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**ANTIBACTERIAL EFFECT OF SEMINAL FLUID AGAINST BACTERIA'S CAUSING BACTERIAL VAGINOSIS**

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**Abstract**

In this study the effect of seminal fluid exposure on vaginosis causing bacteria's; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* were determined using agar disc diffusion method and agar well diffusion method. Clear zone of inhibition were observed against *Staphylococcus aureus* and *Escherichia coli* on the other hand *Pseudomonas aeruginosa* resisted all sort of antibacterial effect successfully. This study opens up the wide possibilities of seminal fluid and its components in therapeutic field. In this current world thriving under the threat of antibiotic resistance and multidrug resistant bugs; any natural substance with stable antibacterial effect is a ray of hope for the scientists. With the results of this study it is clear that human seminal fluid have antibacterial effect on exposure against gram positive organisms like *S.aureus* and gram negative organisms like *E.coli*. The zone of inhibition is comparable to existing antibiotics. This study also clears out various misconceptions of the society.

**Keywords:** *Seminal fluid exposure, vaginosis bacteria, pseudomonas aeruginosa, staphylococcus aureus, Escherichia coli, proteus mirabilis, agar disc diffusion method, agar well diffusion method, zone of inhibition, antibacterial effect, therapeutic field, antibiotic resistance, multidrug resistant bugs, natural substance, gram positive organisms, gram negative organisms.*

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## 1. Introduction

Human seminal fluid is a complex biological fluid formed from various fluids in the male reproductive tract, namely the secretion of the testes, epididymis, seminal vesicles prostate and Cowper's gland along with spermatozoa<sup>1</sup>. Semen, like blood has two types of components i.e. the cellular spermatozoa and the non-cellular seminal plasma contains the combine secretion of all the male accessory sex glands and is of great physiological Significance since it serves as a carrier of sperm to female reproductive tract protected from infectious organisms also.

Possible presence of penicillin in human semen, noticed that semen had an inhibitory effect on bacteria. It was observed more than 80% of the semen samples inhibited the growth *Staphylococcus aureus*<sup>2</sup>. The polyamine spermine and spermidine are some of the constituents of human prostatic fluid which is known to possess an antibacterial activity. Lysozyme occurs in prostatic and seminal fluid<sup>3-5</sup>. Bacterial vaginosis (BV) is a disease of the vagina caused by excessive growth of bacteria or by an imbalance of the naturally occurring bacteria in the vagina. There is a change in the most common type of bacteria and a hundred to thousand fold increase in total numbers of bacteria present<sup>6-7</sup>. Typically, bacteria other than *Lactobacilli* become more common<sup>8</sup>. Common symptoms include increased vaginal discharge that often smells like fish<sup>9</sup>.

The discharge is usually white or grey in colour. Burning with urination may occur. Itching is uncommon. Occasionally, there may be no symptoms. Having BV approximately doubles the risk of infection by a number of other sexually transmitted infections, including HIV/AIDS<sup>10</sup>. It also increases the risk of early delivery among pregnant women<sup>8</sup>. Risk factors include douching, new or multiple sex partners, antibiotics, and using an intrauterine device, among others. However, it is not Considered a sexually transmitted infection<sup>11</sup>.

The aim of this study was to investigate whether seminal fluid exhibited antibacterial effect against bacteria's causing bacterial vaginosis. We isolated *Staphylococcus aureus*, *Eschericiacoli*, *Pseudomonas aeruginosa* and *Proteus spp* from 10 patients with bacterial vaginosis and fresh seminal fluid from 35 healthy men and well diffusion technique was done and observations were recorded.

## 2. Materials and Methods

### 2.1 Materials

Compound microscope (to observe the morphology of the organism), hot air oven and autoclave (for sterilization of articles and Medias), incubator (for incubation of cultures), laminar air flow (For maintaining aseptic conditions while performing various techniques), glassware's (Test tubes of different sizes, glass slides and coverslips, Petri plates, conical flasks of different

Capacities i.e. 500 ml, 250 ml, pipettes of 40 microliter), culture medias (for proper growth of bacteria), Vitec2 compact system

### **2.1 Isolation of clinical sample:**

Vaginal swabs were collected from 10 patients with bacterial vaginosis and were inoculated into Nutrient agar, Blood agar and Macconkey agar and incubated at 37<sup>0</sup>C for 24hr. Identification characterization done manually by Gram's staining, hanging drop method for motility testing, coagulase test, catalase test, OF test, nitrate reduction test, indole test, MR test, VP test, citrate test, sugar fermentation tests, citrate test, urease test. Cultural characters also noted in various culture Medias. Results were confirmed using Vitec2 compactsystem.

