Causative Organisms of Pyospermia in Infertile Male Patients

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ABSTRACT
Background: Male urogenital tract infection is one of the most important causes of male infertility worldwide. Infection processes may lead to impairment of sperm quality, and obstruction of the seminal tract. On the light of this, there is a need to institute a microbiological intervention to detect the probable causative microbial agents.
Objective: The aim of the work was to detect the common bacteria causing pyospermia in a cross-section of infertile men and the sensitive antimicrobials against these bacteria.
Patients and methods: This study included 205 infertile men who were recruited from the outpatient clinic, Andrology Unit, Dermatology and Andrology & STDs Department, Mansoura University Hospital for management of infertility. Patients with grade II or grade III varicocele, more than 60-year, smoker, drug abuser and those who were treated with antibiotics during last 3 months were excluded from the study.
Results: Over the period of the study, out of 205 infertile male patients with documented pyospermia, 95.6 % of semen samples revealed bacteriologic growth. It was obvious that gram positive bacteria (75.1%) were common than the gram-negative bacteria (20.5%). Six bacterial species (Staphylococcus aureus, Streptococci, Enterococci, E. coli, Klebsiella and Pseudomonas) were isolated from semen samples. The most common causative organisms were Staph. Aureus (49.3%) followed by Streptococci (22.4%) then E. Coli (8.3%), Klebsiella (8.3%) then Pseudomonas (3.9%) and finally Enterococci (3.4%).
Conclusion: It could be concluded that semen analysis with peroxidase stain and semen culture are an important diagnostic tool in all patients undergoing fertility investigations to detect genitourinary infections and pyospermia.
Keywords: Causative organisms of pyospermia, Infertile male, Sensitive antimicrobials

INTRODUCTION
Infertility means the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse; it affects approximately 15% of couples [1]. The male factor is the main cause of infertility in 20% of cases and contributes in about 50% [2]. There are many etiologies for male factor infertility; Infectious processes contribute to about 15% of such cases [3].

Pyospermia means the presence of more than one million leukocytes in 1 mL of semen [1]. It has been proposed as an indicator for genital tract infection and/or inflammation [4]. Pyospermia negatively impacts spermatogenesis or sperm maturation and has been linked to a worsening of many qualitative and quantitative sperm parameters. The white blood cells are produced by the body's immune system to fight off invading organisms that cause infection, but when leukocyte count is elevated in semen, male fertility can be compromised due to increase in oxidative stress and decrease in sperm quality [5].

Infertile men have significantly increased ROS levels with a reduction in antioxidant capacity compared with fertile controls [6]. It is postulated that ROS generated by leukocytes are responsible for negatively affecting sperm function [7]. Excessive ROS can induce lipid peroxidation, disrupt DNA, RNA as well as protein functions in the spermatozoa and other testicular cells. Oxidative stress can also decrease success rates of assisted reproduction procedures [8].

There is association between pyospermia and sperm DNA fragmentation in infertile men. Moderately increased leukocytes are also associated with increased levels of cytokines IL-6 and IL-8 in semen [9].

Male genital tract infections are difficult to detect as they are asymptomatic in many cases [10]. A number of patients seeking treatment for impaired fertility are increasing so the diagnosis of "silent" genital tract infections should receive attention as the infection may be linked to asthenozoospermia [11].

Infections are potentially treatable causes of male infertility, but the resistance to common antibiotics and the poor compliance may impede the efficacy of antibiotics in resolving complicated GTI or restoring fertility. In a study on 140 patients with pyospermia, 92 of them (65.7%) yielded bacterial growth with Staphylococcus aureus, Staphylococcus saprophyticus and Escherichia coli with the highest incidence rate by (28.3%), (19.6%) and (13.0%) respectively, then there were Proteus mirabilis, Klebsiella pneumonia and Proteus vulgaris with (10.8% for each). Pseudomonas aeruginosa was (5%) [12].

The aim of the present study was to detect the common bacteria causing pyospermia in a cross-section of infertile men and the sensitive antimicrobials against these bacteria.

PATIENTS AND METHODS

This study included a total of 205 infertile men, attending at Outpatient Clinic, Andrology unit, Dermatology and Andrology & STDs Department, Mansoura University Hospitals for management of infertility. This study was conducted over a period of 12 months between June 2019 to June 2020.

Inclusion criteria: Infertile men with pyospermia (more than one million PMNL in 1 mL of semen) confirmed by peroxidase stain [1], and age group 21-45 years old.

Exclusion criteria: Inability to conceive less than 1 year, grade II and grade III varicocele, cigarette smoking and drug abuser, and patients had taken antimicrobial within last three months.

All patients were subjected to:
Full history taking with stress on the following: Age. Fertility history: duration of infertility, and previous investigations and/or treatment. History of diseases with possible adverse effect on fertility. History of other factors with possible adverse effect on fertility, and sexual history.
General physical examination with stress on the following: Signs of hypogonadism, and gynecomastia and galactorrhea.

