Microbiological profiles of semen culture in male infertility

Original Article

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ABSTRACT

Background: Primary infertility and secondary infertility of men in the reproductive age represent 1.9 and 10.5%, respectively. Many etiological factors are involved, among which urogenital bacterial infections play an important role.

Materials and Methods: Semen analysis, bacteriological culture, and sensitivity analyses were carried out to investigate the effect of genitourinary infections on semen parameters of infertile men.

Results: Staphylococcus aureus was the most common isolated pathogen (46.2%) followed by urogenic gram-negative pathogens (24.1%). The isolated microorganisms are highly sensitive to piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin. These antibiotics could be used empirically while awaiting the results of semen culture.

Conclusion: Semen culture is an important diagnostic tool in all patients undergoing fertility investigations to detect genitourinary infections, pyospermia, and bacteriospermia. Moreover, early treatment should be considered according to the results of culture whenever possible. Wide range of broad-spectrum antibiotics can be used as an empirical treatment for infertile patients to adjust the seminal parameters and reduce the number of leukocytes in semen ejaculates.

Key Words: Antibiotic sensitivity, infertility, semen culture

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INTRODUCTION

Infertility is the inability of a couple to conceive after at least 1 year of unprotected sexual intercourse. Overall, 15% of couples worldwide are affected by infertility. Primary infertility and secondary infertility of men in the reproductive age (20-44 years old) represent 1.9 and 10.5%, respectively. The highest prevalence of infertility is noted in South Asia, Africa, the Middle East, Central/Eastern Europe, and Central Asia[1].

Congenital and hormonal disorders, lifestyle, environmental hazards, and psychological state are all factors contributing to the etiology of infertility[2]. Many factors contribute to male infertility such as immunological conditions, varicocele, endocrine disturbance, ejaculatory failure, and sexual dysfunction[3].

Urogenital infections with infectious agents such as bacteria, virus, fungi, and protozoa contribute to ~15% of male infertility factors[4].

Many studies have tried to link the role of pathogenic bacteria with infertility. Neisseria gonorrhea and Staphylococcus aureus affect certain organs like the testicles, epididymis, and sex hormone production. Ureaplasma urealyticum infections lead to decrease sperm numbers, sperm damage, and invariably impaired sperm motility[5].

Most of genitourinary tract infections are asymptomatic and associated with abnormal semen quality, and the selection of the most appropriate antibiotic is a difficult choice. Therefore, in this study, we determined the microorganism profiles and their antibiotic sensitivity pattern of semen culture among men with primary and secondary infertility.

MATERIALS AND METHODS

A total of 200 seminal fluid samples from men with primary and secondary infertility disorder were investigated. All patients were recruited from the outpatient Clinic of Suez-Canal University. The age of men ranged from 20 to 50 years old.

Semen analysis:

Semen samples were collected in sterile containers by masturbation after 3-5 days of sexual abstinence. No prior antibiotics were taken by patients before culture. Patients were asked to wash hands and external genitalia before ejaculation to avoid possible contamination, and urinate before semen collection in a sterile container. Physical,

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chemical, and microscopic characteristics of semen were recorded according to the methods and standards outlined by WHO guidelines. The semen volume was measured using a graduated disposable pipette and pH checked with the pH paper[6].

The pyospermia or leukocytospermia is a condition in which more than one million white blood cells per milliliter are present in the semen (WHO, 2010). The pus cell count was done for each specimen as follow: 10 µl of each liquefied semen was taken, the mixed seminal sample was mounted on a clean glass slide, covered with a standard cover slip, screened under the high power lens (×40) objective, counted in 10 fields and the average was calculated[7].

Semen culture:

Semen samples were cultured on blood agar. MacConkey agar plate were inoculated within 1 h of specimen collection and incubated aerobically at 37ºC for 24-48 h, whereas chocolate agar cultures were incubated at 5% CO_2 candle jar[8,9]. The bacterial concentration of greater than 10^3CFU/ml for certain pathogens and greater than 10^4 for occasional pathogens was considered as significant, and isolated colonies were identified by colony morphology, gram staining, and biochemical tests[10,11].

