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Bacteriological Profile of Semen Specimens in Infertile Males

Authors
Riddhi Hathiwala[1], Archana Bhimrao Wankhade[2], Poornima Dhandale[3], Siddhi Hathiwala[4]
[1,3]Assistant Professor, Dept. of Microbiology, Chandulal Chandrakar Memorial Medical College, Durg, Chattisgarh
[2]Associate Professor, Dept. of Microbiology, Chandulal Chandrakar Memorial Medical College, Durg, Chattisgarh
[4]Assistant Professor, Dept. of Public Health Dentistry, New Horizon Dental College and Research Institute Bilaspur, Chattisgarh, India
Corresponding Author
Riddhi Hathiwala
Assistant Professor, Dept. of Microbiology, Chandulal Chandrakar Memorial Medical College, Durg, Chattisgarh, India
Email: *riddhihathiwala@gmail.com*, Cont. No.: 9833049504

Abstract
Introduction: *Infertility constitutes a grave emotional and social problem in India. Male urogenital tract infection is one of the most important causes of male infertility and has been associated with 8-35% of male infertility cases. Presence of bacteria in semen samples may compromise the sperm quality.*
Aim: *To study the microbiological profile of the semen specimens collected from adult married males visiting the infertility centres.*
Material and Methods: *In this study, a total of 115 semen samples were collected, after informed written consent, from married males who visited the infertility centres. Semen analysis was carried out according to WHO guidelines (2010). The specimens were processed using standard microbiological procedures for isolating and identifying the organism, followed by antibiotic susceptibility testing. The results were recorded and analysed.*
Results: *A total of 115 semen specimens were cultured, of which 48 (41.7%) showed significant bacterial growth. About 67% isolates were gram positive cocci and 33.3% isolates were gram negative. The commonest isolates were Enterococcus species (25%) and Escherichia coli (23%). Most of the Gram positive cocci were sensitive to Linezolid, Vancomycin, Teicoplanin and Nitrofurantoin. Most of the Gram negative bacilli were sensitive to Amikacin, Piperacillin-Tazobactum, Gentamicin, Nitrofurantoin and Imipenem. The sensitivity to Co-trimoxazole, Penicillin and Ciprofloxacin was quite low. Oligozoospermia was seen in 61 (53%) of the specimens, out of which 30 showed significant bacterial growth.*
Conclusion: *Testing for the bacteriological profile of semen of infertile males should be done routinely as bacteria may affect the quality of semen.*
Keywords: *Semen, Infertility, Bacterial infections, Antibiotic sensitivity, Oligozoospermia.*

Introduction

Infertility constitutes a grave emotional and social problem in societies where great importance is attached to having children. Infertility is defined as inability to achieve conception in a period of 1 year in a couple, despite regular and adequate unprotected sexual intercourse[1]. It is widely accepted that male factor alone accounts for

infertility in about 40% couples facing infertility; female factor alone in 40% of the couples; and in other 20%, there is a combined male and female factor. In India, the prevalence of primary infertility is estimated to be about 10-20% [2].

Semen is a mixture of spermatozoa and fluids derived from the epididymis and the bulbourethral, urethral and prostate glands [3]. The glands and organs that contribute to the semen are considered sterile. The sterility of the internal urethra is maintained by the normal flow of urine; however, the distal urethra is not considered a sterile area. Therefore, the culturing of semen samples usually yields growth of organisms, many of which are considered to be normal flora of the genitourinary tract[4]. Semen contamination arises from the urinary tract of patients or can be sexually transmitted from the partner. The presence of other pathogens in concentrations $\geq 10^3$ bacteria/ ml of ejaculate (bacteriospermia) is clinically regarded as a sign of an active infection[5, 6].

Male urogenital tract infection is one of the most important causes of male infertility worldwide. Genital tract infection and inflammation have been associated to 8-35% of male infertility cases[7-10]. Presence of bacteria in semen samples may compromise the sperm quality, by affecting sperm motility, morphology, or causing deterioration of spermatogenesis, obstruction of the seminal tract, autoimmune processes and dysfunction of accessory sex glands[6,8,10]. Moreover this may lead to transmission of the infection to the female partner as well as the possible illnesses of the offspring. Hence, there is a need to detect the microbial agents present in semen of males with doubtful fertility and their antibiotic susceptibility pattern to control the infection.

Aim

To study the microbiological profile of the semen specimens collected from adult married males visiting the infertility centres.