Genital examination:
- **Penis:** scars, hypospadias and others.
- **Testis:** site, volume, and consistency.
- **Epididymis:** head, body, and tail, thickened, tender or cystic.
- **Vas deferens:** palpable or non-palpable and if palpable weather beaded or not.
- **Scrotal swelling.**
- **Varicocele:** Examination of varicocele was done in both erect and supine positions, during both quiet respiration and valsalva maneuver to detect abnormal visible or palpable veins within the spermatic cord and around the testis with comparing both sides.

Varicocele was graded according to the clinical grading adopted by **Hargreave** [13] as in **table (1).**

Table (1): Clinical grading of varicocele [13].

Grade	Clinical Criteria
Sub clinical	Veins not palpable or visible, with or without Valsalva but can be demonstrated by special means as Doppler examination.
Grade I	Palpable veins only during Valsalva maneuver on testicular examination.
Grade II	Palpable veins at rest but not visible during testicular examination.
Grade III	Palpable and visible veins at rest during testicular examination

Investigations:
Semen collection and processing:
Two hundred and five semen samples were collected from the infertile patients after 3-5 days of sexual abstinence. The patients were advised to urinate then wash their glans penis with regular water and soap then dry it with clean towel. The samples were obtained by masturbation and were ejaculated into sterile containers in a private room near the laboratory. The patients were carefully instructed to avoid contamination of inner containers by fingers or the penis. The semen samples were transferred directly to the laboratory with proper labeling (full name, age, serial number of the patient, date and time of collection).

Computer assisted semen analysis:
Semen samples were examined as soon as they were liquefied. ejaculate volume, pH, concentration, morphology, motility and pyospermia were evaluated according to **WHO** [1] guidelines. Pyospermia is a condition in which more than one million white blood cells per milliliter are present in the semen [1]. 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. Peroxidase stain was done to differentiate pus cells from round cells to confirm pyospermia.

Peroxidase test:
Leukocyte concentrations in semen were quantified by a myeloperoxidase staining test. A 20 ul volume of liquefied semen specimen was placed in a Corning 2.0 mL cryogenic vial with 20 ul of phosphate buffered saline (PBS; pH 7.0) and 40 ul of benzidine solution. The solutions were mixed and allowed to sit at room temperature for 5 minutes. Peroxidase positive leukocytes staining brown were counted by a microcell counting chamber (Conception Technologies. San Diego, CA) under the bright-field objective (magnification, x20). The average of 5-10 fields was calculated. The results after correction for dilution were recorded as 1000000 peroxidase-positive leukocytes per milliliter of semen [14].

Culturing of semen samples:
Confirmed semen sample with pyospermia was inoculated on three types of agar medium plates: nutrient agar, the MacConkey agar and blood agar within 1 hour of semen collocation and incubated aerobically at 37°C for at least 48 hours. Any growth of bacteria ≥ 10,000 colony forming units (CFU/ml) was considered to be significant. The identification of bacterial isolates was done by standard microbiological techniques as described in Bergey's manual of systematic bacteriology which comprises of

studying the colony characters, staining reactions and biochemical tests [15].

Identification of isolated bacterial colonies:
1) **Colony character:** Staph. aureus usually formed gray to deep golden yellow colonies with a smooth, shiny surface on nutrient agar media. Streptococci grow in blood agar media and make hemolysis (alpha, beta, and gamma hemolysis). Enterococci produced compact tiny red colonies either on or beneath the surface of the MacConkey Agar media. E. Coli produced dark pink, dry, donut shaped colonies on the MacConkey Agar media. Proteus produced successive waves to form a thin filmy layer of concentric circles (swarming) colonies in blood agar media. Klebsiella produced large shiny and dark pink colonies. These colonies were mucoid in shape in blood and MacConkey agar media.
2) **Microscopical examination: Gram stain**: It is used to differentiate the organism, whether it is gram-positive or gram-negative. Gram-positive bacteria appeared purple in color and gram-negative bacteria appeared pink.

Biochemical reactions:
1) **The coagulase test**: was used to differentiate between Staphylococcus aureus and other Staphylococcus species. Test tube with pooled human plasma was inoculated with a staphylococcal colony. The tube was incubated at 37 °C for 4 hours.
- Positive test: the plasma will coagulate as in Staph aureus.
- Negative test: the plasma remains liquid as in Staph epidermidis.
2) **Hemolytic reactions of streptococcus:** There are three types of hemolysis alpha, beta and gamma. Alpha hemolysis is a greenish discoloration that surrounds a bacterial colony growing on the agar. Beta hemolysis represents a complete breakdown of the hemoglobin of the red blood cells in the vicinity of a bacterial colony. There is a clearing of the agar around a colony. Gamma hemolysis is a lack of hemolysis in the area around a bacterial colony as in streptococcus faecalis [16].
3) **IMVic (indole, methyl red, Voges-Proskauer, and citrate) tests:** It is used to differentiate between most of gram negative bacteria