Antibiotic susceptibility:

Antibiotic susceptibility testing was performed according to the standard Kirby-Bauer disk diffusion method on Mueller Hinton agar and interpreted according to the Clinical and Laboratory Standards Institute guidelines. Amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline, gentamicin, imipenem, meropenem, ofloxacin, penicillin G, piperacillin/tazobactam, and trimethoprim/sulphamethoxazole were chosen for gram-positive bacteria. Other antibiotics were also tested such as follows: azithromycin, clindamycin, erythromycin, linezolid, teichoplanin, and vancomycin. The antimicrobial discs were purchased from Oxoid (Basingstoke, UK).

Microorganisms classification:

Microorganisms were classified in this study as follows: certain pathogens like some gram-negative bacteria (Enterobacteriaceae such as E. Coli, Proteus, Serratia, Klebsiella, and Pseudomonas spp.); occasional pathogens like gram-positive bacteria, such as Enterococcus spp. and S. aureus; sexually transmitted disease organisms such as N. gonorrhea; and possible pathogens as coagulase-negative Staphylococcus CoNS, such as Staphylococcus epidermidis and Staphylococcus haemolyticus[12].

Compliance with ethical standards:

All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. The authors declare no conflicts of interest. Ethical approval was provided by the Ethical Review Board, Faculty of Medicine, Suez-Canal University, and written informed patient consent was taken.

Data analysis:

Data were analyzed using the statistical package for the social sciences (SPSS; SPSS Inc., Chicago, Illinois, USA) software version16. Descriptive statistics, including frequency, mean, and SD, were done. Analytical statistics included $\chi 2$ to detect significant differences between two groups of qualitative variables, and Student's t-test was used to indicate the presence of any significant difference between the two groups of quantitative variables. Spearman's correlation analysis was used to show the strength and direction of the association between quantitative and qualitative variables. P value was considered a significant difference if *P* value less than *0.05*.

RESULTS

In this study, 200 infertile male patients participated, where 47% of the patients were 26-30 years old, 71% had primary infertility, and 29% had secondary infertility. Overall, 50% had infertility from 1 year to less than 2 years, and 48 patients were smokers. A total of 200 semen cultures were analyzed, and the positive specimens for bacterial growth after 48 h were 54% (108 of 200).

The relationship of semen parameters to bacterial growth shows an insignificant increase in the slow progression movement of sperms in the group with bacterial growth compared with the group without bacterial growth (Table 1).

The relationship of semen parameters and pus cells shows a statistically significant difference with the number of spermatogenic cells, where it increases in patients with pyospermia in comparison with the group with no pus cells. Other parameters show no significance between both the groups (Table 2).

In our study, the microorganism profiles in semen culture showed that group B organisms (59.3%) have been the most frequently presented than group A (24.1%), group C (9.3%), and group D (7.4%) (Table 3). The commonest isolated organisms were S. aureus (46.2%), followed by urogenic gram-negative pathogens (24.1%)

The types of isolated microorganisms had no effect on semen parameters such as sperm count, motility, pus cells, and slow progression (Table 4).

Antibiotic sensitivity pattern in semen cultures shows the microorganisms are highly sensitive to piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin (Table 5).

Group B microorganisms are highly sensitive to linezolid, vancomycin, and clindamycin with high significant difference, whereas group A organisms are sensitive to doxycycline and imipenem, with statistically significant difference (Table 6).

Table 1: Semen analysis of study patients and its relation to bacterial growth

	Without bacterial growth (N=92)	With bacterial growth (N=108)	*P value*
Volume			
<1.5 ml	6	14	0.285
≥1.5 ml	86	94	
Color	Creamy white	Creamy white	–
Viscosity	Normal	Normal	–
Reaction (pH)	Alkaline	Alkaline	–
Sperms count (million/ml)	38.40±33.95	33.08±35.16	0.447
Immediate living (%)	56.06±22.95	46.37±30.79	0.085
Rapid progression (%)	15.13±17.39	15.37±19.56	0.949
Slow progression (%)	16.60±12.67	22.18±11.61	0.057
Sluggish movement (%)	14.25±13.30	18.29±11.38	0.111
Immotile (%)	41.76±22.31	43.72±31.45	0.879
Vitality (%)	66.02±21.50	56.89±32.12	0.082
Abnormal forms (%)	53.29±20.41	45.80±25.93	0.119
Agglutination [n (%)]			
Nil	84 (42)	102 (51)	0.805
+1	6 (3)	4 (2)	
+2	2 (1)	2 (1)	
Pus cells/million [n (%)]			
Normal (<1 million/ml)	28 (14)	46 (23)	0.147
pyospermia (≥1 million/ml)	64 (32)	62 (31)	
Red cells	2.51±3.78	2.75±3.26	0.747
Spermatogenic cells	4.04±3.64	3.12±3.60	0.210

