



THE ANTIBIOTIC RESISTANCE PROFILE OF *STAPHYLOCOCCUS* ISOLATES IN OUTPATIENT INFERTILE SUBJECTS IN ESAN, NIGERIA

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ABSTRACT

This study investigates the antibiogram pattern of *staphylococcus* species isolates from infertile subjects with urinary tract infection in Esan-Nigeria, considering the variation in antibiotics susceptibility pattern based on location, gender and season. It is our assertion that co-morbidity with other health issues can also influences antibiotics susceptibility and resistivity activities. A retrospective study was carried out among individuals with fertility problems and positive for urinary tract infections from three health facilities within the period of January 2013 to December 2013. Collected samples; semen and high

vaginal swab were screened for UTI and antibiotic susceptibility of isolated *Staphylococcus* species pathogens and tested against 21 antibiotics. 220 samples were analyzed and made up of 139 semen and 81 high vaginal swab samples. In the semen samples were isolated 75.54% *Staphylococcus aureus*, 23.02% *Staphylococcus saprophyticus* and 1.44% *Staphylococcus epidermidis* and isolates in the high vaginal swabs were 76.54% of *S. aureus* and 23.46% of *S. saprophyticus*. Cefuroxime (21.10%) and Norfloxacin (40.63%) were the most sensitive against *S. aureus* and *S. saprophyticus* in the semen samples. On the other hand, Cephalexin (25.81%) and Streptomycin (26.32%) were the most sensitive against *S. aureus* and *S. saprophyticus* in the HVS samples. Ampicillin, Cloxacilin, Augmentin, Ampiclox, Co-trimoxazole, Chloramphenicol, Lincocin, Erythromycin, Azithromycin, Tetracycline, Cefuroxime, Norfloxacin, and Ciprofloxacin and Cephalexin were the most resistant against organism in semen and HVS isolates.

KEYWORDS: Infertility, urinary tract infection, *staphylococcus* species, antibiotic susceptibility.

INTRODUCTION

Infertility by the International Committee for Monitoring Assisted Reproductive Technology and the World Health Organization is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.^[1,2] It constitutes a grave emotional and social problem especially in societies where great importance is attached to having children.^[3,4] The contradictory scenario however, is that Sub-Saharan Africa characterized by high fertility rate also has high infertility rate estimates suggest that about 20% to 30% to experience primary and secondary infertility.^[5] Although the most affected areas lie within the central African region “infertility belt”^[5,6], there are indications of high and rising prevalence levels in Nigeria.^[4]

Infertility is generally known to be caused by a variety of factors; male and female alike.^[7] The female factors contribute most (that is 40 to 55%) in the etiologies of infertility followed by male factors (30 to 40%), both partners (10%) and unexplained factor (10%).^[8] Bacteria infections have long been recognized in associated with infertility.^[9,10] However, the significance of these infections in the female and male genital tracts is not well known.^[11,12] It was estimated that about two-thirds of the cases of infertility in Nigeria are attributable to infections.^[13] The laparoscopic investigation of infertility in Nigerian hospitals has demonstrated the presence of pelvic infection and bilateral tubal occlusion in 35% of infertile women in Ibadan^[14], 44% in Ile-Ife^[15] and 65% of women in Jos.^[16]

Staphylococci are ubiquitous human parasites^[17], infecting the skin, the human respiratory tract and urogenital tract.^[18] *Staphylococcus* is a dangerous human pathogen in both community-acquired and nosocomial infections and a fundamental biological property of this bacterium is its ability to asymptotically colonize healthy individuals.^[19] Although it has been reported that *Staphylococcus aureus* is a relatively uncommon cause of UTI^[20,21], currently findings however showed it is the most frequently isolated Gram positive cocci in suspected UTI cases.^[18,22] *Staphylococcus* carriers are at higher risk of infection, and they are presumed to be an important source of the *S. aureus* strains that spread among individuals.^[23] Besides this, *S. aureus* is arguably the dominant organism implicated in primary infertility, among males and females alike.^[24]