Antimicrobial susceptibility testing:
Antibiotic susceptibility test of different isolates was performed by Kirby-Bauer disk diffusion method according to Clinical & Laboratory Standards Institute CLSI recommendations. The antibiotic disks were selected according to the protocol of laboratory, as recommended by the National Committee for Clinical Laboratory Standards **NCCLS** [17]. Mueller Hinton agar (Oxoid, Hampshire, UK) with 5% sheep RBCs plates were inoculated over the entire surface of the medium. The antimicrobial disks were placed using sterilized forceps. The disks were pressed firmly against the agar surface to ensure contact and subsequent antimicrobial diffusion. The plates were then incubated in aerobic environment at 37 C for 24 hours. The diameter of each inhibition zone was measured in mm using ruler on the under surface of the plate and interpreted using the interpretative chart as susceptible or resistant.

Ethical consideration:
An approval of the study was obtained from Mansoura University Academic and Ethical Committee. Written informed consent of all the participants was obtained and submitted them to Mansoura University after IRB approval with code number (MS.19.06.673). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:
The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, Standard deviation ($\pm$ SD) for parametric numerical data; median, range for non-parametric numerical data. Frequency and percentage of non-numerical data. Shapiro test was done to test the normality of data distribution. Significant data was considered to be nonparametric. Student T-test was used to assess the statistical significance of the difference between two study group means. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. P value < 0.05 was considered significant.

RESULTS
The present study was carried out on 205 infertile male patients, with documented pyospermia. The following tables and figures represent the results of the current study.

Mean age of studied cases was 31.1 years. Most of them had secondary infertility (60.5%), while 39.5% had primary infertility. Mean duration of infertility was 3.5 years. Only 17.1% suffered from systemic diseases; 9.3% had DM, 5.4% had hypertension and 2.4% had heart diseases **(Table 2)**.

Table (2): Age and clinical data of all studied cases.

			Cases N=205	
Age (years)		mean±SD	31.1	±5.8
Infertility	Primary	N, %	81	39.5%
	Secondary	N, %	124	60.5%
Duration of infertility (years)		mean±SD	3.5	±1.7
	Primary	mean±SD	2.9	±1.6
	Secondary	mean±SD	3.8	±1.9
Systemic diseases	Absent	N, %	170	82.9%
	Present	N, %	35	17.1%
	DM	N, %	19	9.3%
	Hypertension	N, %	11	5.4%
	Heart disease	N, %	5	2.4%

SD, standard deviation.

Samples with bacterial growth had significantly higher pus cell count and urinary symptoms when compared to samples which revealed no growth. Age, infertility type, duration, systemic diseases, and sperm count did not differ significantly between those with and without bacterial growth **(Table 3)**.

Table (3): Comparison of age, clinical and laboratory data between those with and without bacterial growth.

			No growth N=9		Growth N=196		p
Age (years)		mean±SD	30.9	±4.9	31.1	±5.8	0.930
Infertility	Primary	N, %	4	44.4%	77	39.3%	0.742
	Secondary	N, %	5	55.6%	119	60.7%	
Duration of infertility (years)		Median (range)	3	2-4	3	2-10	0.233
Systemic diseases	Absent	N, %	9	100%	161	82.1%	0.363
	Present	N, %	0	0%	35	17.9%	
Sperm count (million)		mean±SD	60.2	±6.4	40.6	±9.9	0.594
Pus cells (million/mL)		Median (range)	1	1-7	3	1-30	**0.006**
Urinary symptoms		N, %	0	0%	76	38.8%	**0.028**

SD, standard deviation

Gram negative bacilli were significantly associated with secondary infertility, DM, urinary symptoms. Age, sperm count and pus cell count in the semen did not differ significantly between gram positive and gram-negative cultures **(Table 4)**.

Table (4): Comparison of age, clinical and laboratory data between samples with Gram positive and negative stains.

			Gram positive N=154		Gram negative N=42		p
Age (years)		mean±SD	30.9	±5.8	31.5	±5.7	0.563
Infertility	Primary	N, %	68	44.2%	9	21.4%	**0.008**
	Secondary	N, %	86	55.8%	33	78.6%	
Duration of infertility (years)		Median (range)	3	2-9	4	2-10	0.164
Systemic diseases	Absent	N, %	130	84.4%	31	73.8%	0.112
	Present	N, %	24	15.6%	11	26.2%	
	DM	N, %	9	5.8%	10	23.8%	**0.016**
	Hypertension	Median (range)	10	6.5%	1	2.4%	
	Heart disease	N, %	5	3.2%	0	0%	
Sperm count (million)		mean±SD	40.3	±9.9	41.6	±10.1	0.465
Pus cells (million/mL)		Median (range)	3	1-30	3	1-10	0.450
Urinary symptoms		N, %	35	22.7%	41	97.6%	**<0.001**

All studied samples were subjected to antimicrobial sensitivity tests. The most sensitive antibiotics were cefoprazone, rifampicin, amikacin and Cefoperazone/ sulbactam. While the most resistant antibiotics were cotrimoxazole, ceftazidime, piperacillin and ciprofloxacin. Other antimicrobial sensitivity pattern (**Table 5**).