Table 2: Semen analysis of studied patients and its relation to pus cell count

	Normal (<1 million/ml) (N=74)	Pyospermia (≥1 million/ml) (N=126)	*P value*
Volume			
<1.5 ml	10	10	0.369
≥1.5 ml	64	116	
Sperms count (million/ml)	38.33±37.40	33.71±32.97	0.521
Immediate living	49.27±30.13	51.44±26.73	0.709
Rapid progression	16.00±20.35	14.84±17.56	0.765
Slow progression	17.81±13.17	19.79±12.09	0.446
Immotile	37.62±29.51	45.50±26.07	0.168
Vitality	60.48±32.05	61.66±26.33	0.842
Abnormal forms	43.37±26.14	52.46±21.92	0.066
Agglutination			
Nil	68	118	0.918
+1	4	6	
+2	2	2	
Spermatogenic cells	2.37±3.45	4.20±3.58	0.014*

**P≤0.05* is statistically significant

Table 3: Microorganisms profile in semen culture of studied group

Microorganism	n (%)
Group A	26 (24.1)
Group B	64 (59.3)
Group C	10 (9.3)
Group D	8 (7.4)

Group A: certain pathogen (Gram-negative bacteria: E. coli, Klebsiella, Pseudomonas, and Citrobacter). Group B: occasional pathogens (Staphylococcus aureus, Enterococcus, nonhemolytic streptococcus, and S. bovis). Group C: sexually transmitted disease (Neisseria gonorrhea). Group D: skin commensals 'possible pathogens' (Staphylococcus epidermidis and Alpha hemolytic streptococcus).

Table 4: The relation between semen parameters and microorganism groups

	Group A (N=26)	Group B (N=64)	Group C (N=10)	Group D (N=8)	r#	*P value*
Sperm count						
▓<15 millions/ml	16	21	3	3	0.263	0.106
▓>15 millions/ml	10	43	7	5		
Motility (%)						
▓<32	9	19	4	5	0.054	0.749
▓>32	17	45	6	3		
Pus cells						
▓<1 million/ml	15	26	6	2	0.203	0.291
▓>1 million/ml	11	38	4	6		
Slow progression						
▓Mean±SD	17.92±15.62	17.81±11.56	10.40±11.86	11.25±11.08	0.079	0.509

#Spearman's rank correlation

Table 5: Antibiotic sensitivity patterns in semen culture of the studied group

	Sensitive	Intermediate	Resistant
Amikacin	86	18	4
Amoxicillin–Clavulanic acid (Augmentin)	60	14	34
Azithromycin (Zisrocin)	44	2	26
Cefepime	62	8	38
Cefotaxime (Claforan)	66	12	30
Cefoxitin (Primafoxin)	68	0	40
Ceftriaxone (Rociphen)	68	6	34
Chloramphenicol	82	20	6
Ciprofloxacin	68	14	26
Clindamycin	46	0	26
Doxycycline	86	8	14
Erythromycin	46	4	22
Gentamicin	88	18	2
Imipenem (Tienam)	100	6	2
Linezolid	70	0	2
Meropenem	96	8	4
Ofloxacin (Tarivid)	64	22	22
Penicillin G	40	16	52
Piperacillin/Tazobactam	102	4	2
Teichoplanin	44	26	2
Trimethoprim/ Sulphamethoxazole	32	12	64
Vancomycin	64	6	2
Nitrofurantion	82	6	20

