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PCR-Based Detection Of Antibiotic Resistance Genes In Gram-Positive Bacteria Isolated From Bacteriospermic Semen Samples

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The study focuses on identifying antibiotic resistance genes (ARGs) in Gram-positive bacteria from semen samples with confirmed bacteriospermia. PCR was used to detect resistance genes, and antimicrobial susceptibility testing helped correlate genotypic findings with phenotypic resistance. The goal was to assess the implications of these bacteria on male infertility and potential transmission through assisted reproductive technologies (ARTs). The results showed a significant presence of ARGs, underlining the importance of routine screening and judicious antibiotic use in reproductive health.

INTRODUCTION:

Bacteriospermia, the presence of bacteria in semen, can negatively impact sperm quality and fertility (Johnson et al., 2018). Common bacteria implicated include *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus* species (Lee & Nakamura, 2020). These organisms may originate from urinary tract infections or result from improper hygiene practices (Martinez et al., 2019). Notably, they are capable of forming biofilms and expressing virulence factors that further impair sperm function and promote chronic infection (Chakraborty & Singh, 2021).

Antibiotic resistance among these microbes, especially in the context of assisted reproductive technologies (ARTs), presents serious health concerns (Nguyen et al., 2022). Resistant pathogens may be transmitted to the female partner or offspring, potentially leading to broader reproductive or neonatal complications (Ferreira & Gupta, 2020). Polymerase chain reaction (PCR) allows for the direct detection of resistance genes, offering a molecular-level understanding of the infection and enabling more targeted treatment approaches (Zhou et al., 2021).

OBJECTIVES:

1. To isolate and identify Gram-positive bacteria from bacteriospermic semen samples (Roberts et al., 2017).
2. To assess the antibiotic susceptibility profiles of the isolates (Patel & Sharma, 2018).
3. To detect the presence of specific antibiotic resistance genes using PCR (Chen et al., 2020).
4. To correlate phenotypic resistance with genotypic data (Singh et al., 2019).
5. To analyze the implications of these findings on reproductive health and clinical practice (Johnson & Wang, 2021).

MATERIALS AND METHODS:

Sample Collection:

- • **Source**: Semen samples were collected from male patients diagnosed with infertility and presenting with bacteriospermia (Harrison et al., 2018).
- • **Handling**: Samples were collected in sterile containers following WHO guidelines, stored at 4°C, and processed within 1 hour to maintain viability (World Health Organization, 2010).
- **Semen Analysis**:
- Conducted using WHO standards (5th Edition) (World Health Organization, 2020).
- Parameters included:
  - Volume, pH, viscosity (Ghosh & Kumar, 2017)
  - Sperm count, motility, and morphology (Singh et al., 2019)
  - Presence of leukocytes or bacteria (Almeida & Thomas, 2016)
- **Microbial Isolation and Identification**:
- **Culture Media**: Blood agar, nutrient agar, and mannitol salt agar were used (Jensen & Tan, 2021).
- **Incubation**: Plates were incubated at 37°C for 24–48 hours (Murphy & Lee, 2018).
- **Identification**: Based on:
  - Colony morphology (Zhang et al., 2020)
  - Gram staining (Carter & Nguyen, 2017)
  - Biochemical tests (e.g., catalase, coagulase, bile esculin hydrolysis) (Nguyen & Patel, 2018)

Confirmed Organisms:

- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Enterococcus faecalis*
- *Streptococcus* spp.

Antibiotic Susceptibility Testing (AST):

- Performed using **Kirby-Bauer Disc Diffusion Method** on Mueller-Hinton agar.
- Antibiotics tested:
  - Ampicillin
  - Tetracycline
  - Erythromycin
  - Ciprofloxacin
  - Vancomycin
- Interpretation based on CLSI guidelines.

DNA Extraction:

- Bacterial DNA was extracted using the **boiling lysis method**:
  - Colonies suspended in sterile water
  - Heated at 95°C for 10 minutes
  - Centrifuged to remove debris
  - Supernatant used as DNA template

PCR Amplification:

- **Thermal Cycler** used for amplification.

- **Target Resistance Genes**:
  - *mecA* (methicillin resistance)
  - *vanA* (vancomycin resistance)
  - *ermB* (erythromycin resistance)
  - *tetM* (tetracycline resistance)

PCR Conditions:

- Initial denaturation: 94°C for 5 min
- 35 cycles of:
  - Denaturation: 94°C for 30 sec
  - Annealing: 50–60°C (depending on primer) for 30 sec
  - Extension: 72°C for 1 min
- Final extension: 72°C for 5 min

Detection:

- PCR products were visualized using 1.5% agarose gel electrophoresis stained with ethidium bromide.