Table (5): Antimicrobial susceptibility pattern of all studied samples.

	Culture Growth N=196					
	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
Ciprofloxacin	28	14.3%	8	4.1%	160	81.6%
Levofloxacin	86	43.9%	32	16.3%	78	39.8%
Amoxicillin/clavulanic acid	92	46.9%	36	18.4%	68	34.7%
Ampicillin/sulbactam	95	48.5%	38	19.4%	63	32.1%
Tetracyclin	32	16.3%	8	4.1%	156	79.6%
Gentamycin	21	10.7%	29	14.8%	146	74.5%
Piperacillin	34	17.3%	2	1%	160	81.6%
Piperacillin/tazobactam	66	33.7%	23	11.7%	107	54.6%
Cefoperazone/sulbactam	110	56.1%	25	12.8%	61	31.1%
Rifampicin	135	68.9%	14	7.1%	47	24%
Cefotaxime	48	24.5%	14	7.1%	134	68.4%
Amikacin	112	57.1%	19	9.7%	65	33.2%
Cotrimoxazole	17	8.7%	3	1.5%	176	89.8%
Ceftazidime	21	10.7%	0	0%	175	89.3%
Cefoprazone	144	73.5%	10	5.1%	42	21.4%
Ceftrixone	39	19.9%	9	4.6%	148	75.5%

Regarding Gram positive cocci, the most sensitive was rifampicin and cefoprazone, while the most resistant was cotrimoxazole and ceftazidime. Regarding Gram negative bacilli, the most sensitive was amikacin and levofloxacin while the most resistant was piperacillin and ceftazidime. Gram negative bacilli showed significant association with higher frequency of sensitivity towards levofloxacin, amikacin, cefoprazone, higher frequency of resistant towards Amoxicillin/clavulanic acid, ampicillin-sulbactam, gentamycin, piperacillin, rifampicin, ceftazidime when compared to Gram positive cocci (**Table 6**).

Table (6): Comparison of susceptibility patterns between samples with Gram positive and negative stains.

		Gram positive N=154		Gram negative N=42		*p*
		N	**%**	**N**	**%**	
ciprofloxacin	S	18	11.7%	10	23.8%	0.116
	I	6	3.9%	2	4.8%	
	R	130	84.4%	30	71.4%	
Levofloxacin	S	60	39.0%	26	61.9%	**0.021**
	I	29	18.8%	3	7.1%	
	R	65	42.2%	13	31%	
Amoxicillin/clavulanic acid	S	81	52.6%	11	26.2%	**0.001**
	I	31	20.1%	5	11.9%	
	R	42	27.3%	26	61.9%	
Ampicillin-sulbactam	S	83	53.9%	12	28.6%	**<0.001**
	I	33	21.4%	5	11.9%	
	R	38	24.7%	25	59.5%	
Tetracyclin	S	26	16.9%	6	14.3%	0.336
	I	8	5.2%	0	0.0%	
	R	120	77.9%	36	85.7%	
Gentamycin	S	13	8.4%	8	19.0%	**0.005**
	I	18	11.7%	11	26.2%	
	R	123	79.9%	23	54.8%	
Piperacillin	S	33	21.4%	1	2.4%	**0.005**
	I	2	1.3%	0	0%	
	R	119	77.3%	41	97.6%	
Piperacillin/ tazobactam	S	50	32.5%	16	38.1%	0.774
	I	19	12.3%	4	9.5%	
	R	85	55.2%	22	52.4%	
Cefoperazone/sulbactam	S	92	59.7%	18	42.9%	0.126
	I	19	12.3%	6	14.3%	
	R	43	27.9%	18	42.9%	
Rifampicin	S	133	86.4%	2	4.8%	**<0.001**
	I	6	3.9%	8	19.0%	
	R	15	9.7%	32	76.2%	
Cefotaxime	S	41	26.6%	7	16.7%	0.298
	I	12	7.8%	2	4.8%	
	R	101	65.6%	33	78.6%	
Amikacin	S	78	50.6%	34	81.0%	**0.002**
	I	17	11.0%	2	4.8%	
	R	59	38.3%	6	14.3%	
Cotrimoxazole	S	13	8.4%	4	9.5%	0.680
	I	2	1.3%	1	2.4%	
	R	139	90.3%	37	88.1%	
Ceftazidime	S	20	13.0%	1	2.4%	**0.049**
	I	0	0%	0	0%	
	R	134	87.0%	41	97.6%	
Cefoprazone	S	120	77.9%	24	57.1%	**0.013**
	I	8	5.2%/	2	4.8%	
	R	26	16.9%	16	38.1%	
Ceftrixone	S	27	17.5%	12	28.6%	0.103
	I	9	5.8%	0	0%	
	R	118	76.6%	30	71.4%	

