Original Research Article

Isolation, Characterization and Determination of Antibiotic Sensitivity Profile of some Bacteria Associated with Infertility in Male

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Abstract: The study was aimed to isolate, characterize and determination the antibiotic sensitivity profile of some bacteria associated with infertility in male attending Murtala Muhammad Specialist Hospital (MMSH) Kano, Nigeria. Two hundred (200) Semen specimens were collected from males with infertility attending the clinic and General out Patient Department of MMSH Kano. The seminal fluids were diluted with sterile saline, centrifuged and cultured on Nutrient agar, Blood agar, Chocolate agar and Mac Conkey agar then incubated aerobically and in 5% CO_2 at 37°C for 24 hours for the isolation of pathogenic microorganism. Isolates were identified based on Gram's staining, biochemical tests and API 20E Test. The result shows a total of seventy six (76) isolates were obtained, *Staphylococcus aureus* was found to have the highest occurrence of 31 (40.79%), whereas the least was found to be *Mycoplasma* species, 1 (1.32%). Other microorganisms encountered include; Coagulase negative *Staphylococcus* species 14 (18.42%)*, Escherichia coli* 11 (14.47%), *Klebsiella pneumoniae* 6 (7.89%), *Proteus mirabilis* 5 (6.58%), *Pseudomonas aeruginosa* 6 (7.90%) and *Neisseria gonorrhoeae* 2 (2.63%). The isolates were most sensitive to ciprofloxacin and ofloxacin. Ceftriaxone and cefuroxime however showed superior activity against most of the isolates. The activity of the Tetracycline and Augmentin against almost all the isolates was not appreciable. Cotrimoxazole and Gentamicin showed moderate activity against most of the isolates and no activity was observed on Mycoplasma and *Neisseria gonorrhoeae* against most of the antibiotics.

Keywords: Antibiotics, bacteria, infertility, semen, sensitivity pattern.

INTRODUCTION

The emergence of bacterial antimicrobial resistance has made the choice of empirical therapy more difficult and expensive [1]. Hence there is need for regular screening of organisms causing various infections and to characterize their antimicrobial susceptibility pattern to commonly used antibiotics at the hospital, regional, national and global levels to guide the clinicians to select a relevant antimicrobial for empirical treatment of infections. Infertility is the inability of a sexually active, non-contraception couple to achieve spontaneous pregnancy in one year. About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility [2]. One in eight couples encounters problems in attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Both sexes are more or less equally involved in infertility problem [3]. Men either alone or along with their female partners, contribute to 40–45% cases of infertility [4]. Furthermore, infectious aetiology involving bacteria, virus, fungi, and protozoa contribute to 15% of male factor infertility [5].

Microbial infections of the genital tracts or semen are major causes of male infertility [6,7]. According to World Health Organization (WHO) [2], semen consists of concentrated suspension of spermatozoa and the fluid secreted by the accessory sex organs namely prostate gland, seminal vesicles, bulbourethral glands, and epididymides. The fluid secretion is about 90% of semen volume and dilutes the concentrated epididymal spermatozoa at ejaculation. Since the ejaculate is a mixture of secretions derived from the urogenital tract and the male accessory glands, seminal culture identifies the presence of germs in any section of the seminal tract [8].

Male urogenital tract infections are one of the most important causes of male infertility worldwide [9]. Askienazy-Elnhar [10] reported that genital tract infection and inflammation have been associated to 8-35% of male infertility cases and male accessory sex glands infection is a major risk factor in infertility [5]. Studies have also shown that when the characteristics of semen infected and uninfected were compared, semen with micro-organisms had poor indices of fertility [11]. Infertility affecting couples around the World is both a medical as well as social problem particularly in Nigeria [12]. Evidences are being accumulating on the association of asymptomatic bacteriospermia and altered semen quality [13]. There is difference as to the influence of certain microbial infection on male infertility [14]. Urinary tract infections are common in men and clinicians working with infertility frequently encounter patients with these diseases [15]. More than 90% of male infertility cases is due to either low sperm count (oligospermia), no sperm at all (azoospermia) or poor seminal fluid quality or combination of the two and this claimed to the increase prevalence of sexually transmitted diseases (STDs) and urogenital infections alarmed since 1992 [16]. Previous studies have identified *Staphylococcus aureus, E. coli, Citrobacter* species, *Klebsiella* species, *Pseudomonas aeruginosa, Proteus vulgaris, and Proteus mirabilis* with infertility in male partners of infertile couples [7, 9, 17]. The study was aimed to isolate, characterize and determination the antibiotic sensitivity profile of some bacteria associated with infertility in male attending Murtala Muhammad Specialist Hospital (MMSH) Kano, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted at Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH), Kano. Kano state is located in the Northwestern Nigeria with coordinates 11^0 30' N 8^0 30' E. It shares borders with Kaduna state to the West, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the North. It has a total area of $20{,}131 km^2$ (7,777sqm) and population of 11,058,300 [18].