Due to different parental investment of the two sexes, males have evolved a preference for higher baseline (uninfected) promiscuity in humans^[25], as well as in most other species^[26], which may allow for greater gain by manipulating female reproductive behaviour. These asymmetries imply that the benefits of infertility may be greater for the pathogen when imposed on a female, compared with a male host. Remarkably, the higher risk of infertility contrasts with a lower probability of symptomatic infection in women compared with men^[27] this further disconnection between general virulence (symptoms) and infertility may also hint at targeted patho-mechanisms for the latter. However, it cannot be completely excluded that the simple non-selectionist explanation that ascending infection (associated with infertility) is more likely in the female than in the male reproductive system due to anatomical differences. Infertility has often assumed to be a problem that only affects women.^[1,2,28] and the male factor infertility has largely been neglected, but it has been found that factors affecting men account for about 40% of the causes and semen quality is used as a surrogate measure of male fecundity.^[28] In this study, we investigated the antibiogram pattern of *staphylococcus* species isolated from infertile UTI co-infected subjects. The study in male and female was to evaluate if *staphylococcus* species isolated from both sexes response to antibiotics differently in infertile UTI co-infected individuals.

MATERIALS AND METHODS

The study cohorts comprised of subjects having UTI and infertility problems and are in-patients and out-patients of Irrua Specialist Teaching Hospital, Irrua, Esan Central Local Government Area, and Gilead Hospital, Ekpoma and Calvary Medical Centre, Ekpoma, both in Esan West Local Government Area, in Edo State, Nigeria. Two hundred and twenty semen and high vaginal swabs consisting of male (n=139) and female (n=81) subjects were recorded and met the criteria for inclusion. Exclusion criteria were strictly those on antibiotics for conditions related or unrelated to UTIs.

The specimens obtained from these subjects were subjected to antibiotics susceptibility testing which was done by the single disc diffusion agar method.^[29,30] The isolates were subjected to basic antibiotics that as previously reported in Momoh *et al.*^[24] and known to be effective against *Staphylococcus* species such as Amoxicillin, Ampicillin, Cloxacilin, Augmentin, Ampiclox, Flucloxacilin, Gentamicin, Streptomycin, Neomycin, Co-trimoxazole, Chloramphenicol, Tetracycline, Lincocin, Erythromycin, Azithromycin, Cephalexin, Rifampicin, Cefuroxime, Ofloxacin, Noreloxacin, Ciprofloxacin following the Ajumali's

mnemonic coding method as described by Joghi *et al.*^[31] Kirby-Bauer disk diffusion method was adopted. It is a test which uses antibiotics impregnated wafers to test whether a particular bacterium is sensitive or resistant to specific antibiotics.

A 18 – 24 hours old culture of the test organism grown in sterile normal saline was vortexed and with sterile inoculating loop standardized 10^5 cfu/ml of the test isolate was transferred and spread onto Mueller Hilton agar plates. A sterile forcep was used to pick antibiotic discs in the form of thin wafers, transferred to the seeded Mueller Hilton's agar plates and incubated at 37°C for 24 hours. The results of the susceptibility tests were interpreted as sensitive, intermediate or resistance following the criteria described by Stokes and Ribway^[32] and the National Committee for Clinical Laboratory Standard, as described by Akinjogunla and Enabulele^[33], that is, a zone's radius equal to or not more than 3mm smaller than the control was taken as sensitive, a zone's radius more than 3mm smaller than the control and not less than 3mm was reported as intermediate and a zone's radius 2mm or less i.e., no zone of inhibition was reported as resistance.

Antibiogram Mnemonic Typing (Ajumali's Mnemonic Coding) for the determination of the antibiogram types of the isolated bacterial species, the Ajumali's method of mnemonic coding was adapted as described by Joghi *et al.*^[31] Using the mnemonic coding, a sensitivity result was recorded as (+), while a resistant result was recorded as (-). Twenty-one (21) antibiotics were used and these antibiotics were divided into seven (7) groups of three (3) antibiotics each, using their mechanisms of action and clinical indication as criteria for grouping. The three (3) antibiotics in each group were assigned arbitrary values of 1, 2 and 4 for the first, second and third antibiotics respectively. A perfect sensitivity for all three (3) antibiotics was recorded as seven (7), that is, $1 + 2 + 4 = 7$; while complete resistance (no sensitivity) to all three (3) antibiotics was recorded as zero (0), that is, $0 + 0 + 0 = 0$ [34]. All data were analyzed using simple descriptive statistic.