Regarding Staph aureus, the highest sensitivity was attributed to rifampicin followed by cefoperazone. While the highest frequency of resistance was directed to cotimoxazole and ceftazidime. Regarding Streptococci, the highest frequency of sensitivity was towards rifampicin and cefoperazone, while the highest frequency of resistance was towards piperacillin, gentamycin and cotrimoxazole. Regarding enterococci, the highest frequency of sensitivity was towards rifampicin, ampicillin/sulbactam and cefoperazone, while the highest frequency of resistance was towards ciprofloxacin, cotrimoxazole and ceftazidime. Piperacillin showed significant differences between the three strains of Gram positive cocci, as Enterococci showed significant sensitivity to it, while Staphyloccocci showed significant resistance to it **(Table 7)**.

Table (7): Comparison of susceptibility patterns according to organism type in all studied Gram positive isolates.

		Staph aurous N=101		Streptococci N=46		Enterococci N=7		*p*
		N	%	N	%	N	%	
Ciprofloxacin	S	10	9.9%	8	17.4%	0	0%	0.632
	I	4	4%	2	4.3%	0	0%	
	R	87	86.1%	36	78.3%	7	100%	
Levofloxacin	S	31	30.7%	25	54.3%	4	57.1%	0.135
	I	24	23.8%	5	10.9%	0	0%	
	R	46	45.5%	16	34.8%	3	42.9%	
Amoxicillin/clavulanic acid	S	49	48.5%	26	56.5%	6	85.7%	0.378
	I	22	21.8%	8	17.4%	1	14.3%	
	R	30	29.7%	12	26.1%	0	0%	
Ampicillin-sulbactam	S	48	47.5%	28	60.9%	7	100%	0.101
	I	25	24.8%	8	17.4%	0	0%	
	R	28	27.7%	10	21.7%	0	0%	
Tetracyclin	S	20	19.8%	5	10.9%	1	14.3%	0.066
	I	2	2.0%	6	13%	0	0%	
	R	79	78.2%	35	76.1%	6	85.7%	
Gentamycin	S	10	9.9%	1	2.2%	2	28.6%	0.125
	I	11	10.9%	6	13%	1	14.3%	
	R	80	79.2%	39	84.8%	4	57.1%	
Piperacillin	S	24	23.8%	4	8.7%	5	71.4%	**0.003**
	I	2	2%	0	0%	0	0%	
	R	75	74.3%	42	91.3%	2	28.6%	
Piperacillin /tazobactam	S	29	28.7%	17	37%	4	57.1%	0.454
	I	15	14.9%	4	8.7%	0	0%	
	R	57	56.4%	25	54.3%	3	42.9%	
Cefoperazone/sulbactam	S	62	61.4%	24	52.2%	6	85.7%	0.434
	I	14	13.9%	5	10.9%	0	0%	
	R	25	24.8%	17	37%	1	14.3%	
Rifampicin	S	91	90.1%	35	76.1%	7	100%	0.139
	I	2	2%	4	8.7%	0	0%	
	R	8	7.9%	7	15.2%	0	0%	
Cefotaxime	S	30	29.7%	9	19.6%	2	28.6%	0.721
	I	8	7.9%	4	8.7%	0	0%	
	R	63	62.4%	33	71.7%	5	71.4%	
Amikacin	S	55	54.5%	19	41.3%	4	57.1%	0.495
	I	12	11.9%	5	10.9%	0	0%	
	R	34	33.7%	22	47.8%	3	42.9%	
Cotrimoxazole	S	7	6.9%	6	13%	0	0%	0.545
	I	2	2%	0	0%	0	0%	
	R	92	91.1%	40	87%	7	100%	
Ceftazidime	S	13	12.9%	7	15.2%	0	0%	0.697
	R	88	87.1%	39	84.8%	7	100%	
Cefoprazone	S	79	78.2%	34	73.9%	7	100%	0.774
	I	5	5.0%	3	6.5%	0	0%	
	R	17	16.8%	9	19.6%	0	0%	
Ceftrixone	S	18	17.8%	6	13%	3	42.9%	0.159
	I	3	3%	6	13%	0	0%	
	R	80	79.2%	34	73.9%	4	57.1%	

Regarding E. coli, the highest sensitivity was attributed to amikacin and cefoperazone. While the highest frequency of resistance was directed to ceftazidime, cefotaxime, piperacillin and tetracyclin. Regarding Klebsiella, the highest sensitivity was attributed to amikacin and levofloxacin. While the highest frequency of resistance was directed to piperacillin and ceftazidime. Regarding Pseudomnas, the highest sensitivity was attributed to levofloxacin. While the highest frequency of resistance was directed to piperacillin and ceftazidime. Regarding levofloxacin, the highest sensitivity was directed towards pseudomonas. While the highest frequency of resistance was directed to E.coli. Regarding gentamycin, cefotaxime as well as ceftriaxone, the highest sensitivity was directed towards Klebsiella. While the highest frequency of resistance was directed to E. coli (Table 8).

