, and Africa has reported the highest rates. Bacterial semen infections are considered to be one of the significant causes of infertility. Effective antimicrobial therapy is therefore essential in treatment of bacteriospermia. This study aimed to identify the bacterial pathogens present in semen and involved performing the antimicrobial susceptibility testing to improve the clinical management by identifying the antimicrobials that can be the best treatment option.

Methods: Ninety semen specimens were collected from male patients having primary infertility who attended the clinic. Semen was cultured on Nutrient agar, Blood agar, MacConkey agar and Chocolate blood agar using standard bacteriological techniques. Bacterial isolates identification was done by studying the colony characteristics, performing gram staining reactions and biochemical testing. Modified Kirby-Bauer method was used in performing the antimicrobial susceptibility testing.

Result: 51 (56.67%) patients presented with bacteriospermia. Staphylococcus aureus (29.41%) was the most common bacteria isolated, followed by Escherichia coli (23.53%), Coagulase negative staphylococcus species (17.65%), Klebsiella pneumoniae (11.76%), Proteus. mirabilis (7.84%), Pseudomonas. aeruginosa (5.88%) and Neisseria gonorrhoeae (3.92%). Most of the bacteria isolated were highly sensitive to ciprofloxacin, cefotaxime and ofloxacin and moderately sensitive to amikacin, gentamycin and cefuroxime.

Conclusion: Bacteriospermia prevalence rate was 56.67% and was commonly observed in the age group of 30 - 39 years. Staphylococcus aureus was found to be the predominant causative agent of semen infection. The most effective antimicrobials were ciprofloxacin, cefotaxime and ofloxacin and the antimicrobial with least activity was co-trimoxazole.

Keywords: *Male Infertility, Semen Culture, Bacteriospermia, Antimicrobial Susceptibility Patterns*

Introduction

Infertility is incapacity of couples who are sexually active, and not on any form of contraception to successfully achieve pregnancy in a period of one year, as defined by the World Health Organization (WHO) and the International Committee for Monitoring Assisted Reproductive Technology. The highest rates of infertility globally, have been reported in Africa. [1] One out of six couples are affected by infertility worldwide [2] and its diagnosis and treatment require a lengthy time with high costs and this can be frustrating for couples. [2] Genitourinary tract or semen infections are considered to be one of the significant causes of infertility in males. [3] A global decline in the quality of human spermatozoa have been noted over the past few decades. [4] A comparison of semen parameters between the non-infected and infected men reveals that, spermatozoa motility and viability are lowered when an infection is present in the semen. [5] Moreover, evidence that is emerging, is indicating that the presence of microbiome in the semen has major implications for the men's reproductive health, the couple's reproductive health and also the offspring's health, because of transfer of microorganisms to the partner and offspring. [6] There is an escalating research interest in the field of microbiome in the semen. However, this microbial niche in the semen is currently understudied as compared to other areas of research concerning the human microbiome. [6] Lesser is known about the male microbiota and its impact on the reproductive system and male infertility. [7] Preliminary investigations suggest that manipulating the microbiome in humans, may play a role in improving the seminal parameters and male fertility status. [8] Effective antimicrobial therapy is therefore essential in treatment of bacteriospermia. Globally, studies have demonstrated that the antimicrobial resistance is becoming an immense trouble, exacerbated by emergence of bacterial strains that are resistant to multiple drugs, essentially making it extremely hard to provide effective treatment to the patients. [9] Therefore, there is need for continuous

screening and monitoring of the behavioural patterns of microorganisms that have the potential to cause infections, and to identify their antimicrobial susceptibility pattern to the most routinely used antimicrobials at the hospitals at the County, National and Global levels to provide assistance to the healthcare practitioners to determine the most appropriate antimicrobial for the treatment of infections. Semen culture helps in identifying bacteria that are most prominent cause of bacterial semen infections and the antimicrobial sensitivity testing helps in improving the clinical management by identifying the antimicrobials that can be the best treatment options. The aim of this study was to identify the bacterial pathogens that may be present in semen and determine their antimicrobial susceptibility patterns which may help to improve the clinical management by identifying the antimicrobials that are the best treatment option.

