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Characterization and antibiotic susceptibility profile of *Escherichia coli* from semen of male patients with infertility

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Abstract

The study was aimed to characterize and determine the antibiotic sensitivity pattern of *Escherichia coli* isolated from semen of male patients with infertility attending Murtala Muhammad Specialist Hospital (MMSH) Kano, Nigeria. One hundred and sixty five (165) 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 MacConkey 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 pneumonia* 6 (7.89%), *Proteus mirabilis* 5(6.58%), *Pseudomonas aeruginosa* 6 (7.90%) and *Neisseria gonorrhoeae* 2 (2.63%). The sensitivity and resistivity of *E. coli* against the antibiotics, the result showed high sensitivity of isolates to ciprofloxacin (81.8%), ofloxacin (72.7%), co-trimoxazole (63.3%) and gentamicin and augmentin ((54.5%) each. On the other hand, high degree of resistivity rates to tetracycline (81.8%), Cefuroxime (63.6%) and ceftriaxone (54.5%), were detected. It has been concluded that *E. coli* is one of the organism associated with infertility in men.

Keywords: antibiotics, *Escherichia coli*, infertility, semen, resistance

Introduction

Microbial resistance to antibiotics is one of the most serious health problems threatening human well-being today. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic [1]. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria [2]. Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics (antimicrobials used to treat bacterial infections) [3]. This resistance has complicated the selection of antibiotics for the treatment of pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole [4].

Microbial infections of the genital tracts or semen are major causes of male infertility [5, 6]. According to World Health Organization [7], 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. Askienazy-Elnhar [9], 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 [10]. 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 [17, 18].

E. coli resistance to antimicrobials is creating trouble to the

healthcare system worldwide [19]. This complicates treatment outcomes, increases the cost of treatment, and limits the therapeutic options that contribute to the global spectra of a post antimicrobial age in which some of the most effective drugs lose their efficiency [20]. The bacterium is becoming highly resistant to conventionally used antibiotics (to both the newer and older medicines) as evidenced by many previous studies [21–25]. Adaptive resistance was supposed to be the main mechanism for the development of resistance including that to lethal doses of the antimicrobials [26]. The study was aimed to characterize and determine antibiotic susceptibility profile of *Escherichia coli* from semen of male patients with infertility at Murtala Muhammad specialist hospital 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 North-west Nigeria with coordinates 11^0 30 N 8^0 30 E. It shares borders with Kaduna state to the south- west, Bauchi state to the South-East, Jigawa state to the East, Katsina state to the West and Niger republic to the North. It has a total area of 20,131km^2 (7,777sqm) and population of 11,058,300 [27].

Study Population

A total of 165 samples were collected from male patients who had either primary or secondary infertility cases. Inclusion criteria for patients include male patients with any kind of infertility attending MMSH. Exclusion criteria include; Male patients who refused to observed at least three days of sexual abstinence prior to the test and patients who refused to suspend any chemo antibiotic treatment for at least one week prior to sample collection

Ethical clearance

An approval (MOH/off/797/T.I/49) for the study was obtained from Research and Ethic committee Kano state ministry of health. The aim of the study was explained clearly to the clients and informed consent obtained before proceeding to the study.

Determination of sample size

Sample size for the study was determined from a standard formula for the calculation of minimum sample size [28]. Sample size was given by the formula;

$$N = (Z_1\text{-}a)^2 (p) (1\text{-}p) / d^2$$

N = minimum sample size, $Z_{1-}a$ = value of standard normal deviate which at 95% confidence interval has found to be 1.96. P = the best estimate of prevalence obtained from literature review (12.3%) and d = difference between the true population rate and sample that can be tolerated, this is the absolute precision (in percentage) on either side of the population.

N = (1.96)2 (0.123) (1-0.123)/ $(0.05)^2$ = 165.758 as the minimum number of samples for the study. Therefore, a total of 166 were used.

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 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 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.

Isolation and identification of *Escherichia coli*

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.

The preserved isolates were confirmed as *E. coli* using conventional microbiological methods which include Gram staining, lactose fermentation, cystine-lactose-electrolyte deficient (CLED) medium growth and motility test as well as biochemical (Indole, methyl orange, voges proskauer, nitrate reduction and citrate utilization) tests according to the methods described by Holt *et al*. [29].

Antibiotic Susceptibility Testing

Antimicrobial susceptibility test was performed on significant cultures using standard disc diffusion technique using modified Kirby-Bauer method [30]. Using a sterile loop, well isolated colonies of similar appearance was emulsified in 3-4ml 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 Mueller Hinton agar in three directions. The surfaces of the plates were allowed to dry by allowing staying for 5 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. Using interpretative table, the zone sizes of each antimicrobial was measured, reporting the organism as either Sensitive (S) or Resistance (R) [30].

Results

Identification of *Escherichia coli*

The result for identification of *E. coli* is presented in Table 1 below. The isolate was identified based on Gram staining, cultural characteristics and biochemical test. The result showed that the isolate is Gram negative bacteria, positive for indole, MR and motility test but negative for VP and citrate utilization test. The isolate ferment lactose and produce Opaque yellow colony in CLED agar

Table 1: Morphological and Biochemical Characterization of *E. coli*

S/N	Test	Inference
1	Gram staining	-
2	Citrate utilization	-
3	Indole	+
4	Methyl-red	+
5	Voges Proskauer	-
6	Nitrate reduction	+
7	Motility	+
8	Lactose fermentation	+
9	CLED medium growth	Opaque yellow colony

Prevalence of *Escherichia coli*

The microbial isolates obtained from seminal fluids analyzed in laboratory are presented in Table 2. 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 (6.59%), *Pseudomonas aeruginosa* 6 (7.89%) and *Neisseria gonorrhoeae* 2 (2.63%).