Table (8): Comparison of susceptibility patterns according to organism type in all studied Gram negative isolates.

		E coli N=17		Klebsiella spp N=17		Pusdomous spp. N=8		p
		N	%	N	%	N	%	
Ciprofloxacin	S	2	11.8%	6	35.3%	2	25.0%	0.228
	I	0	0%	1	5.9%	1	12.5%	
	R	15	88.2%	10	58.8%	5	62.5%	
Levofloxacin	S	6	35.3%	12	70.6%	8	100.0%	**0.007**
	I	3	17.6%	0	0%	0	0%	
	R	8	47.1%	5	29.4%	0	0%	
Amoxicillin/clavulanic acid	S	7	41.2%	3	17.6%	1	12.5%	0.153
	I	3	17.6%	0	0%	2	25.0%	
	R	7	41.2%	14	82.4%	5	62.5%	
Ampicillin-sulbactam	S	6	35.3%	5	29.4%	1	12.5%	0.596
	I	3	17.6%	1	5.9%	1	12.5%	
	R	8	47.1%	11	64.7%	6	75.0%	
Tetracyclin	S	1	5.9%	2	11.8%	3	37.5%	0.167
	R	16	94.1%	15	88.2%	5	62.5%	
Gentamycin	S	0	0%	7	41.2%	1	12.5%	**0.012**
	I	5	29.4%	5	29.4%	1	12.5%	
	R	12	70.6%	5	29.4%	6	75.0%	
Piperacillin	S	1	5.9%	0	0%	0	0%	0.471
	R	16	94.1%	17	100%	8	100%	
Piperacillin/tazobactam	S	8	47.1%	7	41.2%	1	12.5%	0.485
	I	2	11.8%	1	5.9%	1	12.5%	
	R	7	41.2%	9	52.9%	6	75.0%	
Cefoperazone/sulbactam	S	10	58.8%	4	23.5%	4	50.0%	0.208
	I	2	11.8%	4	23.5%	0	0%	
	R	5	29.4%	9	52.9%	4	50.0%	
Rifampicin	S	0	0%	1	5.9%	1	12.5%	0.107
	I	6	35.3%	1	5.9%	1	12.5%	
	R	11	64.7%	15	88.2%	6	75.0%	
Cefotaxime	S	1	5.9%	5	29.4%	1	12.5%	**0.030**
	I	0	0%	0	0%	2	25.0%	
	R	16	94.1%	12	70.6%	5	62.5%	
Amikacin	S	14	82.4%	14	82.4%	6	75.0%	0.846
	I	0	0%	1	5.9%	1	12.5%	
	R	3	17.6%	2	11.8%	1	12.5%	
Cotrimoxazole	S	1	5.9%	2	11.8%	1	12.5%	0.759
	I	1	5.9%	0	0%	0	0.0%	
	R	15	88.2%	15	88.2%	7	87.5%	
Ceftazidime	S	0	0%	1	5.9%	0	0.0%	0.471
	R	17	100%	16	94.1%	8	100%	
Cefoprazone	S	11	64.7%	9	52.9%	4	50.0%	0.743
	I	0	0%	1	5.9%	1	12.5%	
	R	6	35.3%	7	41.2%	3	37.5%	
Ceftrixone	S	2	11.8%	9	52.9%	1	12.5%	**0.026**
	R	15	88.2%	8	47.1%	7	87.5%	

DISCUSSION

In the current study, the recruited cases were in the age range 21-45 years, and this is similar to **Elgozali and Omer** [18] who studied a group of patients with infertility and pyospermia and found that their age range was 22-45. The range of age showed a little difference from **Bhatt el al.** [15] **and Abdulla** [19] (31- 40 years) and (25- 50 years) respectively.

In our study regarding the type of infertility, 81 cases (39.5%) had the primary type, whereas the remaining 124 cases (60.5%) had the secondary type, these results did not go with **Merino et al.** [20] who noted that samples from 180 infertile patients with pyospermia; primary infertility was 112 (59%) and secondary infertility was 78 (41%) and **Elgozali and Omer** [18] who found that samples from 50 infertile men with pyospermia; 45 men (90%) were primary infertile, while only 5 men (10%) were secondary infertile and **Abbas et al.** [21] who found that 56.3% of infertile patients with pyospermia had primary infertility while 43.8% had secondary type.

In our work, the mean of pus cells was 3.6 million/ml as similar as **Moubasher et al.** [22] who found that the mean of pus cells in 25 infertile men with documented pyospermia was 3.6 million/ml. It showed a little difference from **Oliva and Multigner** [23] who found that the mean of pus cells of 55 infertile men with documented pyospermia was 4 million/ml

In the current study, systemic comorbidities were present in 35 cases (17.1%). Diabetes mellitus was the commonest one (19 cases – 9.3%), followed by hypertension (11 cases – 5.4%), and heart disease (5 cases – 2.4%).