Sample Collection

Semen specimens were collected from males with infertility attending the clinic and GOPD of MMSH Kano. The samples were collected from patients who have had 3-7 days of sexual abstinence from intercourse preferably by masturbation into a sterile clean wide-mouth container. Upon collection, samples were transferred without any delay to the Microbiology Department of MMSH in a nearly as possible to body temperature by placing the container inside a flask containing water at 33-37^0C. Time of collection to the time the samples were received in the laboratory was recorded which must not exceed 45 minutes. Furthermore, analyses of samples with SQA machine were conducted at Microbiology Department, Aminu Kano teaching hospital (AKTH), Kano.

Culture

Culture of seminal fluid samples was done in aseptic condition within one hour of collection in accordance with W.H.O. [2]. The seminal fluids were diluted with sterile saline (1:10) and centrifuged at1500 rpm for 15 min. After removing the supernatant, the sediments were cultured using 10µl calibrated loops on Nutrient agar, Blood agar, Chocolate agar and MacConkey agar which were incubated aerobically and in 5% CO_2 at 37°C for 24 hours for the isolation of pathogenic microorganism [2]. Semino-culture were considered positive when the number of colonies was $\geq$ 10^4 CFU /ml for Gram positive cocci and $\geq 10^5$ CFU/ml for Gram negative rods [19].

Isolation and Identification of Isolates

Nutrient agar plates were prepared for the isolation of pure colonies from the primary plates. A colony was picked and streaked on nutrient agar plates and then incubated at 37^0C for 24 hours. Using a sterile wire loop, a colony from each plate was picked and prepare for Gram's staining and other biochemical tests, some of which include Catalase, Coagulase, DNAse, Triple iron agar, Oxidase and API 20E Test [20].

Gram's stain

The smear was made from the isolate on a clean grease free slide and allowed to air dried and fix. The smear was flooded with crystal violet as a primary stain and was allowed to stain for 2 minutes and rinsed with water. A mordant (Lugol's iodine) was then flooded and allowed to stay for 1 minute and rinsed with water. A smear was then flooded with secondary stain (neutral red) and was allowed to stain for 2 minutes and then rinsed in water and allowed to air dried [21].

Catalase test
Few colonies of the test organisms were emulsified in distilled water on a clean slide. 1-2 drops of 3% H_2O_2 were added and cover with cover slip [20].

Coagulase test
A drop of plasma was placed on a clean dried slide. A drop of saline was place next to the drop of plasma as control. With a loop a portion of the isolated colonies were emulsified in each drop starting with the saline until a smooth suspension was obtained. The suspension was then mixed well, and rocked gently for 5-10 seconds [20].

DNAse test
Using a sterile loop, test and control organism (ATCC 2923) were spot-inoculated and incubated at 35-37 overnight. The surface of plate was covered with 1mol/ml hydrochloric acid solution and excess was tipped off. Clearing around each colony was observed within 5 minutes of adding the acid [20].

Oxidase test
A piece of filter paper was moisture with substrate (1% tetramethyle –p-phenylene-diamine dihydrochloride). A wooden stick was used to remove small portion of bacterial colony and streak across the wetted filter paper Streaked area on wetted filter paper was observed for color change to deep blue [20].

Urease test
The surface of the urea slant agar was streaked with a portion of well isolated colonies. The cap of the slant was left on loose and incubates at $35^{o}c$ for 18-24 hours [20].

Citrate utilization test
The surface of the Simmons citrate agar slant was streaked with a portion of a well isolated colony. The cap of the slant was left on loosely and was incubates at $35^{o}c$ for 18-24 hours [20].

Carbohydrate utilization test
0.1ml of a heavy saline suspension of the test organism was added to each of the four tubes containing glucose, lactose, maltose and sucrose carbohydrate disk and no to the fifth tube and was incubated at $37^{0}C$ for 5 hours. It was examined at 30 minute intervals for up to 5 hours from red to yellow indicating carbohydrate utilization [22].