Materials and Methods

Semen specimen were collected from 90 male partners of couples seeking infertility evaluation. The study was conducted between January and June 2020 at Mombasa Assisted Reproduction Centre (MARC), Mombasa County, Kenya.

Specimen Collection

The semen specimens were provided by male patients who restrained from intercourse for a period of 2 to 5 days and no more than 7 days prior to giving the specimen. Patients were counselled on the procedure of specimen collection. Specimens were self-collected by males by masturbating into a sterile, dry, leak proof, broad-mouth container. To avoid any unnecessary delays in transportation, the specimens were collected at the private specimen collection room situated at MARC.

Isolation and identification of bacterial isolates

All semen specimens were cultured aseptically within one hour of collection. They were diluted with sterile normal saline in the ratio of 1:10 and centrifuged at 1500 rotations per minute for 15 minutes. The supernatant was removed and the sediments were inoculated using a 10µL pre-calibrated loop on blood agar, nutrient agar, chocolate blood agar and MacConkey agar. To ensure that single colony cultures were obtained, the sediments were streaked on the culture plates as per the recommended streaking method. Thereafter the agar plates were incubated at 37°C overnight for 18 to 24 hours, both aerobically and in 5% CO_2 for isolation of pathogenic bacterial microorganism. The bacterial isolates identification was done by studying the colony characteristics, performing gram staining reactions and biochemical testing as per the guidelines provided in the Bergey's manual of systemic bacteriology. [10]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out only on significant cultures by use of standard filter paper disk agar diffusion method using modified Kirby-Bauer method following the definition of Clinical and Laboratory Standards Institute (CLSI), 2018. [11] Isolated colonies of similar features were picked by use of a sterile wire loop and were emulsified in 3 - 4ml of physiological saline. The turbidity formed was adjusted to an equivalent of 0.5 McFarland standards. Using a sterile swab, inoculation was made onto the Mueller Hinton agar plate by streaking the swab in three directions over the surface of the plate. Using a sterile forceps, appropriate antimicrobial discs were placed and distributed evenly on the inoculated plates. Within half an hour, the plates were inverted and incubated appropriately at 35°C for 18 to 24 hours. The zone of growth inhibition was measured in millimetres using a ruler and the zone diameter interpretative table was used to denote the organism either as susceptible, intermediate or resistant. [12]

Quality Control

From each batch of prepared culture media plates, at least three plates were taken and kept overnight in the incubator to ascertain the sterility of the prepared plates. No growth in the plate after 24 hours meant that the culture media plates were sterile and ready for use. Before inoculation, all the culture media plates were also examined visually to ensure that there was no visible change that would suggest contamination or deterioration. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were used as quality control strains for antimicrobial susceptibility testing to ensure the accuracy of the test results obtained.

Data Analysis and Presentation

Data was analyzed using Statistical Package for Social Sciences (SPSS) software (Version 20, SPSS, Inc, Chicago, IL, USA) and presented using tables, graphs and pie charts. Pearson Chi-square test was used to analyze the association between categorical variables and the calculation was carried out at a confidence level of 95% and the p value was considered to be statistically significant if $p < 0.05$

Ethical Consideration

The ethical approval to carry out the study was obtained from the Mount Kenya University, Ethical Review Committee. License to conduct the research was also obtained from National Commission for Science Technology and Innovation (NACOSTI). Authorization to carry out the research was also obtained from Mombasa Assisted Reproduction Centre. The identity of all the patients enrolled in the study and the data collected were

treated in confidence following informed consent from all the patients.

Limitations of the study

In spite of clearly explaining to the male patients on the correct procedure on how to provide a clean analytical semen specimen, the likelihood of ruling out contamination completely, cannot be fully disregarded.