RESULTS

Table 1 showed the distribution of *staphylococcus* isolated from the clinical samples of enrolled cohorts with co-existing urinary tract infections and infertility. Of the 139 semen samples with *staphylococcus* species, 75.54% were *Staphylococcus aureus* and 23.02% and 1.44% were positive for *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* respectively. However, the 81 high vaginal swabs consisted of 76.54% of *Staphylococcus aureus* and 23.46% of *Staphylococcus saprophyticus*.

Table 4.4: Distribution of isolated *staphylococcus* species from the clinical samples of enrolled cohorts co-existing urinary tract infections and infertility

<i>Staphylococcus</i> species	Semen	High vagina swab
<i>Staphylococcus aureus</i>	105 (75.54%)	62 (76.54)
<i>Staphylococcus saprophyticus</i>	32 (23.02%)	19 (23.46)
<i>Staphylococcus epidermidis</i>	2 (1.44%)	0 (0.0%)
TOTAL	139	81

Figure 1 and 2 showed the *in vitro* susceptibility pattern of the various *Staphylococcus* strains isolated from semen samples. Of the 105 isolates of *S. aureus* from semen samples, Cefuroxime presented the most potent antibiotic with sensitivity of 23/109 (21.10%) while Augmentin was the least sensitive (7/105; 6.67%). Strain 40 (Mnemonic code: 0000000), was of significance as it appeared to be a superbug, showing no susceptibility to any of the 21 test antibiotics. Other strains such as strain 26 (Mnemonic code: 0000010), was also highly resistant as it was sensitive to only Cephalexin. Strain 41 (Mnemonic code: 2001124) was one of the least resistant strains, showing susceptibility to five different antibiotics.

S. saprophyticus from semen samples were all sensitive to the antibiotics however, none was 50% sensitive. Overall, Norfloxacin was the most sensitive (13/32; 40.63%) while Cloxacilin was the least sensitive (1/32; 3.13%). On antibiogram types, with the exception of strains 10, 22 and 29 (Mnemonic codes: 0010002, 0000021, 0000011 and 0000022), other strains were susceptible to three or more antibiotics.

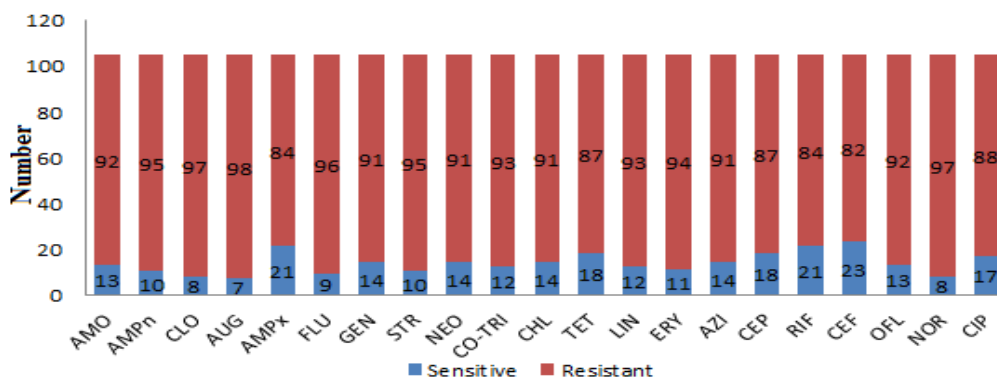


Figure 1: The antibiogram types of *staphylococcus aureus* isolated from semen samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN= Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY= Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Noreloxine, CIP=Ciprofloxacin

