PROC (BAYL UNIV MED CENT)
2018;31(2):165–167

https://doi.org/10.1080/08998280.2018.1444298

Taylor & Francis
Taylor & Francis Group

Time trends for bacterial species and resistance patterns in semen in patients undergoing evaluation for male infertility

G. Luke Machen, MD[a], Erin T. Bird, MD, MBA[a], Monica L. Brown, BGT, MPH[b], Dale A. Ingalsbe, BS[b], Milaida M. East[b], Michelle Reyes, BA, MBA[c], and Thomas J. Kuehl, PhD[b,c]

[a]Division of Urology, Department of Surgery, Scott & White Medical Center and Texas A&M Health Science Center College of Medicine, Temple, Texas; [b]Department of Pathology, Scott & White Medical Center and Texas A&M Health Science Center College of Medicine, Temple, Texas; [c]Department of Obstetrics and Gynecology, Scott & White Medical Center and Texas A&M Health Science Center College of Medicine, Temple, Texas

ABSTRACT

Semen from asymptomatic men who are being evaluated as male partners in interfile couples have been reported to contain a variety of bacteria. Longitudinal studies of the variation of these bacteria over time and their resistance patterns have not been commonly reported. At our institution, residues from semen samples are routinely evaluated for bacteria, including antibiotic sensitivity profiles. We set out to profile the changes in semen bacteria and antibiotic resistance at our institution over time. A total of 72 semen isolates were examined for type of bacteria and sensitivity to a panel of antibiotics. The results were divided into two separate 5-year intervals (the first beginning in 2006, the second in 2011) and compared. The majority of bacteria were skin flora, with *Streptococcus* and *Staphylococcus* being the most prevalent. The resistance data for these two pathogens showed minimal statistically significant difference between the two time periods, although the *Staphylococcus* species did show a trend toward increasing resistance, suggesting that antibiotics currently used in sperm cell preparations may need to be varied.

KEYWORDS Bacterial resistance; infertility; male; semen; semen analysis

Semen from asymptomatic men being evaluated for infertility has been reported to contain a variety of bacteria. Frequently, these results are from contamination, the most likely sources of which are the male urethra or skin secondary to improper collection or colonization.[1,2] Further, incidentally identified semen bacteria in the absence of symptoms are typically considered to have limited, if any, clinical significance.[1,3] These species vary by population, but the most common bacteria are typically gram-positive organisms, such as *Staphylococcus* or *Streptococcus* species.[4] Reports of semen samples positive for bacteria are extremely variable, with rates of 45% to 100%.[5] Longitudinal studies of the variation of these bacteria over time as well as their resistance patterns have not been commonly reported. At our institution, to ensure appropriate processing of semen, residues from semen samples are routinely evaluated for bacteria with antibiotic sensitivity profiles. We set out to profile the changes in semen bacteria and antibiotic resistance at our institution over time.

METHODS

After institutional review board approval was obtained, a retrospective review was undertaken over a 10-year period from 2006 to 2016. Approximately every 2 months, the residues of semen samples collected were sent for microbiology evaluation. The samples were typically leftover portions from freshly collected specimens of men undergoing fertility evaluations. Men were advised to abstain from intercourse 3 to 5 days prior to collection, wash their hands and genitals thoroughly before sample production, and produce the sample into a sterile specimen cup. Isolates were then examined for sensitivity to a panel of antibiotics. The results were divided into two separate 5-year intervals (the first beginning in 2006, the second in 2011) and compared. Results were reported as proportions, and chi-square tests were used in the group comparisons. Student's *t* tests were used to analyze the parametric data.

RESULTS

A total of 75 semen samples were evaluated during a 10-year interval beginning in 2006. Three samples were found

Corresponding author: G. Luke Machen, MD, Division of Urology, Department of Surgery, Scott & White Medical Center and Texas A&M Health Science Center College of Medicine, 2401 S 31st St., Temple, TX 76504 (e-mail: Graham.machen@bswhealth.org)

Received November 3, 2017; Revised January 8, 2018; Accepted January 9, 2018.