Analytical Profile Index (API) 20E test
5 ml ample of API NaCl 0.85% medium was opened. Using a pipette a single well- isolated colony from an isolation plate was removed. It was carefully emulsified in 5 ml ample of API NaCl 0.85% to obtained homogeneous bacterial suspension. Using the same pipette, both tube and cupule of the test CIT, VP and GEL was full with the bacterial suspension. Anaerobiosis was created in the tests ADH, LDC, ODC, H2S, and URE by overlaying with mineral oil. The incubation box was closed and incubated at $36^{0}C$ for 24 hours [20].

Antimicrobial Susceptibility Test
Antimicrobial susceptibility test was performed on significant cultures using standard disc diffusion technique using modified Kirby-Bauer method [23]. Using a sterile loop, well isolated colonies of similar appearance was emulsified in 3-4 ml of sterile physiological saline. In a good light, the turbidity of the standard was match with the turbidity of the standard, which was viewed against a printed card. Using a sterile swab, a plate of Mueller Hinton agar were inoculated (excess fluid was removed by pressing and rotating against the side of the tube). The swab was streak over the surface of the M-H agar in three directions. The surfaces of the plates were allowed to dry by allowing staying for 3-4 minute. An appropriate antimicrobial disk was evenly distributed on the inoculated plates. Within 30 minute of applying the disks. The plates were inverted and incubated appropriately depending on the organism. After overnight incubation, the control and test plates were examined to ensure the growth is confluent or near confluent. Using a ruler a zone of inhibition was measured in mm [23]. Using interpretative table, the zone sizes of each antimicrobial was measured, reporting the organism as either Sensitive (**S**) or Resistance (**R**) [23].

RESULTS

Isolation of Microorganisms
The microbial isolates obtained from seminal fluids analyzed in laboratory are presented in Table 1. The result shows a total of seventy six (76) isolates were obtained, *Staphylococcus aureus* was found to have the highest occurrence of 31 (40.79%), whereas the least was found to be *Mycoplasma* species, 1 (1.32%). Other microorganisms encountered include; Coagulase negative *Staphylococcus* species 14 (18.42%), *Escherichia coli* 11 (14.47%), *Klebsiella pneumoniae* 6 (7.89%), *Proteus mirabilis* 5 (6.58%), *Pseudomonas aeruginosa* 3 (3.95%) and *Neisseria gonorrhoeae* 2 (2.63%).

Table 1: Various organisms isolated and their frequencies of occurrence

Isolates	Number (n)	Percentage (%)
Coagulase negative *Staphylococcus species*	14	18.42
Staphylococcus aureus	31	40.79
Escherichia coli	11	14.47
Klebsiella pneumoniae	6	7.89
Proteus mirabilis	5	6.58
Pseudomonas aeruginosa	6	7.90
Mycoplasma species	1	1.32
Neisseria gonorrhoeae	2	2.63
Total	**76**	**100**

Antimicrobial Susceptibility Test

The susceptibility patterns of the bacterial isolates to various antibiotics are presented in table 2. From the results, the isolates were most sensitive to ciprofloxacin and ofloxacin. Ceftriaxone and cefuroxime however showed superior activity against most of the isolates. The activity of the Tetracycline and Augmentin against almost all the isolates was not appreciable. Cotrimoxazole and Gentamicin showed moderate activity against most of the isolates and no activity was observed on Mycoplasma and *Neisseria gonorrhoeae* against most of the antibiotics.

Table 2: Susceptibility (%) patterns of bacterial isolates to various antibiotics

Organisms	CTR (30µg)	CXM (25µg)	TET (30µg)	AUG (30µg)	COT (25µg)	GEN (10µg)	CIP (10µg)	OFL (5µg)
Staphylococcus aureus	54.8	64.5	9.7	22.6	38.7	22.6	74.2	80.6
CNSS	57.1	64.3	14.3	14.3	35.7	14.3	78.6	64.3
Escherichia coli	63.6	63.6	9.1	18.2	45.5	54.5	109.1	109.1
Proteus mirabilis	60.0	80.0	40.0	60.0	0.00	60.0	80.0	40.0
Klebsiella pneumoniae	83.3	100	0	33.3	50	16.7	66.7	50.0
P. aeruginosa	0.0	33.3	0.0	0.0	0.0	66.7	66.7	66.7
Mycoplasma species	0.0	0.0	0.0	0.0	0.0	0.0	100	100
Neisseria gonorrhoeae	0.0	50.0	0.0	0.0	0.0	0.0	0.0	50.0

Key: CNSS = Coagulase negative *Staphylococcus species*

Susceptibility status of bacterial isolates

The susceptibility status of the bacterial isolates against various antibiotics used is presented in Table 3. Isolates are categorized as Sensitive (S) or Resistant (R) to the antibiotics.

