International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: 10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Bacterial pathogens in semen culture and their antibiotic susceptibility pattern *in vitro*

CP Bhatt[*1], S Mishra[2], AD Bhatt[2], and M Lakhey[1,2]

[1]*Department of Microbiology, Kathmandu Medical College Public Limited, Sinamangal Kathmandu Nepal*
[2]*Medicare National Hospital and Research Center, Kathamndu Nepal*

***Correspondence Info:**
Dr. Chandra Prakash Bhatt,
Associate Professor and HOD,
Department of Microbiology,
Kathmandu Medical College Public Limited, Kathmandu Nepal.
E-mail: drcpbhatt@yahoo.com, drcpbhatt@gmail.com

Abstract

Background: Bacterial infections have been recognized with infertility, bacteria are capable of agglutinating and immobilizing spermatozoa. The aim of present study was to determine bacterial pathogens in semen culture from infertile men and their antibiotic susceptibility pattern in vitro.

Method: Semen samples were collected for routine culture and antibiotic susceptibility test and were processed according to the standard microbiology techniques.

Results: A total of 347 semen specimens were cultured, of which 62 (17.8%) showed significant bacterial growth. Eight different species of bacterial organisms were isolated out of them 37% isolates were gram positive and 62.9% isolates were gram negative. The commonest isolates were *Escherichia coli* (41.9%) followed by *Staphylococcus aureus* (17.7%), *Streptococcus faecalis* (11.2%), *Klebsiella pneumoniae* (9.6%), *Staphylococcus saprophyticus* (8%), and *Pseudomonas aeruginosa* (4.8%). The maximum numbers of culture positive cases (46.7%) were found in patients 31-40 year age group. Both gram positive and gram negative organisms were sensitive to nitrofurantoin (91.5%) and (71.7%) followed by ampicillin-sulbactam (73.9%) and (58.9%), levofloxacin (56.5%) and (71.7%) and gentamycin (56.5%) and (53.8%) respectively. *E. coli* was found susceptible to nitrofurantoin (76.9%), followed by levofloxacin (69.2%), ampicillin sulbactam (57.6%) and gentamycin (61.5%) and co-trimoxazole (50%). *Staphylococcus aureus* was found 81.83% sensitive to nitrofurantoin followed by lavofloxacin (63.6%) and gentamycin (54.5%).

Conclusion: For empirical treatment nitrofurantion seems to be drug of choice followed by lavofloxacin and ampicillin sulbactam. The regular screening of bacterial pathogen in infertile man seems necessary because it affects infertility in several ways.

Keywords: Antibiotic, *Escherichia coli*, Infertility, Semen, *Staphylococcus aureus*

1. Introduction

The spermatozoa are developed in the testis and are present in enormous numbers in seminal fluid.[1] Semen is a mixture of spermatozoa and fluids derived from the epididymis and the bulbourethral, urethral and prostate glands.[2] Each of the areas that contribute to semen samples are considered sterile areas, yet the culturing of semen for bacteria is usually positive.[3] Although sterility of the internal urethra is maintained primarily by the normal flow of urine, the distal urethra is not considered a sterile area. Culturing of semen specimens frequently yields growth of organisms many of which are considered to be normal flora of the genito-urinary tract.[4] For successful fertilization, motility is the most obvious and most essential sperm function and has been repeatedly shown to be predictive of fertilization in vitro.[5] Several studies have shown that the motility characteristics of spermatozoa are of the utmost importance for the men's fertility.[6] Male genital infections are relevant cause in the etiology of infertility due to abnormalities in sperm quality.[7,8] Men may have infections of their reproductive tract, these may include infection of the prostate (prostatitis), of the epididymus (epididymitis), or of the testis (orchitis). The principal microorganism causing prostatitis and epididymitis is *E. coli*.[9] Several reports describe sperm agglutination and immobilization by *E. coli*.[10] Diemer *et al* (1996) reported that *E. coli* inhibits sperm motility by directly adhering to and agglutinating spermatozoa.[11] However, *S. aureus* was observed to adhere only to the tails of the spermatozoa and cause agglutination.[12] Nonspecific seminal tract infection, other than sexually transmitted diseases and tuberculosis, can play an important role in the etiology of male infertility.[13] The comparison of semen characteristics between infected and non-infected men show that motile

spermatozoa and viability are lower when the microorganisms are present in the semen.[14] It appears that bacteria have a direct effect on semen quality with negative consequences in fertility. The purpose of this study was to determine the bacterial pathogens in semen culture from infertile men and their antibiotic susceptibility pattern in vitro that helpful to formulating and monitoring the antibiotic policy and proper empiric therapy.