Table 1. Semen samples studied in the two time periods

Variable	2006–2010	2011–2015	*P* value
Semen samples	39	33	
Sperm concentration (million/mL)	66.4	70.8	0.71
Semen volume (mL)	3.9	3.0	0.06
Progressive motility (%)	42.1	43.7	0.40
Samples without isolates	1 (3%)	1 (3%)	0.90
Number of isolates per sample	2.4	2.4	0.97
Isolates found in samples			
Streptococcus species	27 (69%)	17 (52%)	0.12
Staphylococcus species	25 (64%)	20 (61%)	0.76
Lactobacillus species	3 (8%)	4 (12%)	0.53
Enterococcus species	7 (18%)	8 (24%)	0.51
Corynebacterium species	10 (26%)	11 (33%)	0.47
Others (infrequent)	14 (36%)	11 (33%)	0.82

to have >1 million white blood cells/mL, which could be consistent with an active genitourinary infection. Subsequently, these patients were excluded. Of the 72 remaining samples, 70 (97%) had 1 to 4 isolates of bacteria (mean 2.4). Semen characteristics did not differ between the two groups. *Streptococcus* and *Staphylococcus* were the most prevalent bacteria, growing in 52% to 69% of the specimens *(Table 1)*. No statistically significant differences existed in the types and prevalence of bacteria between the early and late intervals.

The resistance data *(Table 2)* for these two pathogens showed minimal statistically significant difference between the two time periods. In the recent interval, *Staphylococcus* was more susceptible to erythromycin ($P = 0.009$), and a trend toward increasing resistance of *Staphylococcus* to penicillin and ampicillin/sulbactam was observed.

DISCUSSION

During a recent 10-year interval, the majority of a patient subset providing semen for infertility evaluation at our institution was found to have bacterial contamination, which is consistent with reported data.[4] Based on our results, the species of bacteria remained relatively unchanged over time. As mentioned previously, bacterial contamination of semen is common and frequently results from commensal bacteria on the glans, urethra, or hands.[6] In fact, one study found that up to 71% of bacteria that normally colonize the glans are also present in the distal urethra.[7] However, there is some debate as to the clinical implications of bacteriospermia. It is believed that infectious etiologies may be involved in approximately 15% of male subfertility cases,[8] possibly the result of reactive oxygen species,[9] increased rates of DNA fragmentation,[10] and/or alterations in the function and morphology of sperm.[11] Certain types of bacteria, including *Mycoplasma, Ureaplasma, Escherichia coli*, and *Chlamydia trachomatis*, may be more harmful to sperm function than others.[11,12]

Resistance patterns of bacteria in semen samples have not been frequently reported. In one study by Isaiah et al, semen samples of 140 men in Nigeria were examined, and 59% of the

Table 2. Resistance profiles of bacteria found in semen in the two time periods

		2006–2010			2011–2015			
Isolates	Antibiotic family	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	*P* value
Streptococcus	Penicillin	83%	17%	0%	87%	13%	0%	0.81
	Ampicillin/sulbactam	100%	0%	0%	100%	0%	0%	1
	Erythromycin	58%	0%	42%	47%	0%	53%	0.55
Staphylococcus	Penicillin	25%	0%	75%	0%	0%	100%	0.11
	Ampicillin/sulbactam	87%	0%	13%	67%	0%	33%	0.35
	Erythromycin	13%	12%	75%	79%	0%	21%	0.009

bacteria cultured were resistant to oxacillin.[13] Kastrop et al observed microbial contamination in in vitro fertilization culture dishes and found that 20% of the bacteria were resistant to penicillin and that more than 90% of the specimens were resistant to at least one of the antibiotics used in the culture medium.[14]

In our data, the *Staphylococcus* species became more susceptible to erythromycin ($P < 0.05$), and there was a trend toward increasing resistance to penicillin and ampicillin/sulbactam that did not reach statistical significance. These particular antibiotics were chosen because they may be used in initial semen-diluting solutions and are classically associated with resistance development.[5] Of note, though not reaching statistical significance, there was an increase of resistance to penicillin among the *Staphylococcus* isolates, reflecting the increasing incidence of methicillin-resistant *Staphylococcus aureus*. It is estimated that colonization rates of *S. aureus* may approach 20% in the United States and that 1% to 3% of the population is colonized with methicillin-resistant *S. aureus*.[15] Penicillin is currently used in our preparation of sperm cells; given these results, it may be prudent to alter our diluting solutions.