Table 3: Susceptibility status of bacterial isolates to various antibiotics

Organisms	CTR (30µg)	CXM (25µg)	TET (30µg)	AUG (30µg)	COT (25µg)	GEN (10µg)	CIP (10µg)	OFL (5µg)
Staphylococcus aureus	S	S	R	R	R	R	S	S
CNSS	S	S	R	R	R	R	S	S
Escherichia coli	S	S	R	R	R	S	S	S
Proteus mirabilis	S	S	S	S	R	S	S	S
Klebsiella pneumoniae	S	S	R	R	S	R	S	S
P. aeruginosa	R	R	R	R	R	S	S	S
Mycoplasma species	R	R	R	R	R	R	S	S
Neisseria gonorrhoeae	R	S	R	R	R	R	R	S

Key: CNSS = Coagulase negative *Staphylococcus species*

DISCUSSION

The study was aimed to isolate, characterize and determination the antibiotic sensitivity profile of some bacteria associated with infertility in male attending Murtala Muhammad Specialist Hospital (MMSH) Kano, Nigeria. From the results, a total of 76 isolates were obtained and categorized into 8 different isolates. *Staphylococcus aureus* was found to have the highest occurrence, 31 (40.79%), whereas the least isolate was found to be *Mycoplasma* species, 1 (1.32%). Other microorganisms encountered include; coagulase negative *Staphylococcus,* 14 (18.42%)*, Escherichia coli* 11(14.47%)*, Klebsiella pneumoniae* 6(7.89%), *Proteus mirabilis* 5(6.58%), *Pseudomonas aeruginosa* 3 (3.95%), *Neisseria gonorrhoeae* 2 (2.63%). The higher prevalence of *S. aureus* in the study agrees with previous findings [6, 7, 9, 24, 25]. Komolafe and Awoniyi [25] reasoned that, the isolation of *S. aureus* might be associated with body hygiene of

the couples involved.

Moreover, Charanchi *et al.,* [26] contends that, the higher occurrence of *S. aureus* in semen of patients with infertility could be attributed to its minimal growth requirements, high resistance to environmental factors and ability to colonize and establish infection. Conversely, the least occurrence of *Mycoplasma* species in this study disagrees with the work of Domes *et al.,* [24]. Coagulase-negative *Staphylococcus* species are known opportunistic pathogens which are usually involved in Nosocomial and human urinary infections. Since urinary tract always act as locus of infections for the seminal tract, the heterogeneity of microorganisms encountered are capable of causing classical infections of the urogenital tract [27] and according to Ibadin and Ibeh [9] male urogenital tract infections are one of the most important causes of male infertility worldwide.

The susceptibility patterns of the isolates to various antibiotics showed that the isolates were most sensitive to ciprofloxacin and ofloxacin. Ceftriaxone and Cefuroxime however showed superior activity against most of the isolates. The activity of the Tetracycline and Augmentin against the isolates was found be very poor. Cotrimoxazole and Gentamicin showed moderate activity against most of the isolates and no activity was observed on Mycoplasma and *Neisseria gonorrhoeae* against most of the antibiotics. This agrees with the works of Onemu *et al.,* [6] as well as Komolafe and Awoniyi [25] who reported similar findings in their individual researches. The activity of Ciprofloxacin and Ofloxacin against these isolates may be due to the potent inhibition of beta-lactamase produced by most isolates especially *S. aureus* [28]. Similarly, the two antibiotics are Fluoroquinolones known for their high pKa and lipid solubility [8]. Moreover, poor activity of tetracycline and Augmentin may be partly influenced by the widespread misuse of these agents in Kano state as reported in a similar study by Onemu *et al.,* [6]. Resistance to most of these antibiotics by the bacterial isolates could be due to genetic mutations and poor uptake of the antibiotics [25].

CONCLUSION

From the finding of this study, eight (8) different bacterial isolates were recovered from bacteriological analysis of semen of male patients with infertility attending Murtala Muhammad Specialist Hospital (MMSH) Kano. The bacterial isolates include; *Staphylococcus aureus, Mycoplasma* species, Coagulase negative *Staphylococcus* species, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae.* On sensitivity testing, the isolates were most sensitive to ciprofloxacin and Ofloxacin. The activity of the Tetracycline and Augmentin against almost all the isolates was not appreciable and no activity was observed on Mycoplasma and *Neisseria gonorrhoeae* against most of the antibiotics.

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