Interference of Human Spermatozoal Motility by Live *Staphylococcus aureus*

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Abstract

The aim of this work was to investigate the effect of live *Staphylococcus aureus* commonly present in the cervix of females on sperm motility and agglutination in vitro. Highly motile samples of spermatozoa (40 x 10^6 /ml) from normozoospermic males were co-incubated for 4h with 10^7 cells/ml of live *S. aureus*. After 30 minutes, 1, 2 and 4h of incubation, sperm motility and agglutination was checked by light microscopic examination (X 400). From the onset, it was apparent that the live bacteria reduced the motility of spermatozoa. The promptness with which the decreased motility was observed to occur in the live *S. aureus* group was striking. After 30 minutes of mixing the ejaculate with the live bacteria, decrease in motility and clumping of the spermatozoa was noted. Interestingly, no agglutination and immobilization was observed in other experiments in which the bacteria were killed by heat, lysozyme or antibiotics and mixed with the ejaculate. Certainly, it seems evident that live *S. aureus* can significantly depress the motility and agglutinate human spermatozoa in vitro. The mechanism by which this alteration takes place is not obvious, however, detrimental effect on sperm motility by live staphylococci may be an as yet unrecognized component of fertility problems.

Keywords: *Staphylococcus aureus*; sperm immobilization; heat; lysozyme; antibiotics; scanning electron microscopy.

1. Introduction

Infections of the male genitourinary tract represent a significant health care problem and account for almost 15% of cases of male infertility (Gdoura et al., 2007). Infections can affect different sites of the male reproductive tract, such as the testis, the epididymus and male accessory sex glands (Diemer et al., 2000; Golshani et al., 2006). Spermatozoa subsequently can be affected

by infections at different points in their development and maturation. Acute and chronic infections can compromise spermatogenesis, resulting in quantitative and qualitative reductions. Direct interactions with pathogenic bacteria represents another possibility for infectious impact on spermatozoa (Khalili and Yazdi, 2001; Golshani et al., 2006).

Among bacterial species that interact with spermatozoa are well known causative pathogens of genitourinary infections such as *Escherichia coli, Ureaplasma urealyticum, Mycoplasma hominis* and *Chlamydia trachomatis* (Kohn et al., 1998, Hosseinzadeh et al., 2001, Gdoura et al., 2007). *E. coli* probably represents the most frequently isolated microorganism in genitourinary infections (Weidner et al., 1991). *E. coli* rapidly adheres to human spermatozoa in vitro, resulting in agglutination of spermatozoa (Diemer et al., 2003). Agglutination of spermatozoa was produced only by live pathogenic *E. coli* whereas killed bacteria failed to do so. Liu et al. (2002) while studying other uropathogenic microorganisms found significant decrease in sperm motility when spermatozoa were coincubated with *S. aureus*. Jiang and Lu (1996) reported *S. aureus* as the predominant flora in infertile men with a significant decrease in sperm motility. Based on these observations and our observation that *Staphylococcus aureus*, one of the predominant flora in the infertile men and commonly present in the cervix of females immobilizes the spermatozoa (Ohri and Prabha, 2005), we devised a series of simple experiments to determine whether decrease in motility is associated with live bacteria or killed bacteria can also bring about the same change.

2. Materials and methods

2.1 Semen samples

Spermatozoa were obtained from 10 healthy donors and men undergoing evaluation of fertility at PGIMER, Chandigarh, India, by masturbation following a 24h continence period. Only ejaculates showing normal sperm parameters according to World Health Organization criteria (1999) were used.