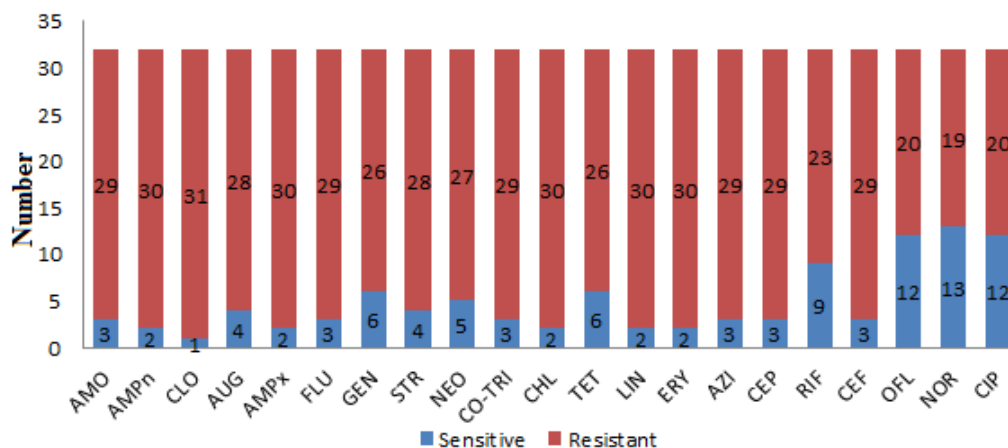


Figure 2: The antibiogram types of *Staphylococcus saprophyticus* isolated from semen samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx=Ampiclox, FLU=Flucloxacilin, GEN= Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY=Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Norfloxacin, CIP=Ciprofloxacin

The pattern of the antimicrobial profile of *S. aureus* and *S. saprophyticus* isolated from the HVS samples are as depicted on figure 3 and 4. The general antibiogram profiles of *S. aureus* strains were poor as no one antibiotic was sensitive to half of the test antibiotics. The most sensitive antibiotic was Cephalexin (16/62; 25.81%) while the least was Ampicillin (3/62; 4.84%). The Ajumali's mnemonic typing shows strains 25 (Mnemonic code: 2000000) and strain 38 (mnemonic code: 0200000) were the most resistant of the isolated strains, as they were both susceptible to only one antibiotics. Other isolated strains showed susceptibility to two or more different antibiotics, for emphasis, strain 6 (Mnemonic: 1002042) was susceptible to four antibiotics.

S. saprophyticus strains isolates were sensitive to all the antibiotics to varying extent. Overall, Streptomycin was the most sensitive (5/19; 26.32%) while Tetracycline and Norfloxacin were the least sensitive (3.13%). The Ajumali's mnemonic typing of these strains revealed that strain 11 (Mnemonic code: 0100004) and strain 15 (Mnemonic code: 0020020) were the most resistant of the isolates. While these two strains were sensitive to only two antibiotics each, other strains such as strains 2,4,16 and 18 were sensitive to three or four antibiotics.

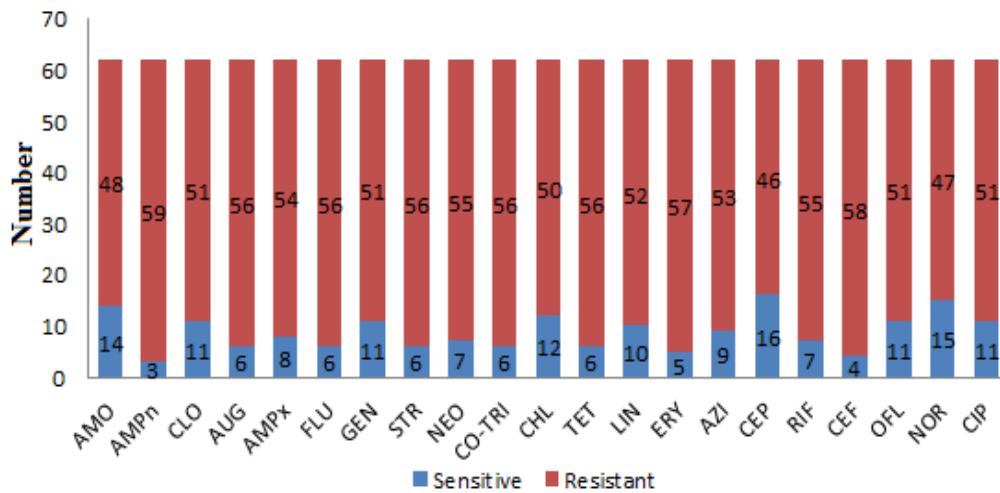


Figure 3: The antibiogram types of *Staphylococcus aureus* isolated from high vagina swab samples.