Results

Seminal culture was performed on all the 90 specimens, out of which 51 (56.66%) showed significant bacterial growth, where 27 were gram negative and 24 were gram positive bacteria (figure 1). *Staphylococcus aureus* (29.41%) was the commonest bacteria isolated, followed by *Escherichia coli* (23.53%), Coagulase negative staphylococcus species (17.65%), *Klebsiella pneumoniae* (11.76%), *Proteus mirabilis* (7.84%), *Pseudomonas aeruginosa* (5.88%) and *Neisseria gonorrhoeae* (3.92%) (Table 1). The highest occurrence of bacteriospermia was observed in the age group of 30 - 39 (47.06%), followed by 40 - 49 (35.29%) (Table 2).

Table 3 contains the antimicrobial susceptibility status of the various isolated bacteria, against the several antimicrobials that were used. Ciprofloxacin, cefotaxime and ofloxacin exhibited greater antimicrobial action against *S. aureus* whereas co-trimoxazole exhibited the least action. Ciprofloxacin, amikacin and ofloxacin showed high degree of antimicrobial action against the isolated *E. coli*, however amoxicillin/clavulanic acid and co-trimoxazole displayed the least action.

As contained in figure 2, most of the bacteria isolated from the present study showed high degree of susceptibility to ciprofloxacin (76.47%), cefotaxime (74.51%) and ofloxacin (76.47%). Moderate sensitivity was exhibited by amikacin (68.63%), gentamycin (60.78%) and cefuroxime (68.83%). However, the antimicrobial activity of amoxicillin/clavulanic acid (52.94%) and co-trimoxazole (43.14%) was not much appreciable.

Discussion

Male urogenital tract infections caused by bacteria have been linked to subfertility and infertility. [13] Toxins released by

Table 1: Distribution of bacterial isolates and their frequency of occurrence.

Bacterial Isolate	Number of isolates (n=51)	Percentage (%)
Staphylococcus aureus	15	29.41%
Escherichia coli	12	23.53%
Coagulase negative staphylococcus species	9	17.65%
Klebsiella pneumoniae	6	11.76%
Proteus mirabilis	4	7.84%
Pseudomonas aeruginosa	3	5.88%
Neisseria gonorrhoeae	2	3.92%

Table 2: Bacteriospermia distribution in relation to age groups.

Age Group	Positive bacterial culture (n = 51)	Negative bacterial culture (n = 39)	P-value
20 - 29	5 (9.80%)	9 (7.69%	
30 - 39	24 (47.06%)	18 (46.15%)	
40 - 49	18 (35.29%)	9 (23.07%)	.453
50 - 59	3 (5.88%)	2 (5.13%)	
60 - 69	1 (1.96%)	1 (2.56%)	

**P value was determined by Pearson Chi-square test*

Table 3: Antimicrobial sensitivity patterns of individual bacterial isolates from seminal culture.

Bacterial isolate	Isolate numbers	Pattern	CIP (5µg)	AK (30µg)	GM (10µg)	CXM (30µg)	CTX (30µg)	AMC (30µg)	OFL (5µg)	SXT (25µg)
S. aureus	15	S	11	10	7	10	12	7	12	5
		I	1	3	4	2	3	0	1	4
		R	3	2	4	3	0	8	2	6
E. coli	12	S	9	8	7	7	7	4	8	4

Bacterial isolate	Isolate numbers	Pattern	CIP (5µg)	AK (30µg)	GM (10µg)	CXM (30µg)	CTX (30µg)	AMC (30µg)	OFL (5µg)	SXT (25µg)
		I	1	1	1	1	2	3	2	1
		R	2	3	4	4	3	5	2	7
CoNS	9	S	6	4	3	3	4	5	6	2
		I	1	1	2	2	2	2	1	2
		R	2	4	4	4	3	2	2	5
K. pneumoniae	6	S	4	3	2	5	4	2	3	2
		I	1	1	1	1	1	2	1	1
		R	1	2	3	0	1	2	2	3
P. mirabilis	4	S	3	2	2	1	2	2	2	1
		I	0	1	0	1	0	0	1	0
		R	1	1	2	2	2	2	1	3
P. aeruginosa	3	S	1	1	2	1	1	0	1	0
		I	0	0	0	0	0	0	0	0
		R	2	2	1	2	2	3	2	3
N. gonorrhoeae	2	S	1	0	0	1	0	0	1	0
		I	0	0	0	0	0	0	0	0
		R	1	2	2	1	2	2	1	2
Total number of isolates	51									
		S+I	39	35	31	35	38	27	39	22
		R	12	16	20	16	13	24	12	29