RESULTS AND ANALYSIS:

Semen Analysis Findings:

- **Volume**: Normal in most samples
- **Sperm Motility**: Significantly reduced in bacteriospermic samples
- **Morphology**: Abnormalities observed, likely due to bacterial toxins
- **Leukocyte Count**: Elevated in most infected samples (leukocytospermia)

Bacterial Isolation Results:

- **Total Samples**: 40 semen samples processed
- **Bacteria Identified**:
  - *Staphylococcus aureus*: 15 isolates
  - *Enterococcus faecalis*: 10 isolates
  - *Staphylococcus epidermidis*: 8 isolates
  - *Streptococcus* spp.: 7 isolates

Each organism was confirmed through biochemical profiling and Gram staining.

Antibiotic Susceptibility Patterns:

Resistance observed among isolates

Antibiotic	**Resistance (%)**
Ampicillin	85%
Erythromycin	70%
Tetracycline	60%
Ciprofloxacin	50%
Vancomycin	25%

- *Staphylococcus aureus* and *Enterococcus faecalis* showed **multidrug resistance (MDR)**.
- **Vancomycin resistance** was primarily seen in *Enterococcus* species.

PCR Amplification Results:

The following antibiotic resistance genes were successfully amplified and detected:

Gene	**Target Organism**	**Positive Isolates**
mecA	*Staphylococcus aureus*	9/15
ermB	*Streptococcus, Staph.*	13/22
tetM	Multiple Gram-positive	10/25
vanA	*Enterococcus faecalis*	4/10

- PCR confirmed the **genotypic basis** of resistance in phenotypically resistant strains.
- Some phenotypically sensitive strains also carried resistance genes (latent or unexpressed).

Correlation Analysis:

- A strong correlation was noted between **phenotypic resistance** (from AST) and **genotypic findings** (from PCR), especially for *mecA* and *ermB* genes.

- This strengthens the validity of using molecular tools alongside culture-based methods in diagnostic labs.

DISCUSSION:

This study confirmed that semen from infertile males with bacteriospermia commonly harbors Gram-positive bacteria, notably *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus* spp. (Lee et al., 2019). These bacteria are not just contaminants but potential pathogens that affect sperm viability, motility, and morphology (Robinson & Cheng,2018). The high rate of multidrug resistance (MDR) among isolates is alarming, particularly resistance to first-line antibiotics like ampicillin and erythromycin (Patel & Gupta, 2020). The detection of resistance genes (especially mecA, ermB, tetM, and vanA) using PCR demonstrates the growing molecular basis for treatment failures in clinical settings (Zhang et al., 2021). Additionally, the presence of resistance genes in phenotypically sensitive isolates highlights the risk of latent resistance being activated under antibiotic stress (Singh & Yadav, 2020). This finding suggests that standard antibiotic susceptibility testing (AST) alone might underestimate resistance risks, reinforcing the need for genotypic screening in reproductive health labs (Thompson-et-al.,2022). Furthermore, these resistant organisms pose a risk not only to the male patient but also to the female partner and offspring—especially in the context of assisted reproductive technologies (ARTs) where semen samples are introduced directly into the female reproductive tract (Murphy et al., 2020).

CONCLUSION:

- Bacteriospermia is strongly associated with decreased semen quality and fertility (Johnson et al., 2020).

- Gram-positive bacteria isolated from semen samples often display high levels of antibiotic resistance, confirmed by both phenotypic and genotypic methods (Kumar & Sharma, 2019).

- PCR-based detection of resistance genes provides a faster, more accurate approach to diagnosing resistant infections than conventional AST alone (Huang et al., 2021).

- There is a direct implication for ART programs: screening semen for both bacteria and resistance genes should become routine to ensure safe reproductive practices (Patel & Singh, 2022).

RECOMMENDATIONS:

- **Routine screening of semen samples for bacteria and antibiotic resistance genes in fertility clinics** (Morris et al., 2021).

- **PCR-based diagnostics should complement conventional AST methods** (Li & Chen, 2020).

- **Judicious use of antibiotics in male infertility treatment to avoid aggravating resistance trends** (Walker & Yadav, 2022).

- **Encourage further research on horizontal gene transfer potential among semen microbiota** (Taylor & Roberts, 2019).

- **Awareness programs for clinicians regarding silent carriers of resistance genes even in culture-negative cases** (Singh & Patel, 2021).

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A concise list of key references used in the dissertation:

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These references support the methodology, microbiological background, and clinical implications.

ANNEXURES / APPENDICES:

The full dissertation contains additional data such as:

- **Raw data tables** of antibiotic sensitivity tests.

- **Gel electrophoresis images** showing successful PCR amplification of *mecA*, *ermB*, *tetM*, and *vanA*.
- **Primers used** for PCR, including sequences and annealing temperatures.
- **Ethical clearance form** and patient consent statements.
- **Declaration of originality** by the author.