The limitations of this study include its retrospective nature and small sample size. Furthermore, though semen suspension media may include other antibiotics, such as streptomycin or gentamicin, it may be of benefit to perform similar analyses that include these antibiotics. Thus, though further studies are needed, this study adds to the existing body of evidence regarding resistance patterns of bacteria in sperm and suggests that it may be prudent to take into account the increasing resistance of *S. aureus* in the processing of semen samples.

FUNDING

The study was supported by the TJK Noble Centennial Chair Endowment for Research in Obstetrics and Gynecology.

1. Bar-Chama N, Fisch H. Infection and pyospermia in male infertility. *World J Urol.* 1993;11:76–81. doi:10.1007/BF00182033.
2. Keck C, Gerber-Schäfer C, Clad A, Wilhelm C, Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. *Hum Reprod Update.* 1998;4:891–903. doi:10.1093/humupd/4.6.891.
3. Krissi H, Orvieto R, Ashkenazi J, et al. Effect of contaminated preprocessed semen on fertilization rate and embryo quality in assisted reproductive techniques. *Gynecol Endocrinol.* 2004;18:63–67. doi:10.1080/09513590310001651821.
4. Abeysundara PK, Dissanayake D, Wijesinghe PS, Perera R, Nishad A. Efficacy of two sperm preparation techniques in reducing non-specific bacterial species from human semen. *J Hum Reprod Sci.* 2013;6:152–157. doi:10.4103/0974-1208.117169.
5. Dissanayake DM, Amaranath KA, Perera RR, Wijesinghe PS. Antibiotics supplemented culture media can eliminate non-specific bacteria from human semen during sperm preparation for intra uterine insemination. *J Hum Reprod Sci.* 2014;7:58–62. doi:10.4103/0974-1208.130859.
6. Cottell E, Harrison RF, McCaffrey M, et al. Are seminal fluid microorganisms of significance or merely contaminants? *Fertil Steril.* 2000;74:465–470. doi:10.1016/S0015-0282(00)00709-3.
7. Willen M, Holst E, Myhre EB, Olsen AM. The bacterial flora of the genitourinary tract in healthy fertile men. *Scand J Urol Nephrol.* 1996;30:387–393. doi:10.3109/00365599609181315.
8. Diemer T, Huwe P, Ludwig M, Hauck EW, Weidner W. Urogenital infection and sperm motility. *Andrologia.* 2003;35:283–287. doi:10.1111/j.1439-0272.2003.tb00858.x.
9. Fraczek M, Szumala-Kakol A, Jedrzejczak P, Kamieniczna M, Kurpisz M. Bacteria trigger oxygen radical release and sperm lipid peroxidation in in vitro model of semen inflammation. *Fertil Steril.* 2007;88(Suppl 4):1076–1085. doi:10.1016/j.fertnstert.2006.12.025.
10. Domes T, Lo KC, Grober ED, Mullen JB, Mazzulli T, Jarvi K. The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. *Fertil Steril.* 2012;97:1050–1055. doi:10.1016/j.fertnstert.2012.01.124.
11. Moretti E, Capitani S, Figura N, et al. The presence of bacteria species in semen and sperm quality. *J Assist Reprod Genet.* 2009;26:47–56. doi:10.1007/s10815-008-9283-5.
12. Rybar R, Prinosilova P, Kopecka V, et al. The effect of bacterial contamination of semen on sperm chromatin integrity and standard semen parameters in men from infertile couples. *Andrologia.* 2012;44(Suppl 1):410–418. doi:10.1111/j.1439-0272.2011.01198.x.
13. Isaiah IN, Nche BT, Nwagu IG, Nnanna II. Current studies on bacterospermia the leading cause of male infertility: a protégé and potential threat towards mans extinction. *N Am J Med Sci.* 2011;3:562–564.
14. Kastrop PM, de Graaf-Miltenburg LA, Gutknecht DR, Weima SM. Microbial contamination of embryo cultures in an ART laboratory: sources and management. *Hum Reprod.* 2007;22:2243–2248. doi:10.1093/humrep/dem165.
15. O'Reilly EB, Johnson MD, Rohrich RJ. Comprehensive review of methicillin-resistant *Staphylococcus aureus*: screening and preventive recommendations for plastic surgeons and other surgical health care providers. *Plast Reconstr Surg.* 2014;134:1078–1089. doi:10.1097/PRS.0000000000000626.