CIP = Ciprofloxacin, AK = Amikacin, GM = Gentamicin, CXM = Cefuroxime, CTX = Cefotaxime, AMC = Amoxicillin/clavulanic acid, OFL = Ofloxacin, SXT = Co-trimoxazole, S = Susceptible, I = Intermediate and R = Resistant

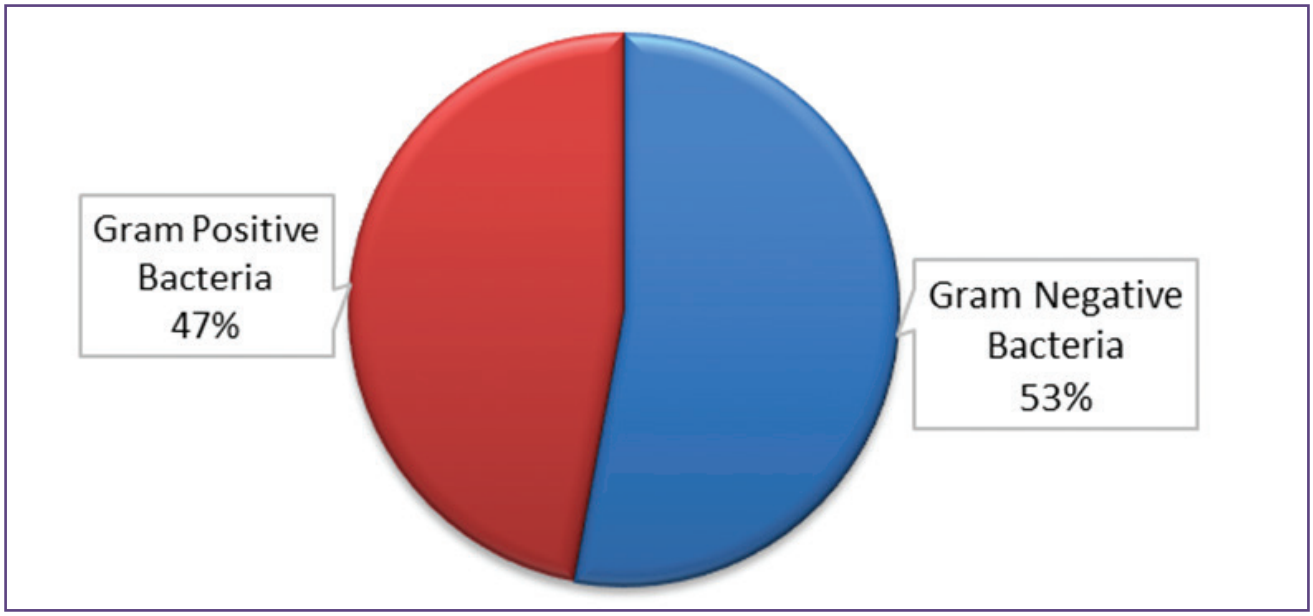

Fig. 1: Distribution of gram positive and gram negative bacterial isolates.