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN= Gentamicin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY=Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Norfloxacin, CIP=Ciprofloxacin

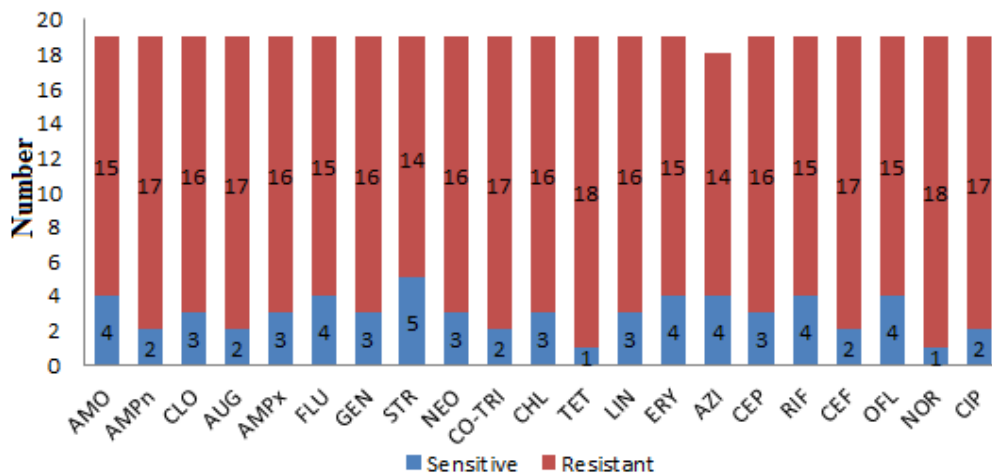


Figure 4: The antibiogram types of *Staphylococcus saprophyticus* strains isolated from high vagina swab samples

Key: AMO=Amoxicillin, AMPn=Ampicillin, CLO=Cloxacilin, AUG=Augmentin, AMPx= Ampiclox, FLU=Flucloxacilin, GEN=Gentamycin, STR=Streptomycin, NEO=Neomycin, CO-TRI=Co-trimoxazole, CHL=Chloramphenicol, TET=Tetracycline, LIN=Lincocin, ERY=Erythromycin, AZI=Azithromycin, CEP=Cephalexin, RIF=Rifampicin, CEF=Cefuroxime, OFL=Ofloxacin, NOR=Norfloxacin, CIP=Ciprofloxacin.

DISCUSSION

The high rate and still growing rate of infertility is one of the most important and underappreciated reproductive health problems.^[35,36] Bacteria infections have long been recognized in association with infertility^[9,10] but the significance of these infections in the female and male genital tracts is not well known.^[11,12] In our present study, we observed staphylococcus infestation in semen and high vaginal swab samples from infertility subjects in Esan, Nigeria. Specifically, *S. aureus* was the most prevalent staphylococcus spp. isolated and accounted for 76.54% and 75.54% in the HVS and semen samples respectively. *S. saprophyticus* was also present and accounted for 23.02% and 23.46% in the semen and HVS samples while *S. epidermidis* was only isolated in the semen sample. Similar to our findings on semen, Ibeh *et al.*^[37] reported that of a total number of 140 semen specimen processed, 92 (65.7%) yielded bacterial growth with *Staphylococcus aureus*, *S. saprophyticus* and *Escherichia coli* having the highest incidence rate of 28.3%, 19.6% and 13.0%, respectively. In a study by Okonofua *et al.*^[38], *Candida albicans* (25%), *S. aureus* (21.7%) and *Neisseria gonorrhoeae* (17.4%) were the most commonly isolated microorganisms in the lower genital tract infections in infertile Nigerian women. A variety of bacteria have been isolated from the endometrium of infertile women.^[39] According to Anchana *et al.*^[8], high vaginal cultures of infertile women yielded only bacteria flora similar to fertile women but being complicated by dissemination of quiescent sepsis of the endometrial or vagina. The high rate of staphylococcus in our study indicated the significance of staphylococcus spp. in asymptomatic bacteriuria in infertility. In accordance with our findings, several previous studies have documented *S. saprophyticus*^[40-42], *S. aureus* as the most prevalent causative agent of asymptomatic bacteriuria.^[43,44]