2. Materials and Methods

A prospective study was conducted in Medicare National Hospital and Research Centre Kathmandu during April 2011 to May 2012. The research objective and methods were explained to the patients and informed consent was taken from each patient before collection of specimen. In this study semen sample from infertile patients was collected for routine culture and antibiotic susceptibility test and processed according to the standard laboratory methods. Semen was collected after 2-3 days of sexual abstinence in aseptic condition in clean dry, sterile and leak proof container. The sample was taken to the laboratory for further analysis without any delay. The sample collected was evaluated in terms of its acceptability, proper labeling (full name, age, serial number of the patient, date and time of collection). The semen samples were cultured onto the MacConkey agar and blood agar plates by the semi-quantitative culture technique using a standard calibrated loop. Known volume (0.001 ml) of mixed un-centrifuged semen was inoculated on the surface of MacConkey agar (MA) and blood agar (BA). The plates were then aerobically incubated at 37^0C 24 hours. Any growth of bacteria ≥ 10,000 colony forming units (CFU/ml) on the 5% blood agar was considered to be significant.[4] The identification of bacterial isolates was done by standard microbiological techniques as described in Bergey's manual of systemic bacteriology which comprises of studying the colony characters, staining reactions and biochemical tests.[15] Antibiotic susceptibility test of different isolates were performed by Kirby-Bauer disk diffusion method according to CLSI recommendations.[16] The antibiotic discs used for the susceptibility test was from Hi-Media Laboratories Pvt. Limited such as gentamycin (10µg), co-trimoxazole (25µg), ofloxacin (5µg), cephalexin (30µg), levofloxacin (5µg), amoxicillin (10µg) ampicillin- sulbactam (10µg) and nitrofurantoin (300µg). Data was analyzed by EPI-Info and presented by tables and diagrams.

3. Results

Out of 347 semen samples, 62 (17.8%) samples showed significant bacterial growth (Fig- 1). Among the bacterial culture positive cases 39 isolates were from gram negative and 23 isolates were from gram positive bacterial organism (Fig- 2). Culture positive case was commonly found in age group 31- 40 (46.7%) followed by age group 21-30 (29%) and 41-50 (24%) (Fig- 3). In gram negative, *E. coli* was the predominant isolate followed by *K. pneumoniae*, and *P. aeruginosa*. In gram positive, *S. aureus* is predominant organism followed by *S. faecalis* and *S. saprophyticus* (Table- 1). Among gram positive organism, *S. aureus* was found 81.83% sensitive to nitrofurantoin followed by lavofloxacin (63.6%) and gentamycin (54.5%). In case of *S. sapro*phyticus nitrofurantoin was the drug of choice showed (100%) sensitive followed by ampicillin- sulbactam (80%). S. *faecalis* was 100% sensitive to nitrofurantoin followed by lavofloxacin, ampicillin- sulbactam and gentamycin (71.4%) (Table- 2). Among over all gram positive bacterial isolates, nitrofurantoin was found to be (91.5%) sensitive, followed by ampicillin-sulbactam (73.9%), levofloxacin and gentamycin (56.5%) (Table- 3). Among gram negative bacterial isolates, *E. coli* was found highly susceptible to nitrofurantoin (76.9%), followed by levofloxacin (69.2%), ampicillin sulbactam (57.6%) and gentamycin (61.5%) and co-trimoxazole (50%). *K. pneumoniae* was equally sensitive to levofloxacin, ampicillin sulbactam and ofloxacin (83.3%). *P. aeruginosa* showed (33.3%) sensitive to nitrofurantoin, levofloxacin, ofloxacin and gentamycin (Table- 4). In over all gram negative bacterial isolates, nitrofurantoin and levofloxacin was found to be (71.7%) susceptible followed by ampicillin sulbactam (58.9%), gentamycin (53.8%) and ofloxacin (51%) (Table- 5).