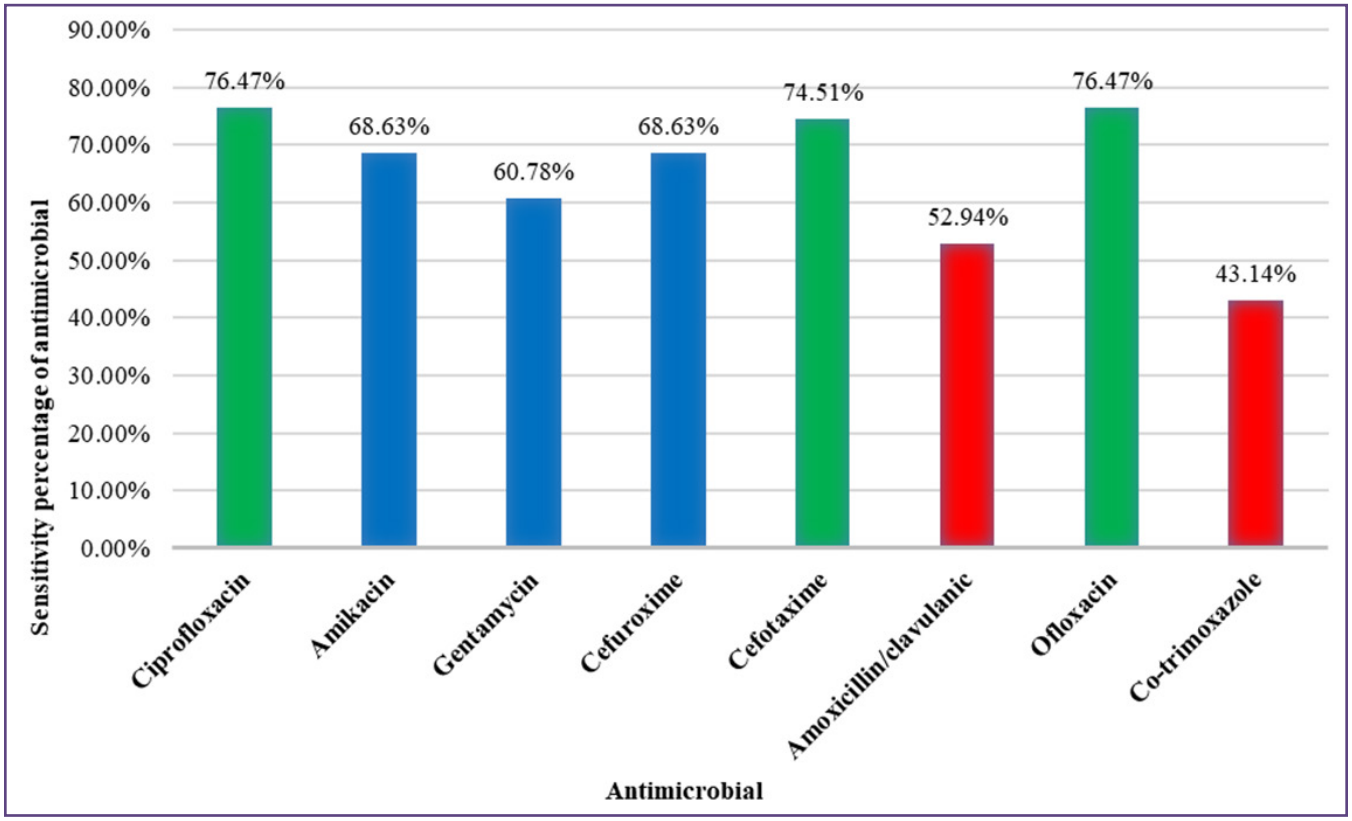

Fig. 2: Overall antimicrobial susceptibility (%) testing for bacterial isolates.