2.2 Microorganism

The bacterial isolates used in the present study were taken from the cervices of 10 women with unexplained infertility who were seen at the Department of Obstetrics and Gynecology, Government Multi Speciality Hospital, Sector-16, Chandigarh, India. Before taking the samples, the patients' recent medical history was taken into consideration. The cervical samples were taken from only those females who had not had any antibiotic intake for at least a week. The sample was taken only at that time when there was no clinical incidence of cervical infection. Therefore, all the cervical isolates which were obtained were considered to be normal flora. Swab samples were taken from the cervical area by the standard technique (Hok et al., 1967). The samples were directly spread by streaking on sheep blood agar plates and the plates were incubated aerobically at 37°C for 24-48h. In total 10 isolates were obtained. The isolates were identified according to Bergey's Manual of Determinative Bacteriology (1994). Screening of 10 isolates for interaction with human sperm identified only one isolate, *S. aureus* as an inducer of sperm agglutination. Therefore, further experiments were carried out with this isolate.

2.3 Killing of bacteria

Killing of bacteria was carried out by using three different methods viz. heat, lysozyme and antibiotic treatment.

Heat treatment: Aliquots of 2 ml of 72h old culture of *S. aureus* were exposed to different temperatures viz. 40, 50, 60, 80 and 100°C for 10 and 30 minutes and checked for non- viability by subsequent culturing on nutrient agar plates.

Lysozyme treatment: Aliquots of 2 ml of 72h old culture of *S. aureus* were incubated with different concentrations (10-1000 μg ml^{-1}) of hen egg white lysozyme (Hi-Media Laboratories, Mumbai, India) for different time intervals (2-24h) at 37°C in order to find out the best dose time combination for killing of bacteria (Millar, 1987). After completion of incubation, the culture was checked for non- viability by subsequent culturing on nutrient agar plates.

Antibiotic treatment: Eleven antibiotics were chosen for the study according to their common

use in research and human medicine. They belonged to following groups β-lactams or penicillins: amoxicillin (Maxheal pharmaceuticals, Maharashtra, India), ampicillin (Maxheal pharmaceuticals, Maharashtra, India), penicillin G (Hindustan Antibiotics Ltd., India), methicillin; macrolides: erythromycin (Shalina Laboratories Ltd., Mumbai, India); aminoglycosides: gentamicin (Maxheal pharmaceuticals, Maharashtra, India), streptomycin (Synbiotics Ltd., Vadodra, India); quinolones: ciprofloxacin (Shalina Laboratories Ltd., Mumbai, India); glycopeptides: vancomycin (VHB Life Sciences, Kerala, India); lincosamides: clindamycin (Pfizer Manufacturing, Belgium) and chloramphenicol (Maxheal pharmaceuticals, Maharashtra, India). Stock solutions (1mg ml^{-1}) of each antibiotic were prepared using water for injection. MBC (Minimum Bactericidal Concentration) for each antibiotic was obtained using the standard protocol (Scott, 1989). MBC was defined as a 99.99% reduction of cell viability with respect to that of initial inoculum.

2.4 Effect of live and dead bacteria on sperm agglutination

0.1 ml volumes of fresh ejaculate were mixed with 0.1 ml of each of the following:

(1) Phosphate Buffered Saline (50 mM, pH 7.2).

(2) 72h old live culture of *S. aureus.*

(3) 72h old culture of *S. aureus* exposed to 100°C for 10 minutes.

(4) 72h old culture of *S. aureus* pre-incubated with lysozyme at a concentration of 1mg ml^{-1} for 4h at 37°C and after incubation removal of lysozyme from the culture by centrifugation and washing the cell pellet twice with PBS (50 mM, pH 7.2) under cold conditions and pellet resuspended in same volume of PBS (Reddy et al., 2004).

(5) 72h old culture of *S. aureus* pre-incubated with the MBC of each of antibiotic for 24h and after incubation removal of antibiotic from the culture by centrifugation and washing the cell pellet twice with PBS (50 mM, pH 7.2) under cold conditions and pellet resuspended in same volume of PBS (Reddy et al., 2004).

Following mixing, a drop each of the suspension was placed on a glass slide and examined under the microscope to determine immobilization/agglutination. These examinations were repeated at different intervals for 4h.