### **2.1 Semen collection and preparation**

Semen was obtained by masturbation after atleast 3 days of abstinence from 35 healthy donors with normal sperm characteristics. It is then centrifuged at 10,000 rpm for 10 min to separate spermatozoa from seminal plasma.

### **2.2 Antimicrobial susceptibility testing**

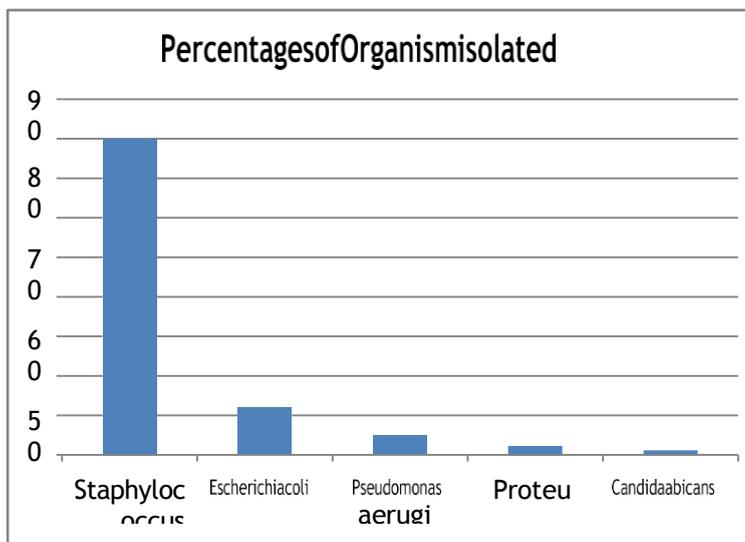
Agar well diffusion method was done using Muller Hinton Agar (MHA) which is a nonselective, non-differential medium containing 2.0g beef extract, 17.5 casein hydrolysate, 1.5g starch and 17.0g agar dissolved in 1 liter of distilled water, pH adjusted to neutral

At 25<sup>0</sup>C. Media was prepared beforehand using Himedia. Isolated bacteria's were inoculated into a nutrient broth Culture and at required turbidity it was lawn cultured on to the MHA plates using sterile swab and wells were punched using a well cutter. 100µl of the freshly collected seminal fluid samples were inoculated in to the well using a micro pipette. Plates were then carefully placed in an incubator overnight; afterwards clear zones of inhibition were seen and the measurements were recorded. Spermidin was bought online and the same procedure was repeated and observations were noted.

### **Results**

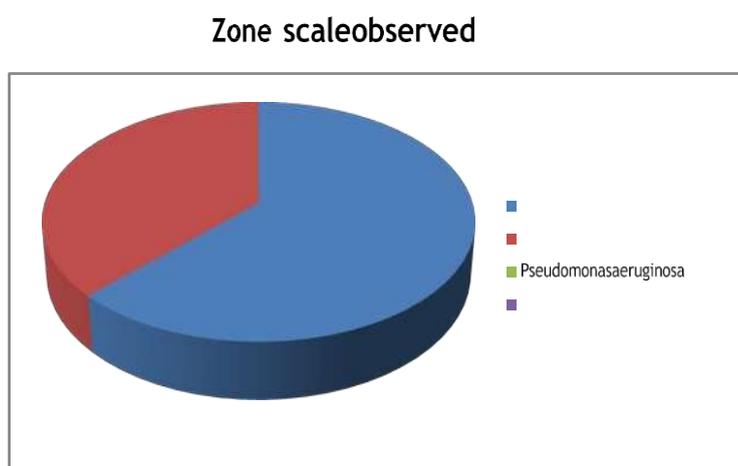
From the 10 vaginal swabs collected, five different organisms were isolated comprising 80% of *Staphylococcus aureus*, 12% *Escherichia coli*, 5% *Pseudomonas aeruginosa*, 2% *Proteus spp* and 1% *Candida albicans*. From which four organisms with higher percentage of aetiology was taken for study. Clear zone of inhibition was observed in some plates while some organisms successfully resisted the action of seminal fluid.