Table 1: Species wise distribution of semen pathogens

Pathogens	Number	Percentage
Gram negative	39	62.9%
Escherichia coli	26	41.9%
Klebsiella pneumoniae	6	9.6%
Pseudomonas aeruginosa	3	4.8%
Proteus mirabilis	2	3.2%
Proteus vulgaris	2	3.2%
Gram positive	23	37%
Staphylococcus aureus	11	17.7%
Streptococcus faecalis	7	11.2%
Staphylococcus saprophyticus	5	8%
Total	62	100

Table 2: Antibiotic sensitivity pattern of gram positive bacterial organisms

Organism	*S. aureus* (N=11)			*S. saprophyticus* (N=5)			*S. faecalis* (N=7)		
Antibiotic	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Gentamycin	54.5	18	27	40	0	60	71.4	0	28.5
Co-trimoxazole	36	18	45.4	40	0	60	14	0	85.7
Ofloxacin	45.4	9	45.4	20	40	40	57	14	28.5
Cephalexin	36	0	63.6	0	40	60	0	0	100
Levofloxacin	63.6	9	27	20	20	60	71.4	14	14
Amoxicilin	36	0	63.6	0	20	80	42.8	14	42.8
Ampicillin- sulbactam	27.7	0	27	80	0	20	71.4	14	14
Nitrofurantoin	81.8	0	18	100	0	0	100	0	0

S = Sensitive, I = Intermediate, R = Resistant

Table 3: Antibiotic sensitivity pattern of overall gram positive bacterial organisms

Gram positive bacterial organisms						
Antibiotic	**Sensitive**	**Percentage**	**Intermediate**	**Percentage**	**Resistant**	**Percentage**
Gentamycin	13	56.5	2	8.6	8	34.7
Co-trimoxazole	7	30.4	2	8.6	14	60.8
Ofloxacin	10	43.4	4	17.3	9	39
Cephalexin	5	21.7	2	8.6	16	69.5
Levofloxacin	13	56.5	3	13	7	30.4
Amoxicilin	7	30.4	2	8.6	14	60.8
Ampicillin- sulbactam	17	73.9	1	4.3	5	21.5
Nitrofurantoin	21	91.3	0	0	2	8.6

Table 4: Antibiotic sensitivity pattern of gram negative bacterial organisms

	E. coli (N=26)			*K. pneumoniae* (N=6)			*P. aeruginosa* (N=3)			*P. vulgaris* (N=2)			*P. mirabilis* (N=2)		
Antibiotic	S%	I%	R%	S %	I%	R%	S %	I%	R %	S%	I%	R%	S %	I%	R%
Gentamycin	61.5	3.8	34.6	33.3	0	66.6	33.3	0	66.6	50	0	50	50	0	50
Co-trimoxazole	50	7.6	42.3	33.3	0	66.6	0	33.3	66.6	50	0	50	50	0	50
Ofloxacin	46	3.8	50	83.3	0	16.6	33.3	0	66.6	50	0	50	50	50	0
Cephalexin	30.7	3.8	65.3	0	0	100	0	0	100	0	0	100	0	0	100
Levofloxacin	69.2	15.3	15.3	83.3	0	16.6	33.3	0	66.6	100	0	0	100	0	0
Amoxicilin	38.4	3.8	61.5	0	16.6	83.3	0	0	100	0	50	50	50	0	50
Ampicillin sulbactam	57.6	0	42.3	83.3	0	16.6	0	0	100	50	50	0	100	0	0
Nitrofurantoin	76.9	3.8	19.2	66.6	0	33.3	33.3	0	66.6	50	0	50	100	0	0