Table 6 :Antibiotic sensitivity patterns of isolated microorganisms

	Group A (N=26)	Group B (N=64)	Group C (N=10)	Group D (N=8)	*P value*
Amikacin	20	52	6	8	0.451
Amoxicillin–Clavulanic acid (Augmentin)	6	42	6	6	0.142
Azithromycin (Zisrocin)	0	36	0	8	<0.001*
Cefepime	14	40	6	2	0.590
Cefotaxime (Claforan)	10	46	8	2	0.125
Cefoxitin (Primafoxin)	18	44	4	2	0.233
Ceftriaxone (Rociphen)	10	48	6	4	0.322
Chloramphenicol	18	48	8	8	0.174
Ciprofloxacin	14	40	10	4	0.281
Clindamycin	0	42	0	4	<0.001*
Doxycycline	24	56	4	1	0.011
Erythromycin	0	38	0	8	<0.001*
Gentamicin	20	52	8	8	0.321
Imipenem (Tienam)	22	62	8	8	0.041*
Linezolid	0	62	0	8	<0.001*
Meropenem	20	58	10	8	0.759
Ofloxacin (Tarivid)	14	36	8	6	0.145
Penicillin G	6	24	4	6	0.010*
Piperacillin/ tazobactam	22	62	10	8	0.305
Teichoplanin	0	38	0	6	<0.001*
Trimethoprim/ sulphamethoxazole	12	14	0	6	0.064
Vancomycin	0	56	0	8	<0.001*
Nitrofurantion	14	54	8	6	0.410

**P≤0.05* is statistically significant

DISCUSSION

Urogenital tract infections are an important cause of infertility in couples. Bacteriospermia contributes to ~15-20% of male infertility causes[13]. Studies found that men with these infections have increased sperm agglutination, acrosome reaction impairment, and abnormal cell morphology[14].

Our study depicts that 47% of men were between the ages of 26 and 30 years old, where most were undergoing infertility evaluation. Approximately 63% of the semen samples were pyospermic, and it is observed among 59.4% of occasional pathogen group (S. aureus, Enterococcus, and nonhemolytic Streptococcus) but with no statistically significant difference.

Berger *et al.*[15] and Jarvi and Noss[16] found that pyospermia affects sperm penetration assays, sperm parameters, count, and motility. Kjaergaard *et al.*[17] related semen quality with pyospermia and concluded that fertility decreased in 43% of patients who were pyospermic.

Ricci *et al* [18] found a negative influence of leukocytes on sperm function and fertilization rates as leukocytes represent the main source of reactive oxygen species in both seminal plasma and sperm suspensions. The urogenital tract inflammatory process passes in different phases; the presence of bacteria and leukocytes in semen causes oxidative imbalance, and the accumulation of pus cells leads to the initiation of phagocytosis[18].

Activation of proinflammatory cytokines modulates the prooxidative and antioxidative system, thus promoting (reactive oxygen species) burst, leading to spermatozoon peroxidative damage. Remnants of the oxidative stress process might persist in semen for a longer time after removing the infectious agent, finally resulting in spermatozoa damage[19].

In our study, there was no statistical difference between the number of pus cells and any of the semen parameters except for the increased number of spermatogenic cells, which might be owing to a compensatory mechanism to the increased spermatozoa destruction; however, further studies are needed to prove this result.

The prevalence of bacteriospermia in this study was 54%. Similar high prevalence rates of 51.7, 52.5, 65.7, 68, 79, and 97% in other studies were described by Cottell *et al*., Alekwe *et al*., Isaiah *et al*., Elgozali *et al*., Damirayakhian *et al*., and McGowan *et al*., respectively [7,9,20-23].

In the current study, S. aureus is the commonest organism isolated (46.2%); this result is similar to the other studies conducted by Owolabi and colleagues who reported that S. aureus represented 72.9% and 44.4% of isolated organisms from infertile men, respectively[21,24].

In this study, the urogenic gram-negative pathogens were detected in 24.1% of semen samples. Ochsendorf [4] concluded that the urinary tract is the origin of organisms infecting the semen. Keck *et al.*[25] and Diemer *et al.*[26] reported that E. coli was found to be the most common organism infecting male genital tract with a negative influence on sperm quality and motility[25,26].