the bacteria may cause detrimental effects to the ejaculated sperm cells and enhance the negative effects of leukocyte-generated oxidative stress causing peroxidative damage to the spermatozoa cell membrane to a level beyond which the ability of the sperm cell to fuse with the oocyte can be significantly compromised. [13] Bacteriospermia prevalence in this study was highest amongst men aged between 30 - 39 years. This results echos with the findings of Nwofor *et al* [14], who observed the same for the males aged between 31 - 40 years. Bacteriospermia was prevalent in 51 (56.67%) patients, and 39 (43.33%) patients exhibited no bacterial growth even after 48 hours of incubation of the culture media plates, this can be attributed owing to manifestation of a different type of causative agent that requires special techniques for detection such as viruses, Chlamydia or Mycoplasma or it may be due to absence of any microorganism in the semen specimen. These results were corresponding to results observed by Nasrallah *et al* [15] and Al-Jebouri *et al* [16] where the bacteriospermia prevalence rate was 54.0% and 50.0% respectively. The findings of this study showed a higher prevalence of bacteriospermia as compared to other similar studies conducted in Germany by Zeyad *et al* [17] and India by Vilvanathan *et al* [18] which showed a prevalence of 30% and 35.30% respectively. However, studies conducted by Isaiah *et al* [19] and Nwofor *et al* [14] showed a higher bacteriospermia prevalence rate of 65.7% and 83.8% respectively as compared to the findings of this study.

Previous studies have shown that *S. aureus* can affect the semen parameters in various ways by causing a reduction in the sperm count, reduce sperm motility and affect the sperm morphology. [19,20] The prevalence of *S. aureus* in this study was 29.41%, similar rates of 30.0% and 28.3% were observed by Al-Jebouri *et al* [16] and Isaiah *et al* [19] respectively. Higher prevalence rates of 40.79% and 46.2% were observed by Nasiru *et al* [21] and Nasrallah *et al* [15] respectively. The prevalence of *Escherichia coli* in this study was 23.53%, similar as compared to a study done by Al-Jebouri *et al* [16] which observed a prevalence of 23.30%. However, lower prevalence rates of *Escherichia coli* were observed by Nasiru *et al* [21] and Vilvanathan *et al* [18] as 14.47%, and 10.0% respectively. Prevalence of Coagulase negative staphylococcus species in this study was 17.65%, similar to 18.42% observed by Nasiru *et al*. [21] However, Vilvanathan *et al* [18] observed a slightly higher prevalence of 23.33%. The prevalence of *Klebsiella pneumoniae* in this study was 11.76%. Similar studies conducted by Al-Jebouri *et al* [16] and Nasiru *et al* [21] detected a prevalence of 10.0%

and 7.89% respectively. Prevalence of *Proteus mirabilis* in this study was 7.84%. Slightly lower prevalence rates of 6.6% and 6.58% were detected by Al-Jebouri *et al* [16] and Nasiru *et al* [21] respectively. *Pseudomonas aeruginosa* prevalence in this study was 5.88%. Other similar studies conducted by Isaiah *et al* [19] and Nasiru *et al* [21] observed prevalence rates of 6.5% and 7.9% respectively. The prevalence of *Neisseria gonorrhoeae* in this study was 3.92%. However, Nasiru *et al* [21] detected a slightly lower prevalence of 2.63% and Al-Jebouri *et al* [16] detected a higher prevalence of 6.6%.

The antimicrobial susceptibility testing results showed that most of the bacteria were highly susceptible to ofloxacin, ciprofloxacin and cefotaxime, which correlates to similar findings observed by Nasiru *et al* [21] where ciprofloxacin and ofloxacin were the most sensitive antimicrobials. Moderate sensitivity was exhibited by amikacin, gentamicin and cefuroxime. This is in contrary to the findings observed by Vilvanathan *et al* [18] in a similar study that showed 100% sensitivity of amikacin to *E. coli*, *K, pnuemoniae*, Proteus spp. and Citrobacter spp. This can be attributed to the fact that bacteria undergo mutation which makes their susceptibility patterns to vary from one geographical region to another. [22] Some clinical strains of *S. aureus* isolated from the study, exhibited resistance to aminoglycosides such as amikacin and gentamycin, this can be attributable to acquisition of cytoplasmic aminoglycoside modifying enzymes encoded by mobile genetic elements. [23]

However, the antimicrobial activity of amoxicillin/clavulanic acid was not that appreciable. Amoxicillin/clavulanic acid resistance mechanisms might be favoured by increased consumption of amoxicillin/clavulanic acid in the community. Resistance to amoxicillin/clavulanic acid in bacteria is caused due to overproduction of β-lactamase enzyme, increased production of AmpC cephalosporinase enzyme and inhibitor-resistant penicillinases. [24] Nevertheless, amoxicillin/clavulanic acid exhibited reasonable antimicrobial action against majority of the bacterial isolates, but failed to exhibit any activity against *N. gonorrhoeae* and *P. aeruginosa*. The activity of co-trimoxazole against most of the isolates was found to be immensely poor. This was similar to a study conducted Nwofor *et al*, [14] where co-trimoxazole showed increasing resistance to most of the bacteria.