The treatment of bacterial infections is increasingly complicated because of the ability of bacteria to develop resistance to antimicrobial agents. The case of UTI among subjects with fertility problem is even alarming considering the results of this study. The present study showed that semen *S. aureus* and *S. saprophyticus* samples were highly resistant to the 21 antibiotics investigated as none of the antibiotic was 50% susceptible to either *S. aureus* or *S. saprophyticus*. Ampiclox, Tetracycline, Cephalixin, Rifampicin, Cefuroxime and Ciprofloxacin were the most susceptible antibiotics against semen *S. aureus* isolates with susceptibility potencies ranging from 21.10% to 16.19% while Ofloxacin, Norfloxacin and Ciprofloxacin were the most susceptible antibiotics against semen *S. saprophyticus* isolates and have potencies ranging from 40.63% to 37.50%. Comparatively, a study by Ibeh *et al.*^[37]

has documented microbiologically isolates of *S. aureus* and *S. saprophyticus* were highly susceptible to Ceftazidime, Ceftriaxone, Gentamycin, vancomycin, Cefotaxime, and Imipenem but highly resistant to ofloxacin in adult married infertile males in Benin metropolis.

In female, the present study showed that Amoxicillin, Chloramphenicol, Cephalexin and Norfloxacin were the antibiotics with greater susceptibility against *S. aureus* with potencies ranging from 25.81% to 19.36% while Amoxicillin, Flucloxacilin, Streptomycin, Erythromycin, Azithromycin, Rifampicin and Ofloxacin were the most susceptible against *S. saprophyticus* with potencies ranging from 26.32% to 21.05%. The study by Anchana *et al.*^[8] has reported *S. aureus* from infertile women to be resistance to Amoxycilin, Ampicilin, Penicillin, Piperacillin, Cephalothin /Cephalexin, Ceftazidime, Tobramycin, Nalidixic acid, Cefoperazone + Sulbactam and Ceftazidime + Clavulanic acid. Our findings disagree with that of Anchana *et al.*^[8] in-terms of Amoxicillin and Cephalexin but agree in terms of the other antibiotics. Antimicrobial susceptibility testing of pregnant women with UTI has indicated *S. saprophyticus* isolates were highly sensitive to Amikacin, Gentamicin, Cefotaxime, Imipenem, Ciprofloxacin, Nitrofurantoin, and Ofloxacin.^[45] While the Ofloxacin was sensitive in this study and partly correspond with the report by Iliyasu *et al.*^[45], the other antibiotics were not in line.

Overall, the resistance rates recorded in this study were higher than those we observed in urinary tract infected subject in same area^[46] (article under review). Based on our findings, Ciprofloxacin seem to be a better antibiotic of therapy for UTI of staphylococcus origin in male with fertility problem while Amoxicillin showed better potency for UTI of staphylococcus origin in female with fertility problem. The sensitivity of these antibiotics may be due to the fact that they are comparatively less frequently used as compared to Tetracycline, Augmentin and Ampicillin that may be commonly and indiscriminately used. The findings of the present study showed that Staphylococcus infections associated with infertility is relatively high in the area and that antibiotic susceptibility pattern of the isolated *S.aureus* and *S. saprophyticus* are associated with multidrug resistance to many of the commonly recommended antibiotics and varies with gender. Thus, infertility may lead to decreased susceptibility of the commonly used antibiotics and subsequently, screening appears to be a likely cost effective approach. Considering that appropriate antimicrobial use is only possible after culture and sensitivity testing, it is therefore recommended that routine urinalysis, and semen and HVS MIC be emphasize on in subject with infertility problem.

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