Material and Methods

This study was conducted in a tertiary care teaching institute, located in Central India during April, 2015 to March, 2016. The procedure and purpose of the study was explained and written informed consent was taken from all the participants. Semen sample of 115 adult married men attending the infertility clinics were collected by masturbation, after 3-5 days of abstinence period. The patients were advised not to consume any antibiotic for about a week before collection of semen sample. They were advised to wash their hands and genital area with soap and water and to urinate before collection of semen sample, to prevent contamination. Samples were collected into sterile universal containers.

All specimen collected were immediately transferred to Microbiology Laboratory after collection. Semen analysis was carried out according to WHO guidelines (2010) after liquefaction for 30 min at 37 °C, for assessing pH, volume, presence of pus/red blood cells, sperm motility, sperm concentration and abnormal morphology[5]. All the specimens were processed using standard microbiological procedures for isolating and identifying the organisms. They were cultured by the semi-quantitative culture technique using a standard calibrated loop on Blood, MacConkey and Chocolate agar. Samples were incubated in a microaerophilic (5% CO_2) and aerobic conditions at 37 °C overnight [11]. Culture for strict anaerobic organisms was not a part of the study and was not performed. Seminoculture was considered positive when the number of colonies was $\geq 10^3$ bacteria/ ml of ejaculate[5,6]. Mixed growths were considered as urethral contamination and hence, were not processed further. The identification of bacterial isolates was made using standard microbiological techniques as described in Bergey's Manual of Systemic Bacteriology which comprise of studying the colony characters, staining reactions and biochemical tests[12]. Antibiotic susceptibility testing was performed by Kirby Bauer disk diffusion method as per the CLSI (2015) recommendations [13]. The antibiotic

disks used for the susceptibility testing were procured from Hi-Media Laboratories Pvt. Limited. The results were recorded and analysed for descriptive details.

Results

A total of 115 semen specimens were included in this study. Out of them, 48 (41.7%) showed significant bacterial growth i.e. $\geq 10^3$ bacteria/ml of semen ejaculate. About 32 (67%) isolates were Gram positive cocci (GPC) and 16 (33.3%) isolates were Gram negative bacilli (GNB). The commonest isolates were Enterococcus species (25%), followed by Escherichia coli (23%), Coagulase Negative Staphylococcus species, Staphylococcus aureus, Proteus species and Pseudomonas aeruginosa. (Table 1)

Among the GPC isolated, all were sensitive to Linezolid, Vancomycin and Teicoplanin, and most of them were sensitive to Nitrofurantoin (87.5%). (Table 2) Among the GNB isolated, most were sensitive to Amikacin (87.5%) and Piperacillin-Tazobactum (87.5%), and lesser sensitivity was seen for Gentamicin, Nitrofurantoin and Imipenem (70%). (Table 3) The sensitivity to Co-trimoxazole (44%), Penicillin (40%) and Ciprofloxacin (36.2%) was quite low.

Of all the 115 semen specimens studied, 61 (53%) were oligozoospermic (20-60 million/ml), 42 (36.5%) were normozoospermic (>60 million/ml), and 12 (10.4%) patients had azoospermia (<20 million/ml). Of the 48 significant bacterial growth recovered, 30 (62.5%) were recovered from the oligozoospermic patients, 14 (21.16%) from the normozoospermic and 4 (8.3%) from azoospermic cases. (Table 4)

The most common age group among the participants was 25-30 years (42.6%), followed by 31-35 (37.5%). The maximum numbers of culture positive cases (48%) were found in patients 25-30 year age group. (Table 5)

Table 1 Distribution of organisms in Semen according to their species

Pathogens	Number (N = 115)	Percentages (%)
Gram Positive Cocci	**32**	**27.82**
Enterococcus species	12	10.43
Staphylococcus aureus	06	5.21
CoNS	13	11.3
Streptococcus species	01	0.87
Gram Negative Bacilli	**16**	**13.9**
Escherichia coli	11	9.56
Proteus species	03	2.6
Pseudomonas aeruginosa	02	1.73
Contaminants	**10**	**8.7**
No Growth	**57**	**49.56**