2.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to investigate the adherence of live and dead bacteria on spermatozoa. The sample processing was done according to the standard method (Hafez and Kanagawa, 1973) with slight modifications. 200µl of washed sperm suspension was incubated with 200µl of each live and killed (heat/lysozyme/antibiotic) *S. aureus* for 4h. To each tube 4 ml of 2.5% phosphate buffered gluteraldehyde was added gently with a pasteur pipette. After 30 minutes samples were centrifuged at 500 x g for 10 minutes and washed twice in PBS (50mM, pH 7.2). One drop of fixed and washed spermatozoa was placed on a silver painted adhesive tape mounted on brass stubs and air dried. 100Å gold coating was done on Jeol fine coat ion sputter (JFC-1100) and the specimens were observed.

3. Results and discussion

There is disagreement as to the influence of certain microbial infection on male infertility. Several investigators have reported different types of organisms in seminal fluid specimens depending on the methods of examination. Detection of bacteria in semen does not necessarily suggest infection. Since bacterial isolates in seminal fluid may represent contamination or colonization of the uretheral orifice. *Staphylococcus aureus* has been isolated from 68.2% of the seminal fluids (Okon et al., 2005) in Maiduguri. Most practitioners discuss the presence of *S. aureus* as mere contamination which is assumed to be of no significance. However, Emokpae et al. (2009) reported that presence of *S. aureus* should not be ignored as it can lead to decrease in the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity. Earlier work done by Jiang and Lu, (1996) and Liu et al. (2002) has also shown *S.*

aureus to be the dominant flora in infertile men with a significant decrease in sperm motility.
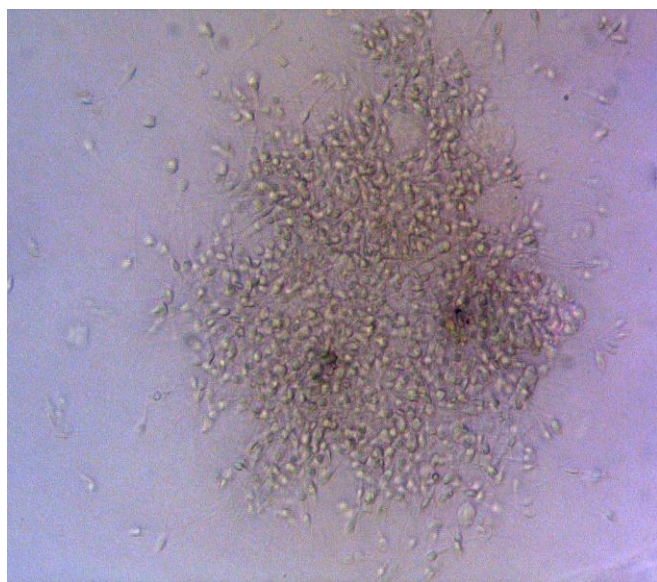
Figure 1a
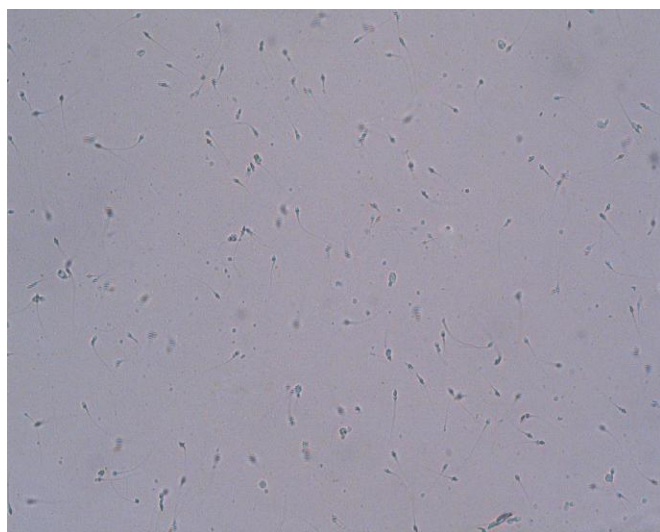
Figure 1b

Figure 1: (a) Agglutination of motile spermatozoa by live *S. aureus* (x 400). **(b)** No agglutination by dead *S. aureus* (x 200).