S = Sensitive, I = Intermediate, R = Resistant

Table 5: Antibiotic sensitivity pattern of overall gram negative bacterial organisms

Gram negative bacterial organisms						
Antibiotic	Sensitive	%	Intermediate	%	Resistant	%
Gentamycin	21	53.8	1	2.5	17	43.5
Co-trimoxazole	17	43.5	3	7.6	19	48.7
Ofloxacin	20	51	2	5	17	43.5
Cephalexin	8	20.5	1	2.5	30	76.9
Levofloxacin	28	71.7	4	10	7	17.9
Amoxicilin	11	28	3	7.6	26	66.6
Ampicillin sulbactam	23	58.9	1	2.5	15	38.4
Nitrofurantoin	28	71.7	1	2.5	10	25.6

Fig 1: Growth profile of semen sample

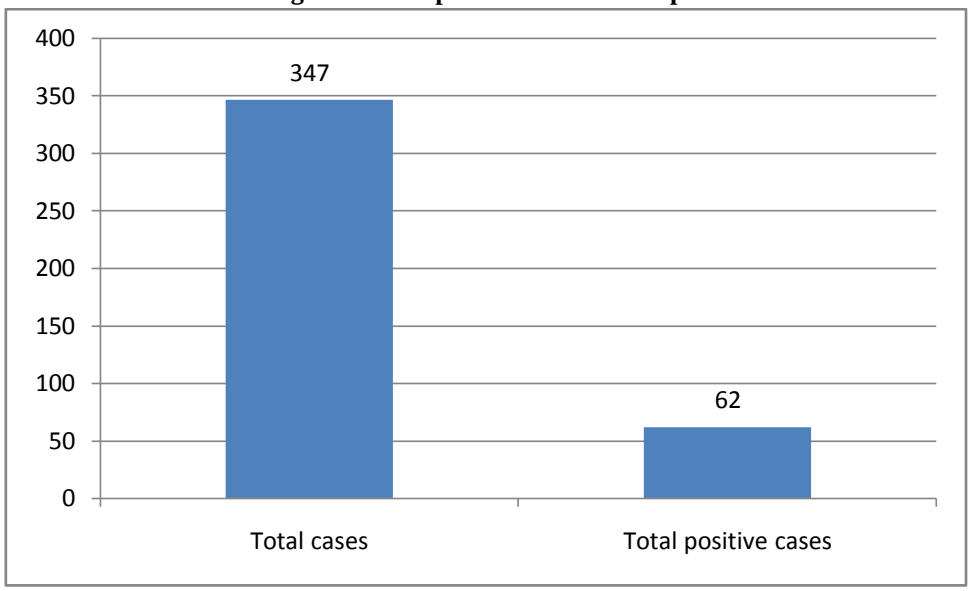


Fig 2: Distribution of gram positive and gram negative bacterial organisms

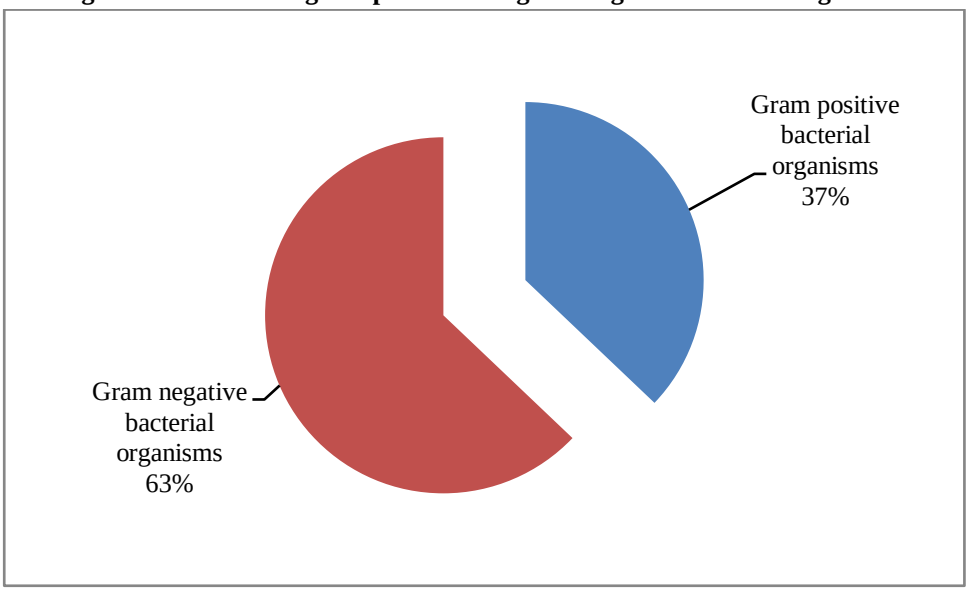
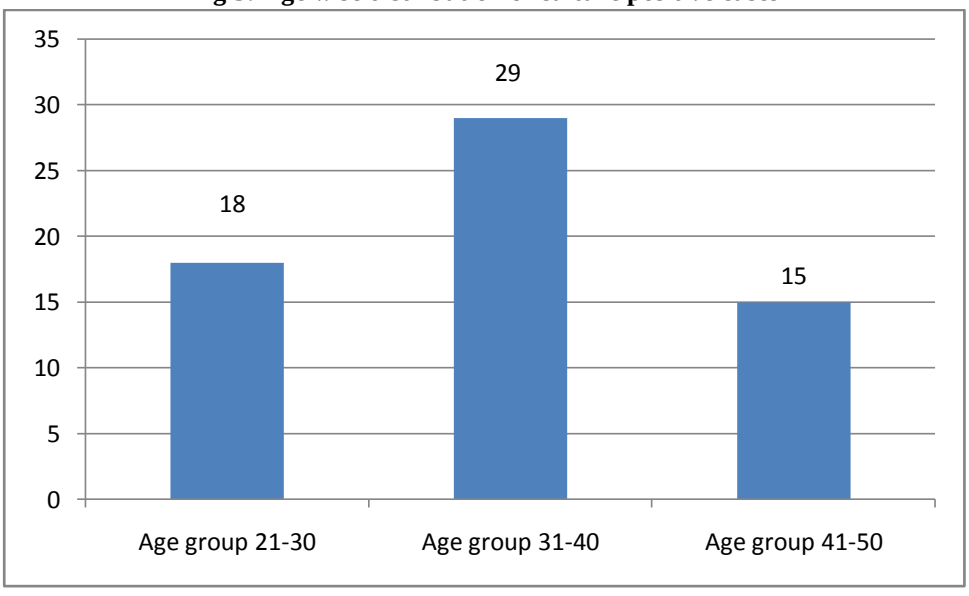


Fig 3: Age wise distribution of culture positive cases



4. Discussion

Bacterial infection of the reproductive tract organs can impair the production of sperm or cause scarring and blockage of the tubules that transport sperm, causing infertility. Infection of the testis can shut down the production of sperm by blocking the tiny testicular tubules in which sperm are produced called seminiferous tubules. Freshly produced sperm are temporarily stored in the epididymis, an organ alongside the testis consisting of coiled sperm ducts in which sperm undergo final maturation as they slowly move through the sperm ducts. Infections in the epididymis may interfere with the proper maturation of sperm and can block sperm transport. Because the prostate produces a large portion of the fluid in the ejaculate, infection in the prostate may block the release of fluid from the prostate, reducing the volume of ejaculate.[17]