Table 2 Antibiotic Sensitivity pattern of Gram Positive organisms in Semen

Organisms	Staphylococcus aureus (N = 6)		Enterococcus species (N = 12)		CoNS (N = 13)		Streptococcus species (N = 1)		Gram Positive Cocci (N = 32)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Cefoxitin	33.3	66.6	-	-	30.76	69.23	100	00	35	65
Linezolid	100	00	100	00	100	00	100	00	100	00
Vancomycin	100	00	100	00	100	00	100	00	100	00
Teicoplanin	100	00	100	00	100	00	100	00	100	00
Penicillin	33.3	66.6	75	25	7.69	92.3	100	00	40.6	59.4
Nitrofurantoin	83.33	16.66	91.66	8.33	92.3	7.69	100	00	90.6	9.4
Ciprofloxacin	33.3	66.6	25	75	30.76	69.23	-	-	28.1	71.9
Co-trimoxazole	50	50	-	-	38.46	61.53	100	00	45	55
Gentamicin	50	50	66.66	33.33	61.53	38.46	-	-	61.2	38.8

* S = Sensitive, R = Resistant

Table 3 Antibiotic Sensitivity pattern of Gram Negative organisms in Semen

Organisms	Escherichia coli (N = 11)		Proteus species (N = 3)		Pseudomonas aeruginosa (N = 2)		Gram Negative Bacilli (N = 32)	
Antibiotics	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	90.9	9.09	100	00	50	50	87.5	12.5
Gentamicin	63.63	36.36	66.66	33.33	100	00	68.75	31.25
Imipenem	63.63	36.36	66.66	33.33	100	00	68.75	31.25
Piperacillin-Tazobactum	72.72	27.27	100	00	100	00	81.25	18.75
Nitrofurantoin	72.72	27.27	-	-	50	50	70	30
Ciprofloxacin	36.36	63.63	100	00	50	50	50	50
Co-trimoxazole	45.45	54.54	33.33	66.66	-	-	42.85	57.15

* S = Sensitive, R = Resistant

Table 4 Distribution of the Semen isolates according to sperm count

Organisms	Oligozoospermia	Normozoospermia	Azoospermia
Enterococcus species	8	3	1
Escherichia coli	6	4	1
Staphylococcus aureus	5	1	0
CoNS	7	5	1
Proteus species	1	1	1
Pseudomonas aeruginosa	2	0	0
Streptococcus species	1	0	0
Total	30	14	4

Table 5 Age-wise distribution of the study participants

Age groups (Years)	No. of participants (%) (N = 115)	Culture Positive (%) (N = 48)
25-30	42.6	47.9
31-35	37.4	29.16
36-40	8.69	8.33
41-45	7.82	10.4
> 46	3.47	4.16

Discussion

Infertility is a globally increasing problem with increasing use of Assisted Reproductive Techniques. Since male genital tract infections are often linked to poor sperm motility and function, proper bacteriological testing of the semen including antibiotic resistance should also be included in andrological diagnostic workup for infertility testing. By doing so, a significant number of patients can be treated, as these infections are potentially treatable with an appropriate antibiotic therapy. This in turn will help in preventing the transmission of the infection to the female partner as well as the possible illnesses of the offspring due to infection [14].

The source of microorganisms in semen specimen is doubtful as the glands and organs that contribute to the semen are considered sterile, but normal microflora exists in urethra and on genital skin [15, 16]. In this study, all attempts were made to minimise the contamination of the specimens from the skin and urethra by washing the hands and genital organ with soap and water and urinating before collection of semen sample. These measures might be a reason that culture was found to be sterile in 57 (49.5%) of specimens, and contaminant growth was found only in 10 (8.7%) of the specimens in this study.

In our study, the significant bacterial growth was seen in 41.6% of semen specimens. Literature shows a wide variation of isolation; Moretti, et al. reported 33.2% isolation, Enwuru, et al. reported 70.4% isolation, Bhatt, et al. reported 17.8% isolation, while Mogra, et al. found 42.9% significant bacterial growth [17-20].

In this study, about 32 (67%) isolates were GPC and 16 (33.3%) isolates were GNB. Moretti, et al. isolated 64% and 36%, respectively, which was similar to our study results. Different results were found in other studies, where Enwuru, et al. isolated 49.4% and 21%, respectively; Bhatt, et al. isolated 37% and 63% respectively and Isaiah, et al. isolated about 48% of GPC and 52% of GNB [17-19, 21].

E. coli (10%) and Enterococci (10.5%) were detected as the most common isolates from the semen specimen in this study. They are the common bacteria of the intestinal tract. Hillier, et al. detected both the types of bacteria in 13% of their patients. Moretti, et al. isolated in 20.3% and 32%, respectively; Mogra, et al. isolated 17.1% and 31.4%, respectively; Enwuru, et al. reported 10.5% of E. coli and 29.6% of Staphylococcus species; while in study done by Bhatt, et al. the commonest isolates were E. coli (41.9%) and S. aureus (17.7%) [17-20, 22].