Table 2: Various organisms isolated and their frequencies of occurrence

Isolates	Number (n)	Prevalence (%)
Staphylococcus aureus	31	40.79
Coagulase negative Staphylococcus	14	18.42
Escherichia coli	11	14.47
Pseudomonas aeruginosa	6	7.89
Klebsiella pneumoniae	6	7.89
Proteus mirabilis	5	6.59
Neisseria gonorrhoeae	2	2.63
Mycoplasma species	1	1.32

Antibiotic Sensitivity Testing

The sensitivity and resistivity of *E. coli* against the antibiotics used is presented below (Table 3). Some of the antibiotics were active against the isolates while resistance to others. The result showed high sensitivity of isolates to ciprofloxacin (81.8%), ofloxacin (72.7%), co-trimoxazole (63.3%) and gentamicin and augmentin ((54.5%) each. On the other hand, high degree of resistivity rates to tetracycline (81.8%), Cefuroxime (63.6%) and ceftriaxone (54.5%), were detected.

Table 3: Sensitivity and resistivity of *E. coli* against some antibiotics

Antibiotics	Concentration (µg)	Sensitive (n) (%)	Resistance (n) (%)
Ceftriaxone	30	5 (45.5)	6 (54.5)
Cefuroxime	25	4 (36.4)	7 (63.6)
Tetracycline	30	2 (18.2)	9 (81.8)
Augmentin	30	6 (54.5)	5 (45.5)
Co-trimoxazole	25	7 (63.6)	4 (36.4)
Gentamicin	10	6 (54.5)	5 (45.5)
Ciprofloxacin	10	9 (81.8)	2 (18.2)
Ofloxacin	10	8 (72.7)	3 (27.3)

Discussion

E. coli is one of the most widespread bacteria throughout the world. Some strains of *E. coli* can cause serious illness for humankind including urinary tract infections, bloodstream infections, skin infection, otitis media, and diarrhea. 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, (40.79%), whereas the least isolate was found to be *Mycoplasma* species, (1.32%). Other microorganisms encountered include; coagulase negative *Staphylococcus*, (18.42%), *Escherichia coli* (14.47%), *Klebsiella pneumoniae* (7.89%), *Proteus mirabilis* (6.58%), *Pseudomonas aeruginosa* (3.95%), *Neisseria gonorrhoeae* (2.63%). The higher prevalence of *S. aureus* in the study agrees with previous findings [31 ,32, 33, 34, 35]. Komolafe and Awoniyi [35] reasoned that, the isolation of *S. aureus* might be associated with body hygiene of the couples involved. Moreover, Charanchi *et al*. [36] 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*. [34]. 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 [37] and according to Ibadin and Kai [33] male urogenital tract infections are one of the most important causes of male infertility worldwide.

In this study, the overall resistance of *E. coli* to antimicrobials was low. The result showed high sensitivity of isolates to ciprofloxacin (81.8%), ofloxacin (72.7%), co-trimoxazole (63.3%) and gentamicin and augmentin ((54.5%) each but high degree of resistivity rates to tetracycline (81.8%), Cefuroxime (63.6%) and ceftriaxone (54.5%). The result is consistent with the findings of previous studies. The resistance rate in this study was in conformity with that of Khan *et al*. [38]. High resistance rate of *E. coli* to Cefuroxime and ceftriaxone in this study justify the study conducted by Sabir *et al*. [39] who reported 100% resistant of *E. coli* to beta lactam drugs. Resistance to tetracycline in this study was in line with the study conducted by Nas *et al*. [40], Andargachew *et al*. [41] and Petkovsjek *et al*. [42] in Nigeria, Sudan and Slovenia respectively. One of the major reasons for this high resistance can be co-expressed resistance mechanisms in the species. Increasing rates of antimicrobial resistance among *E. coli* is a growing concern worldwide. Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries. The higher resistance against the above antimicrobials could be as a result of repeated or prolonged use or exposure of pathogens to antibiotics repeated use of antibiotics can damage urethral flora, allowing pathogens to colonize and subsequently to infect male reproductive tract, leaving clinicians with very few choices of drugs for the treatment of infertility. Moreover, this condition enables bacteria to exchange their genetic material through horizontal gene transfer resulting in resistant gene that confer resistance to

particular antibiotics [43]. In this study, *E. coli* isolates were sensitive to ciprofloxacin, ofloxacin, gentamicin, and co-trimoxazole. Similar studies conducted by Okonko *et al*. [44] in Nigeria have reported comparable susceptibility rates. High sensitivity to ciprofloxacin and gentamicin was also recorded from previous studies conducted in India [45].

Conclusion

Escherichia coli are one of the causes of infertility in humans. The finding of this study revealed that *E. coli* was observed as the most common etiologic agent of infertility. Its prevalence accounted for 14.47% of the semen samples examined. The result showed high sensitivity of isolates to ciprofloxacin, ofloxacin and co-trimoxazole but high degree of resistivity rates to tetracycline, Cefuroxime and ceftriaxone. The judicious use of antibiotic is recommended which will help to limit the increasing rate of drug resistance in the pathogens.

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