In this study 17.8% of semen culture was found significant bacterial growth. The commonest isolates were *E. coli (*41.9%) followed by *S. aureus* (17.7%), *S. faecalis* (11.2%) *K. pneumoniae* (9.6%), *S. saprophyticus* (8%), *P. aeruginosa* (4.8%), *P. mirabilis* (3.2%), and *P. vulgaris* (3.2%). *S*imilar studies conducted by other researchers found higher bacterial growth rate than this study. Mogra *et a1,* found that 42.9% significant bacterial growth, the type of organisms isolated were *S. faecalis* (31.4%), *E. coli* (17.1%), and coagulase negative Staphylococci (14.3%).[18] Orji *et al,* reported *S. aureus* was the highest prevalent bacteria isolated (37.1%).[19] Rehewy *et al,* obtained 73% positive bacterial cultures. The most common aerobic organisms grown were Corynebacterium, *S. aureus*, *S. epidermidis*, *E. coli*, *P. mirabilis*, *K. pneumoniae* and Mycoplasma.[20] Ekhaise *et al,* found that *S. aureus* 7 (77.8%) was the most predominant isolate.[21] However, it has been stated that the presence of bacteria in semen may affect fertility in several ways including damage of spermatozoa, hampering their motility, altering the chemical composition of the seminal fluid.[6] Seminal infection could also be the cause of the chronicity of urinary tract infection by acting as the reservoir of infection.[18] In many cases, opportunistic microorganisms cause such classical infections of the urogenital tract as epididymits and prostatitis as well as subclinical reproductive tract infections. Some possible pathophysiological mechanisms that lead to the development of infertility are linked to the presence of microorganisms in the ejaculation.[20] Microbial infections of the semen are major causes of male infertility.[22] The viability and structural integrity of the semen lies on its characteristic feature of mobility.[23] The negative influence of these microorganisms towards sperm reproductive potential has been revealed in cases of infection with *E. coli* and *S. aureus* amongst other microorganisms.[24] Presence of *E. coli* in semen decreases sperm motility.[25] Bacteria affect sperm motility by adherence, agglutination and dialyzable factors. It is known that there is a significant negative effect of *E. coli, Streptococci* and coagulase negative *Staphylococci* towards sperm motility, morphology and viability.[26]

In this study *E. coli* was found susceptible to nitrofurantoin (76.9%), followed by levofloxacin (69.2%), ampicillin sulbactam (57.6%) gentamycin (61.5%) and co-trimoxazole (50%). *S. aureus* was found 81.83% sensitive to nitrofurantoin followed by lavofloxacin (63.6%) and gentamycin (54.5%). Mogra *et al,* reported maximum number of strains of *Staphylococcus albus*, *Streptococcus faecalis* and *E. coli* were sensitive to ampicillin, followed by trimethoprim-sulphamethoxazole (co-trimoxazole), nitrofurantoin, erythromycin and chloramphenicol.[18] The development of bacterial resistance against ciprofloxacin, ofloxacin, cephalexin and gentamicin perhaps reflects upon the practice of too frequent and indiscriminate use of these antibiotics. Inadequate anti-microbial treatment is an important factor in the emergence of antibiotic resistant bacteria.[27]

The finding of this study showed that 17.8 % semen culture was found to be bacterial pathogens. *E. coli* was found commonest organism followed by *S. aureus.* Culture positive case was commonly found in 31-40 year age group. For empirical treatment nitrofurantion seems to be drug of choice followed by lavofloxacin and ampicillin sulbactam. The regular screening of bacterial pathogen in infertile man seems necessary because it affects infertility in several ways.

References

[1] Gray's anatomy, descriptive and applied. Thirty second edition. Edited by Johnston TB, Davies DV, Davies F. Longmans, Green and Co London, New York Torento 1958.

[2] Mawhinney M. Male accessory sex organs and androgen action in Lipshultz L and Howards S (eds), Infertility in the Male Churchill Livingstone, New York, 1983 pp 135-165.

[3] Hilher S, Rabe L, Muller C. Relationship of bacteriologic characteristics to semen indices men attending an infertility clinic. *Obstet Gynecol* 1990; 75: 800-04.

[4] Shalika S, Smith KDRD, Padilla SL. The effect of positive semen bacterial and ureaplasma cultures on in vitro fertilization successes. *Human reproduction* 1996; 12: 2789-2792.

[5] Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB *et al*. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 1998: 352: 1172-1177.

[6] Karpuz V, Gokturk A, Meral Koyuturk M. The effects of sperm morphology and motility on the outcome of intracytoplasmic sperm injection. *Marmara Med J* 2007; 20: 92-97.