Patients suffering from diabetes mellitus are prone to a higher occurrence of certain infections compared with the general population. Indeed, diabetes is considered a risk factor for urinary and genital tract infections, particularly in the setting of uncontrolled hyperglycemia [24].

Regarding microbiological profile of the included cases, bacterial growth was detected in 196 cases (95.6%), while no growth was detected in the remaining 9 cases (4.4%). **Al-Dahmoshi et al.** [25] also found that 61(87.1%) semen specimens of 70 infertile men with documented pyospermia revealed positive bacterial culture. In another study, out of 120 seminal fluid samples collected from infertile men with pyospermia, 74(61.66%) of samples revealed positive significant growth of bacteria on culture media, while 46(38.33%) with no growth [26].

On comparing cases with and without bacterial growth (196 and 9 cases respectively) in our study, no significant difference was detected between the two groups in age, type and duration of infertility and sperm count. This is agreement with **Elgozali and Omer** [18] that also reported no difference between cases with and without bacterial growth.

Nevertheless, in our study the group with bacterial growth had significantly more pus cells and the prevalence of urinary tract infections was higher in cases with bacterial growth. **Kim et al.** [27] reported that genital tract infections may arise from organism spread form the urinary tract. It should be noted that presence of urogenital tract infection may pose a danger to married couples and it should be eradicated by thorough antibiotics and anti-inflammatory therapy [28].

In our study, when comparing gram positive and negative cases, gram negative bacilli were significantly associated with secondary infertility. Besides, urinary symptoms were more prevalent in gram negative cases. Also, **Uneke and Ugwuoru** [29] reported that all the subjects with genital infections also had urinary tract infection (UTI) and the commonest bacteria implicated were Proteus species and E. coli. As UTI is commonly caused by gram-negative organisms, it is expected to encounter more urinary symptoms in cases with genital infections.

In the current study, Staph. Aureus was the commonest gram-positive organism (49.3%), followed by streptococci (22.4%), and enterococci (3.4%). Furthermore, E. coli together with Klebsiella were the commonest gram-negative organism (8.3% for each), followed by Pseudomonas (3.9%). It is similar to **Nasralla et al.** [30] that found the commonest isolated organisms were Staph. Aureus (46.2%) and **Elgozali and Omer** [18] that found the commonest organism isolated was Staph. Aureus (61.7%) followed by Escherichia coli (35.3%), and Proteus mirabilis (2.9%). Also, the frequency rate of staphylococcal aureus infection was 62.5% in seminal fluid infection [30]. Moreover, **Isaiah et al.** [12] found that out of a total number of 140 specimen, 92 (65.7%) yielded bacterial growth with Staphylococcus aureus, S. saprophyticus and Escherichia coli having the highest incidence rate of 28.3%, 19.6% and 13.0%, respectively.

Conversely, another study reported much less prevalence of staph aureus infections. Significant growth of positive isolates was Enterococcus faecalis (30%), coagulase positive staphylococci (20%), Escherichia coli (13.3%), Pseudomonas aeruginosa (10%), and Klebsiella pneumoniae (10%) [19]. Another study reported that pathogens detected in the semen of infertile males were as follows; E coli (26.9%), proteus (25%), staph aureus (15%), streptococci (11.5%), klebsiella (11.5%), and pseudomonas (9.6%) [29]. Also **Sasikumar et al.** [32] noticed that the dominant isolated bacteria were E. coli (40%), S. aureus (28%), Pseudomonas aeruginosa (14%), and Proteus mirabilis (8%). Moreover, **Bhatt el al.** [15] 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%).

All studied samples were subjected to antimicrobial sensitivity tests. The most sensitive

antibiotics were cefoperazone (73.5%), rifampicin (68.9%), amikacin (57.1%) and Cefoperazone/sulbactam (56.1%). While the most resistant antibiotics were cotrimoxazole (89.8%), ceftazidime (89.3%), piperacillin (81.6%) and ciprofloxacin (81.6%). Conversely **Nasralla *et al.*** [30] found that piperacillin/tazobactam, imipenem, meropenem, amikacin, gentamicin, doxycycline, and nitrofurantoin were the most sensitive antibiotics. While **Elgozali and Omer** [18] found that most of the tested strains were susceptible to azithromycin (97.1%), ciprofloxacin (94.1%), ofloxacin (94.1%), and sparfloxacin (94.1%).Also, Another study reported that most of the tested strains were susceptible to ciprofloxacin, cefloxacin, cephaloridine, ceftazidime, ceftriaxone, and erythromycin [19].

The difference in antibiotic sensitivity results could be explained by different microbiological profiles and the tested antibiotics between different studies. This may explain why our susceptibility results were not similar to previous studies in the literature.