E. coli isolated from this study showed significant levels of resistance to gentamicin, amoxicillin/clavulanic acid, and co-trimoxazole. This mechanisms of resistance in gram-negative bacteria can be due to efflux pump; membrane permeability; β-lactamases production; modification of antibiotic target sites; and, acquisition of alternative metabolic pathways to those inhibited by the antibiotics. [25]

Antimicrobial sensitivity and resistance patterns vary from one community to another and from one hospital to another. This dissimilarity in the pattern of antimicrobial drug resistance could be as a result of the emergence of antimicrobial resistant strains and can also be attributed to indiscriminate use of the antimicrobial drugs. [26]

Conclusion

This study concludes that the prevalence of bacteriospermia amongst the patients who visited the Mombasa Assisted Reproduction Centre clinic for fertility evaluation was 56.67%. Bacteriospermia was most commonly observed in the age group of 30 - 39 years. *Staphylococcus aureus* was found to be the predominant causative agent of semen infection followed by *Escherichia coli*. Ciprofloxacin, cefotaxime and ofloxacin exhibited excellent antimicrobial action against most of the bacterial isolates. Whereas, amikacin, gentamycin and cefuroxime exhibited moderate susceptibility. Co-trimoxazole exhibited the poorest antimicrobial activity. Therefore, it is highly recommended that these drugs which exhibit the least antimicrobial activity should not be used as first line treatment drugs and should only be used after ascertaining the antimicrobial susceptibility testing.

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Competing Interests

The authors declare that there are no competing interests

Reference

1. Inhorn, M. C., & Patrizio, P. (2015). Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Human reproduction update, 21(4), 411–426.
2. Cariati, F., D'Argenio, V., & Tomaiuolo, R. (2019). The evolving role of genetic tests in reproductive medicine. Journal of translational medicine, 17(1), 267.
3. Ekhaise, F. O., & Richard, F. R. (2011). Common bacterial isolates associated with semen of men attending the fertility clinic of the university of Benin teaching hospital (U.B.T.H), Benin City, Nigeria. African Journal of Microbiology Research, 5, 3805–3809
4. Sengupta, P., Borges, E., Jr, Dutta, S., & Krajewska-Kulak, E. (2018). Decline in sperm count in European men during

the past 50 years. Human & experimental toxicology, 37(3), 247–255.