**Figure 1: Measurements of zone of inhibition observed are:**



1	<i>Staphylococcus aureus</i>	20mm
2	<i>Escherichia coli</i>	12mm
3	<i>Pseudomonas aeruginosa</i>	no zone of inhibition
4	<i>Proteus mirabilis</i>	no zone of inhibition

**Figure 2: Representation of susceptibility of organisms:**



Seminal fluid have significant antibacterial effect on both gram positive as well as gram negative bacteria's comparable to existing antibiotics. Clear zone of inhibition was observed on plates of *Staphylococcus aureus* and *Escherichia coli* while *Pseudomonas aeruginosa* and *Proteus mirabilis* successfully resisted the action of seminal fluid.

### 3. Discussion

Human semen and expressed prosthetic fluid are said to exhibit an antibacterial activity in vitro<sup>2</sup>. The current study also shows that seminal plasma have significant antibacterial activity against both gram positive as well as gram negative organisms. From the sample collected, 80% of *Staphylococcus aureus*, 12% *Escherichia coli*, 5% *Pseudomonas aeruginosa*, 2% *Proteus spp* and 1% *Candida* Plasma had inhibitory effect on *Staphylococcus aureus* and *Escherichia coli* while on *Proteus mirabilis* and *Pseudomonas aeruginosa* it had effect. A related condition was observed by Hirsch and Dubos; they found that although spermin had no effect on *Mycobacterium tuberculosis*, an oxidation product of spermin was a potent inhibitor; same also inhibited the Growth of *Trypanosom equiperdum*, *S. aureus*. and *E.coli*. It is therefore probable that an oxidation product formed via bacterial metabolism may be a factor responsible for its bactericidal effect<sup>12</sup>. At about time Gurevitch et al reported that spermine had antibacterial activity<sup>12</sup>.

Subsequently human serum and prosthetic fluid were shown to inhibit the effect of *S.aureus*, *S.albus*, *E.coli*, *Bacillus cereus*, *Neisseria meningitides*, *Neisseria gonorrhoea*, *Shigella flexneri*, *Salmonella typhi* *murium* etc<sup>13</sup>. These studies on spermin, lead to investigations of its antibacterial effect on *S. aureus*, *E. coli*, *Lactobacillus caesei*, *N.gonorrhoeae*, *Bacillus anthrax*, *N. meningitides*, *S. flexneri*, *Streptococcus haemolyticus*<sup>13</sup>. Rusk and collaborators (1973) tested 97 human ejaculates and found these inhibit growth of *S. albicus*, *S. aureus* and *E.coli*. Purified spermin has an antibacterial effect particularly on gram positive species.

The action of spermine is antagonized by hydrogen ion<sup>2</sup>. Seminal fluid also contain several proteolytic enzymes<sup>15</sup>. The presence of antibacterial molecules in seminal plasma has been suspected for more than five decades. In 1949 it has been first reported also. Apart from Sexually Transmitting Diseases (STD's) male and female body is naturally capable of preventing infections for themselves and for one another. Due to lack of knowledge or scientific proof coitus is still a taboo during minor inflammations or infections like bacterial vaginosis; while ironically seminal plasma being an apt remedy.

Mild infections like bacterial vaginosis shouldn't be a barrier to sexual intercourse as it may have advantageous effects instead. So the typical concept of restricting direct intercourse during such condition is a misconception. In a country where 1 out of 10 married women suffering from bacterial vaginosis once or repeatedly; this study holds relevance in dwelling into the natural antibacterial capability of seminal fluid against bacteria's causing bacterial vaginosis. This study also opens up the window to the wide range of application of factors behind this antibacterial effect of seminal plasma in therapeutically areas that too when the medical field is in a crisis of antibiotic resistance. Relying more upon natural medication provides us with stability and close to no side effects. This study could also clear out many misconceptions of society also.

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