Escherichia coli was the most frequently isolated microorganism in male patients with genital tract infections or semen contamination. The deleterious effect of E. coli on male fertility and specifically sperm quality is due to its effects on sperm motility [23] and the impairment of acrosome reaction [16]. A report by Moretti, et al. [17] suggested that bacterial flagella and pili (contact accessory structures) of E. coli could be an important determinant of pathogenicity. The possible mechanism of sperm damage, in addition to the adhesion of pili, consists of the production of toxins and metabolic products that induce apoptosis, resulting in a breakdown in the mitochondrial membrane potential [24].

In this study, about 32 (67%) isolates were GPC. Mehta, et al. reported that aerobic cocci are present in about 50% of semen samples of male partners in infertile couples. Enterococcus faecalis was isolated from 53% of patients. The influence of gram-positive uropathogenic bacteria on sperm morphology and function has not been completely investigated until now. Genito-urinary infections caused by E. faecalis are associated with compromised semen quality in terms of sperm concentration and morphology [25]. In an in vitro study, the researchers described a negative influence on membrane integrity of human sperm head, neck and mid-piece, probably mediated by hemolysin, a well-known virulence factor of enterococci [26].

In this study, most of the GPC were found to be sensitive to Linezolid, Vancomycin, Teicoplanin and Nitrofurantoin and most of the GNB were found to be sensitive to Amikacin, Piperacillin-Tazobactum, Gentamicin, Imipenem and Nitrofurantoin. The sensitivity to Co-trimoxazole, Penicillin and Ciprofloxacin was quite low (≈40%). Similar results were reported by other authors [19, 20, 27]. The decreasing sensitivity of the bacterial isolates against Co-trimoxazole, Penicillin, Ciprofloxacin and Gentamicin reflects upon the practice of too frequent, inadequate and indiscriminate use of these antibiotics for treatment [28].

The results of our study showed that 61 (53%) specimens were oligozoospermic, 42 (36.5%) normozoospermic, and 12 (10.4%) patients had azoospermia. This was similar to other studies done for semen analysis. Enwuru, et al. reported 52.5%, 33.3% and 14.2%, respectively. They had the opinion that the bacterial contamination of semen affects sperm quality in the form of oligozoospermia and hence cause infertility. They reported that semen contamination by GPC was associated with overall lower mean sperm concentration [18]. This was in accordance with the report of in vitro study conducted by Qiang, et al. [26]. The relatively increased count of oligozoospermic and azoospermia specimens (63.4%) seen in this study, can be attributed to the increased ratio of GPC as compared to GNB (67%: 33.3%).

In this study, the maximum number of the participants (42.6%) and culture positive specimens (48%) were from the 25-30 years age group. The study conducted by Bhatt, et al. reported maximum culture positivity in specimens from 31-40 years age group (46.7%), while 21-30

years age group had only 29% of culture positivity [19]. The differences could be due to demographic factors.

Many studies have examined the impact of genital tract infections and bacterial semen contamination in male fertility; however, the detrimental effect of bacteria on the sperm quality is still controversial [29, 30]. There are studies which suggest that presence of bacteria in semen samples may compromise the sperm quality, by affecting sperm motility, morphology, spermatogenesis, obstruction of the seminal tract, and autoimmune processes [6, 8, 10]. There are other studies which report that the bacteria isolated from the genitourinary tracts of men have no effect on semen quality in normozoospermic males; however, in infertile men, it is possible that bacteria further deteriorate the whole quality of the seminal plasma [17, 31]. Several investigations which assessed in vitro fertilization indicated that oocyte fertilization was reduced in the presence of pathogenic organisms in semen [32] and concluded that semen bacterial contamination reduces semen quality and interferes with fertilization. Enwuru, et al. had the opinion that bacterial contamination of semen affects sperm quality and hence causes infertility. They reported that Gram negative organisms' presence in semen may affect the quality in terms of motility while Gram positive bacteria resulted into overall lower mean sperm concentration [18].

Conclusion

This study done on semen specimens of males with complaint of infertile showed that about 42% had bacterial pathogens. Enterococcus species were found to be the commonest organism followed by E.coli. Nitrofurantoin was active against most of the organisms isolated and can be used as drug of choice for genito-urinary tract infections; but most of the organisms were resistant to Co-trimoxazole, Penicillin and Ciprofloxacin. Most of the semen specimens collected from 25-30 years age group were found to be culture positive. It is so, highly recommended that the semen of infertile males should be tested routinely for their bacteriological profile as bacteria may affect the quality of semen.

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