5. Menkveld, R., Wong, W. Y., Lombard, C. J., Wetzels, A. M., Thomas, C. M., Merkus, H. M., & Steegers-Theunissen, R. P. (2001). Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: an effort towards standardization of in-vivo thresholds. Human reproduction (Oxford, England), 16(6), 1165–1171.
6. Altmäe, S., Franasiak, J. M., & Mändar, R. (2019). The seminal microbiome in health and disease. Nature reviews. Urology, 16(12), 703–721.
7. Tomaiuolo, R., Veneruso, I., Cariati, F., & D'Argenio, V. (2020). Microbiota and Human Reproduction: The Case of Male Infertility. High-throughput, 9(2), 10.
8. Lundy, S. D., Vij, S. C., Rezk, A. H., Cohen, J. A., Bajic, P., & Ramasamy, R. (2020). The microbiome of the infertile male. Current opinion in urology, 30(3), 355–362.
9. Dinah Moraa, Dr. Scholastica Mathenge, Arodi Washington, Torome Tom, Oliver Mbuthia, Martin Kinyua (2019). Antibiotic Susceptibility Pattern among Male Patients with Urinary Tract Infection in Special Treatment Centre, Nairobi County, Kenya. Int. J. Adv. Multidiscip. Res. 6(2): 36-41.
10. Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H. & Whitman, W.B. (2009). Bergey's Manual of Systematic Bacteriology, 2nd ed., vol. 3, Springer-Verlag, New York, NY
11. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance Standards for Antimicrobial Susceptibility Testing. 28th Edition. CLSI supplement M100
12. Granato PA, Morton V, Morello JA. (2019) Laboratory Manual and Workbook in Microbiology applications to patient care 12th Edition, McGraw-Hill Education pages 82-84.
13. Fraczek, M., & Kurpisz, M. (2015). Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. Folia histochemica et cytobiologica, 53(3), 201–217.
14. Nwofor C. N, Ogbulie J. N, Obiajuru I. O. C and Elezua N (2016). Antibiotic susceptibility pattern of bacterial isolated from semen of men with fertility problem in Imo State, Nigeria. African Journal of Medicine and Medical Sciences. 4. 020 - 023.
15. Nasrallah, Y., Anani, M., Omar, H., Hashem, A. (2018). Microbiological profiles of semen culture in male infertility. Human Andrology, 8(2), 34-42.
16. Al-Jebouri, M. and Mdish, S. (2019) Tracing of Antibiotic-Resistant Bacteria Isolated from Semen of Iraqi Males with Primary Infertility. Open Journal of Urology, 9, 19-29.
17. Zeyad, A., Hamad, M. F., & Hammadeh, M. E. (2018). The effects of bacterial infection on human sperm nuclear protamine P1/P2 ratio and DNA integrity. Andrologia, 50(2)
18. Vilvanathan, S., Kandasamy, B., Jayachandran, A. L., Sathiyanarayanan, S., Tanjore Singaravelu, V., Krishnamurthy, V., & Elangovan, V. (2016). Bacteriospermia and Its Impact on Basic Semen Parameters among Infertile Men. Interdisciplinary perspectives on infectious diseases, 2016, 2614692.
19. Isaiah, I. N., Nche, B. T., Nwagu, I. G., & Nnanna, I. I. (2011). Current studies on bacterospermia the leading cause of male infertility: a protégé and potential threat towards mans extinction. North American journal of medical sciences, 3(12), 562–564.
20. Sanocka-Maciejewska, D., Ciupińska, M., & Kurpisz, M. (2005). Bacterial infection and semen quality. Journal of reproductive immunology, 67(1-2), 51–56.
21. Nasiru A S, Farouk S N, Sani U D, Muhammad A. (2018) Antibiotic Sensitivity Pattern of Bacteria Isolated from Semen of Male Patients with Infertility Attending Murtala Muhammad Specialist Hospital Kano, Nigeria. Advancements in Bioequivalence & bioiavailability. 1(4). ABB.000519.2018
22. Gupta, K., Hooton, T. M., & Stamm, W. E. (2001). Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Annals of internal medicine, 135(1), 41–50.
23. Jensen, S. O., & Lyon, B. R. (2009). Genetics of antimicrobial resistance in Staphylococcus aureus. Future microbiology, 4(5), 565–582.
24. Miró, E., Navarro, F., Mirelis, B., Sabaté, M., Rivera, A., Coll, P., & Prats, G. (2002). Prevalence of clinical isolates of Escherichia coli producing inhibitor-resistant beta-lactamases at a University Hospital in Barcelona, Spain, over a 3-year period. Antimicrobial agents and chemotherapy, 46(12), 3991–3994.
25. Matzov, D., Bashan, A., & Yonath, A. (2017). A Bright Future for Antibiotics? Annual review of biochemistry, 86, 567–583.
26. Khan S, Singh P, Siddiqui Z, Ansari M. Pregnancy-associated asymptomatic bacteriuria and drug resistance. Journal of Taibah University Medical Sciences. 2015 Sep 1;10(3):340-5.

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