In the present studies we have also found that *S. aureus* isolated from the cervix of a woman with unexplained infertility produces profound depression in the motility of human spermatozoa in vitro. Within 30 minutes of mixing of semen with live *S. aureus*, clumping of spermatozoa and dampening of motility was observed. At 4h there was complete absence of motility and significant agglutination of spermatozoa (Figure 1a). Interestingly, no agglutination or dampening of motility was noted in those cases (Figure 1b) where the bacteria were killed before mixing with the ejaculate by treatment with heat at 100°C for 10 minutes (Figure 2a), lysozyme at a conc. of 1mg ml^{-1} for 4h (Figure 2b) and various antibiotics (MBC, Table 1).

Table 1: Minimum bactericidal concentration of various antibiotics used to kill bacteria

| Antimicrobial	MBC (μg/ml)
Ampicillin	6
Amoxycillin	20
Ciprofloxacin	40
Chloramphenicol	6
Clindamycin	8
Erythromycin	10
Gentamicin	4
Methicillin	6
Penicillin G	10
Streptomycin	10
Vancomycin	10

Further to ascertain the mechanism underplaying the agglutination and dampening of spermatozoal motility, scanning electron microscopy was performed. The electron micrographs showed close association between live *S. aureus* and spermatozoa. The bacteria were seen to adhere to tail and head (Figure 3a). Along with bacterial adherence, a prominent morphological defect i.e. the curling of tail was also observed. These results are in accordance to earlier studies carried out by Diemer et al. (2000) wherein they have also reported the adherence of

E. coli to both heads and tails of spermatozoa. Further, electron microscopic evaluation of sperm-*E. coli* interactions revealed multiple and profound alterations involving all superficial structures of spermatozoa, indicating that morphological defects might be accounting for the immobilization of spermatozoa (Diemer et al., 2000).
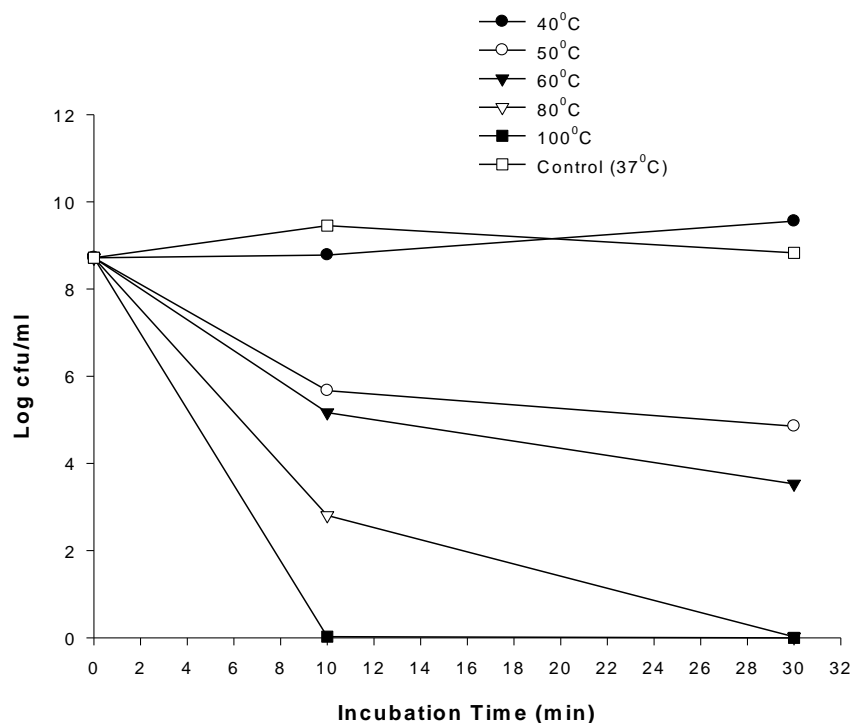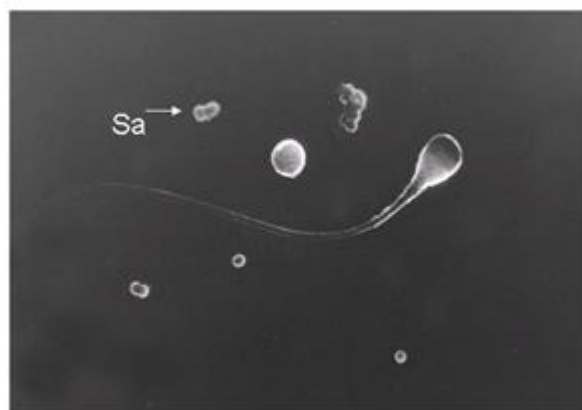