In this study, the most sensitive antibiotics for gram positive organisms were rifampicin and cefoperazone, while the most resistant antibiotics were cotrimoxazole and ceftazidime. Gram negative pathogens were sensitive to amikacin and levofloxacin whereas they were resistant to piperacillin and cotrimoxazole. Conversely, **Nasralla *et al.*** [30] found that both gram-positive and gram-negative bacteria were highly sensitive to piperacillin/tazobactam, imipenem, meropenem, gentamicin, doxycycline, amikacin, and nitrofurantoin. The gram-positive bacteria are highly sensitive to linezolid, vancomycin, azithromycin, clindamycin, teichoplanin, erythromycin, and azithromycin, and **Bhatt el al.** [15] 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).

As regard gram-positive organisms in our study, staph. Aureus was more sensitive to rifampicin, followed by cefoperazone, while it was resistant to cotrimoxazole and ceftazidime. Streptocooci were sensitive to rifampicin and cefoperazone, whereas it was resistant to piperacillin, cotrimoxazole. Moreover, enterococci expressed sensitivity towards rifampicin, ampicillin-sulbactam, and cefoperazone, while the highest frequency of resistance was towards ciprofloxacin, cotrimoxazole, ceftazidime, but **Isaiah *et al.*** [12] found that Staphylococus aureus and Staphylococcus saprophyticus was sesnstive to Imipenem and vancomycin, while **Uneke and Ugwuoru** [29] reported that staph aureus was more sensitive to nitrofurantoin and perfloxacin, while it was resistant to ampicillin, penicillin, and

chloramphenicol. Moreover, streptococci was sensitive to cotrimoxazole and tetracycline, whereas it was resistant to ampicillin and penicillin. In another study, staph aureus was more sensitive to azithromycin, ofloxacin, and sparfloxacin, while it was resistant to cephalexin and cotrimoxazole [14]. While **Abdulla** [19] reported that staph aureus was sensitive to ciprofloxacin and cephaloridine, while it was resistant to penicillin. Furthermore, enterococci was more sensitive to ciprofloxacin and cephaloridine compared to staph, whereas it was resistant to both penicillin and erythromycin.

Regarding gram-negative organisms in the current study, E coli expressed high sensitivity for amikacin and cefoperazone, whereas it was resistant to ceftazidime, cefotaxime, piperacillin, tetracyclines. Klebsiella was sensitive to amikacin while it was resistant to piperacillin, ceftazidime. Besides, Pseudomonas was sensitive to levofloxacin, while being resistant to piperacillin, ceftazidime, but in another study, E coli was sensitive to azithromycin, while it was resistant to cotrimoxazole. Moreover, Proteus mirabilis expressed almost resistance to azithromycin, ofloxacin, sparfloxacin, cephalexin and cotrimoxazole [14]. Also **Uneke and Ugwuoru** [29] reported that E.coli was more sensitive to erythromycin, while it was resistant to perfloxacin. Besides, klebsiella was sensitive to nitrofurantoin, whereas it was resistant to cotrimoxazole and penicillin. Pseudomonas was sensitive to erythromycin, chloramphenicol, tetracycline, and **penicillin**, while it showed resistance to nitrofurantoin and perfloxacin. While **Abdulla** [19] reported that all gram-negative pathogens including E coli, Pseudomonas, and Klebsiella were sensitive to ciprofloxacin, while they were resistant to both erythromycin and penicillin.

Generally, there is a large variation in the antimicrobial sensitivity and resistance in the literature. Hence, it is recommended to perform culture and sensitivity for all cases presented with pyospermia, rather than following sensitivity parameters reported in the existing literature.

CONCLUSIONS

It could be concluded that semen analysis with peroxidase stain and semen culture are an important diagnostic tool in all patients undergoing fertility investigations to detect genitourinary infections and pyospermia.

95.6 % of semen samples revealed bacteriologic growth. Gram positive bacteria were more common than gram negative bacteria in semen culture.

The most common gram-positive bacteria of pyospermia in infertile men were Staph Aureus. The most common gram-negative bacteria of pyospermia in infertile men were E. Coli and Klebsiella.

The most sensitive antibiotics were cefoprazone, rifampicin, amikacin and Cefoperazone/sulbactam.

The most sensitive antibiotics for gram positive bacteria were rifampicin and cefoperazone.

The most sensitive antibiotics for gram negative bacteria were amikacin and levofloxacin.

RECOMMENDATION

Large sample size is recommended to detect the most common organisms causing pyospermia in a wider range. Additional studies with another multiple cultures to detect more bacteria causing pyospermia.

Using PCR for detecting viral infections and atypical bacteria. Using more antibiotic discs in disc diffusion method to detect more sensitive antibiotics. Frequent use of broad spectrum antibiotics should be avoided and antibiotic susceptibility testing should be performed to prevent more resistance.

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