In a study conducted by Uneke and Ugwuoru[27], the Enterobacteriaceae members were the commonest bacteria isolated including E. coli (26.9%), Proteus spp. (25%), and Klebsiella spp. (11.5%). Moreover, S. aureus (15.4%) and Streptococcus spp. (11.5%) were identified from the semen of infertile men. Another study was carried by Sasikumar *et al.*[28] who noticed that the dominant isolated bacteria were E. coli (40%), S. aureus (28%), Pseudomonas aeruginosa (14%), and Proteus mirabilis (8%). Moreover, Bhatt *et al*[29] noticed that the commonest isolates were E. coli (41.9%) followed by S. aureus (17.7%), Streptococcus faecalis (11.2%), Klebsiella pneumoniae (9.6%), Staphylococcus saprophyticus (8%), and Pseudomonas aeruginosa (4.8%).

In our study, a wide number of infectious bacterial agents were detected in semen culture with the highest percentage of occasional pathogens at 59.3%, 24.1% of certain pathogens, and lower percentage of sexually transmitted diseases and skin commensals at 9.3 and 7.4%, respectively.

In this study, all the semen parameters were not significantly affected owing to bacteriospermia. Similarly, Domes *et al.*[10], Cottell *et al*.[20], and Sangeetha *et al.*[30] found that sperm concentration, morphology, and motility were not significantly affected in bacteriospermic samples.

Moreover, Sangeetha *et al.*[30] observed similar results, as they reported a lack of significant association between altered semen quality among different studied bacterial species. Conversely, Sasikumar *et al.*[28] confirmed that a higher percentage of nonmotile sperms and the morphologically abnormal sperms was found in infertility cases with bacteriospermia, whereas Sanocka-Maciejewska *et al*.[31] and Fraczek *et al.*[32] reported a negative influence of bacteria on sperm motility. Moretti *et al.*[33] in their study suggested that sperm motility was significantly reduced in most studied bacterial species (except for in those with Streptococcus agalactiae and Streptococcus anginosus), and they explained that the bacterial flagella and pili have a role in this pathogenicity.

Villegas *et al.*[34] hypothesized that the mechanisms of sperm damage caused by bacteria passes through the

expression of the adhesive properties of the flagella and pili to mannose receptors which are present on the surface of human spermatozoa[35].

In our study, from 108 isolates, 72 (66.6%) isolates are gram-positive bacteria and 36 (33.3%) isolates are gram-negative bacteria; both gram-positive and gram-negative bacteria were highly sensitive to piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin. The gram-positive bacteria (S. aureus, Streptococcus spp., and CoNS) are highly sensitive to linezolid, vancomycin, azithromycin, clindamycin, teichoplanin, erythromycin, and azithromycin.

A study carried by Uneke and Ugwuoru[27] demonstrated that half of the S. aureus and Streptococcus isolates were sensitive to erythromycin and amoxicillin–clavulonic acid, but they were completely resistant to penicillin and ampicillin. All Streptococcus strains were sensitive to clotrimoxazole and tetracycline, and half of S. aureus strains were sensitive to clotrimoxazole and tetracycline. Pseudomonas strains are susceptible to penicillin, chloramphenicol, and tetracycline. E. coli are highly susceptible to chloramphenicol, whereas Klebsiellla strains are susceptible to clotrimoxazole and penicillin[27].

Bhatt *et al.*[29] reported that both gram-positive and gram-negative organisms were sensitive to nitrofurantoin (91.5% and 71.7%, respectively) followed by ampicillin–sulbactam (73.9% and 58.9%, respectively), levofloxacin (56.5% and 71.7%, respectively), and gentamicin (56.5% and 53.8%, respectively). Specifically, E. coli and S. aureus isolates were more susceptible to nitrofurantoin (76.9% and 81.8%, respectively) followed by levofloxacin (69.2% and 63.6%, respectively) and gentamicin (61.5% and 54.5%, respectively).

A meta-analysis conducted by Skau and Folstad revealed a significant positive effect of antibiotic treatment on the sperm parameters (sperm volume, sperm concentration, sperm morphology, and sperm motility) and reduction of the number of leukocytes in ejaculates of leukocytospermic infertile men[36].

CONCLUSION

Semen culture is an important diagnostic tool in all patients undergoing fertility investigations to detect genitourinary infections, pyospermia, and bacteriospermia. Moreover, early treatment should be considered according to the results of culture whenever possible. Wide range of broad-spectrum antibiotics can be used as an empirical treatment for infertile patients like piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin. This could be beneficial to adjust the seminal parameters and reduce the number of leukocytes in semen ejaculates.

CONFLICT OF INTEREST

There are no conflicts of interest.

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