Figure 2a
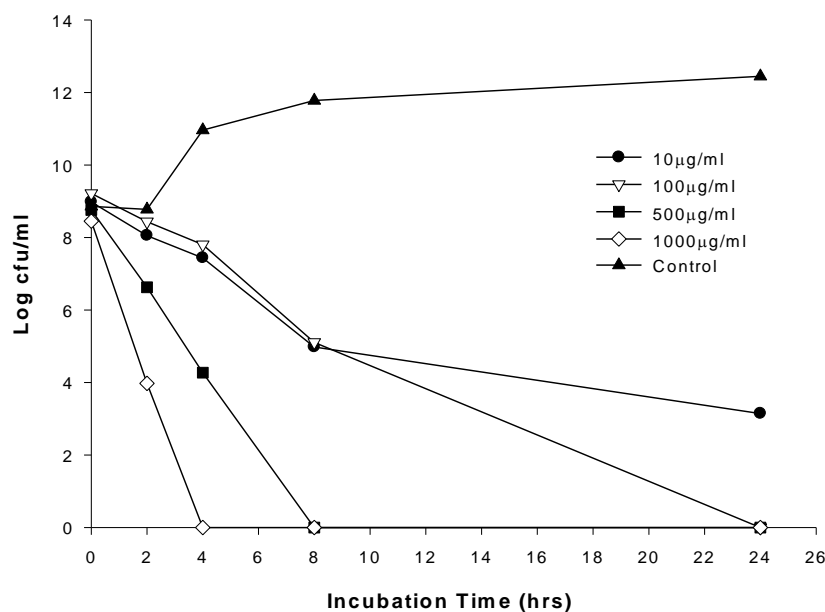
Figure 2b

Figure 2: Viable count plating after treatment of *S. aureus* with **(a)** heat and **(b)** lysozyme.

Figure 3a

Figure 3b

Figure 3: Scanning electron micrograph showing **(a)** adherence of live *S. aureus* (Sa) (x 8,500) and **(b)** non-adherence of dead *S. aureus* (Sa), to human sperm (x 2,700)

The dead *S. aureus* neither adhered to spermatozoa nor induced any changes in the morphology (Figure 3b) which might be the reason of causing no immobilization of the spermatozoa. In an earlier study, Hosseinzadeh et al. (2001) have also shown that live Chlamydial elementary bodies (EBs) can have a direct and detrimental effect on sperm physiology whereas heat treatment abolishes the same.

The absence of immobilization and agglutination of spermatozoa upon incubation with killed *S. aureus* could be suggestive of some labile factor present on bacterial cells. However, this mechanism clearly requires further investigation.

4. Conclusion

Live *S. aureus* obtained from cervical cultures agglutinates and immobilizes human spermatozoa in vitro whereas killed bacteria fail to do so.

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