[7] Golshani M, Taheri S, Eslami G, Suleimani Rehber AA, Fallah F, Goudarzi H. Genital tract infection in asymptomatic infertile men and its effect on semen quality. *Iranian J Publ Health* 2006; 35: 81-84.

[8] Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G Armanini D. Genital tract infections and

infertility. *Eur J Obstet Gynecol Reprod Biol:* 2008; 140(1): 3-11.

[9] Giamarellou H, Tympanidis K, Bitos NA, Leonidas E, Diakos GK. Infertility and chronic prostatitis. *Andrologia 1984;* 16: 417-422.

[10] Khalili MB, Sharifi-Yazdi MK. The effect of bacterial infection on the quality of human's spermatozoa. *Iranian J Publ Health* 2001; 30: 119-122.

[11] Diemer T, Weidner W, Michelmann HW, Schiefer HG, Rovan E, Mayer F. Influence of *Escherichia coli* on motility parameters of human spermatozoa *in vitro*. *Int J Androl* 1996; 19: 271-277.

[12] Ohri M, Prabha V. Isolation of sperm– agglutinating factor from *Staphylococcus aureus* isolated from a woman with unexplained infertility. *Fertil Steril* 2005; 84: 1539-41.

[13] Homonnai Z, Sasson S, Paz G. Improvement of fertility and semen quality in men treated with a combination of anticongestive and antibiotic drugs. *Int J Fertil* 1975; 20: 45-49.

[14] Menkveld R, Wong WY, Lombard CJ, Wetzels AMM, Thomas CMJ, Merkus HMWM *et al*. Semen parameters, including WHO and strict criteria morphology, in a fertile and sub-fertile population: An effort towards standardization of *in-vivo* thresholds. *Hum Reprod* 2001; 16: 1165-71.

[15] Geoge MD, David RB, Richard WC. Bergey's manual of systemic bacteriology 2nd ed. Springer, NY; 2001.

[16] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-third information (supply) (M100-S23). Wayne PA: Clinical and Laboratory Standards Institute: 2013.

[17] Wegner C. Male infertility and bacterial infections. 2013 [www.livestrong.com.]

[18] Mogra N, Dhruva A, Kothari LK. Non-specific seminal infection and male infertility: A bacteriological study. *J Post Grad Med* 1981; 27(2): 99-104.

[19] Orji I, Georg E, Sabinu AE, Florence O. Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality: A study on 100 infertile men. *Pak J Med Sci* 2007; 23 (6): 885-88.

[20] Rehewy MSE, Hafez ESE, Thomas A, Brown WJ. Aerobic and anaerobic bacterial flora in semen from fertile and infertile groups of men. *Arch. Andrology* 1979; 2: 236-38.

[21] Ekhaise FO, Richard FR. 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. *Africa J Microbi Resear* 2011; 5(22): 3805-09.

[22] Gomez CI, Stenback WA, James AN, Criswell BS, Williams RP. Attachment of *Neisseria gonorrhoea* to human sperm. Microscopical study of the effect of trypsin and iron. *Brit J Ven Dis* 1979; 55: 245 – 55.

[23] Stephen PB, Rusell OD, David FK. Current problems in obstetrics, gyneacology and fertility. Yearbook Medical Publishers, Incorporated, 1989; p. 199.

[24] Sanocka – Macleiewska D, Ciupinska M, Kurpis ZM. Bacterial infection and semen quality. *J Reprod Immunol* 2005; 67(1-2): 51-56.

[25] Auroux MR, Jacques L, Mathieu D, Auer J. Is the sperm bacterial ratio a determining factor in impairment of sperm motility: an *In-vitro* study in man with *Esherichia coli*. *Internat J Androl* 1991; 14(4): 264-70.

[26] Esfandiari N, Saleh RA, Abdoos M, Rouzrokh A, Nazemian Z. Positive bacterial culture of semen from infertile men with asymptomatic leukocytospermia. *Int J Fertil Women's Med* 2002; 47: 265-70.

[27] Marin HK. Inadequate anti-microbial treatment and an important determinant of outcome for hospitalized patients. *Clin Infect Dis* 2000; 31 (supply): 5131-8.