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Full length research paper

The antibiogram types of auto-agglutinating *Staphylococcus aureus* strains isolated from the semen samples of males with infertility problems in Edo state, Nigeria.

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There are several causes of infertility in males, including bacterial and other pathogenic infections. *Staphylococcus aureus* is a frequent isolated pathogen of the reproductive tract. In this work, a total of 431 males were enrolled for the study, with semen samples taken from all of them. *Staphylococcus aureus* with a total of 105(47.73%) isolates had the highest prevalence of organisms isolated. Others were *Escherichia coli* 74(33.64%), *Staphylococcus saprophyticus* 32(14.55%), *Proteus mirabilis* 4(1.82%), *Klebsiella* species 3(1.36%) and *Staphylococcus epidermidis* 2(0.91%). The isolates were inoculated on Manitol Agar Salt (MSA) and Nutrient Agar. Isolates with opaque golden yellow colour with diameter 3 – 5mm were presumably identified as *Staphylococcus aureus* while, colonies with white to cream colours on Nutrient Agar were tested for coagulase activity, catalase test, staphyloslide test, hemolysis test, sensitivity to Novobiocin and Deferrioxamine to differentiate them. Using the single disc diffusion method, the antibiogram typing of the *Staphylococcus aureus* isolates were done with 21 different antibiotics of various chemotherapeutic groups, following the Ajumali's mnemonic typing. This revealed strain 40 (Ajumali's mnemonic code: 0000000) to be completely resistant to the 21 test antibiotics. The auto-agglutinating strains of *Staphylococcus aureus* are strongly associated with infertility with a statistical significance (X^2=10.83; P<0.001)

Keywords: Ajumali's Mnemonic typing; Antibiogram; Auto-agglutinating; Male infertility; *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus has been implicated in a number of disease conditions including male infertility (Momoh *et al.,* 2009). Male infertility is an important issue, especially in Nigeria. Though, it is a problem occurring worldwide, it is still a neglected health issue in Nigeria (Okonofua et al., 2005). Reports indicate that male factors accounts for 20 – 50% of the causes of infertility in different parts of Nigeria (Chukwudebelu et al., 1979; Esimai et al., 2002).

Male infertility is actually the inability of a couple to achieve pregnancy, despite regular unprotected intercourse, usually after a period of 12 months, with detected factors attributed to the male. Sexually transmitted diseases especially those attributed to bacterial infections may also be associated with male infertility (Nwabuisi and Onile, 2001; Onemu and Ibeh, 2001).

Recent studies revealed that various microbial infections reduce the viability of semen, with consequent reduction in motility of sperms. There is also a concomitant leukocytospermia associated these microbial infections (Momoh et al., 2009).

Comhaire et al., 2008 reported that the diagnosis of male adnexitis is difficult and the influence of this condition on fertility is still a matter of debate. Though many studies have been devoted to the question whether infection of the accessory sex glands can cause male sub-fertility, results are however discrepant and no final

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Table 1: Distribution of males with complaints of UTI having positive bacteria cultures from semen samples according to the age ranges.

Age group in years	No of positive bacteria cultures
15 – 19	21
20 – 24	19
25 - 29	20
30 - 34	23
35 - 39	25
40 - 44	26
45 - 49	22
50 - 54	20
55 - 59	21
60+ years	23
Total	220

conclusion has yet been reached (Comhaire *et al.,* 2008). Indeed no control study has been performed on sub-fertile men with infection, comparing the fertility out come with or without treatment. This may be attributed to the confusion concerning the diagnosis of infection. Some investigators diagnosed infection as soon as pathogenic bacteria are cultured in the seminal plasma (Okon et al., 2005). Some consider then number of bacteria per ml of ejaculate as an important diagnostic criterion whereas, some other investigators considered certain changes in sperm morphology and in the biochemical composition of the seminal plasma of great importance. Suffice to add that since infection is defined on the basis of such different criteria, the reported incidence in sub fertile men greatly varies from one investigation to another and the results of treatment are very divergent.

The aim of this study is to report the results of the investigations on the antibiogram types of auto-agglutinating strains of *Staphylococcus aureus* isolated from the semen samples of males with UTI and of sub-fertile males with a possible association between the presence of these pathogens in the semen and subfertility.

MATERIALS AND METHODS

A total of 431 males were enrolled for this study and semen samples taken from all of them. All the semen samples were collected by masturbation method. The specimens were sent to the laboratory for analysis within 15minutes of collection and all specimens were from males with complaints of UTI and infertility (primary) following the WHO criteria (WHO, 1999) for semen analysis, i.e, collection of semen by masturbation into sterile specimen bottles, following abstinence from intercourse 2 – 3 days prior to collection. Immediate
transfer of samples to the laboratory for analysis, a wet preparation made and quick assessment of motility done, sperm morphology viewed, other microscopic examinations followed by culture and sensitivity.

Isolation and identification

All samples were inoculated onto Manitol Salt Agar and Nutrient Agar plates by streaking. Inoculated plates were then incubated aerobically at 37^0C for 24 hours, thereafter; discrete colonies were picked from the growth and Gram stained, while further sub-culturing were done to obtain pure cultures for biochemical tests. Bacterial isolates were identified using the method described by Bauer *et al.,* 1996.

Antibiogram mnemonic typing

For the determination of the antibiogram types of the isolated *Staphylococcus aureus* strains, the Ajumali's method of mnemonic coding was adapted as described by Joghi *et al.,* (1984). Using this mnemonic coding, a sensitive result was recorded as (+), while a resistance result was recorded as (-). 21 antibiotics were used and these antibiotics were divided into 7 groups of 3 antibiotics each using their mechanism of action and clinical applications as criteria for grouping.

The 3 antibiotics in each group were assigned arbitrary value of 1, 2 and 4 for the first, second and third antibiotics respectively. A perfect sensitivity for all 3 antibiotics was recorded as 7, i.e 1+2+4=7. While complete resistance (no sensitivity) to all 3 antibiotics was recorded as 0, that is, 0+0+0=0 (Orhue, 2004)

RESULTS

Table 1 below shows the age distribution of males with positive semen cultures. The age range of 40 – 44 as well as 35 – 39 recorded slightly higher positive bacteria cultures. Other age ranges also recorded positive bacteria cultures.

Table 2 shows the various isolated bacterial species. From a total of 431 semen samples analysed, 220(51.04%) returned positive bacterial cultures. *Staphylococcus* species accounted for 139 (63.18%) of the isolates. Furthermore, some enrolled cohorts with complaints of infertility also had staphylococcus species as monomicrobial infections following semen cultures.

TABLE 2: Prevalence of isolated bacterial strains from semen of males with UTI.

Isolated Microorganism	No of isolates
Staphylococcus aureus	105 (47.73%)
Staphylococcus epidermidis	2 (0.91%)
Staphylococcus saprophyticus	32 (14.55%)
Escherichia coli	74 (33.64%)
Proteus mirabilis	4 (1.82%)
Klebsiella species	3 (1.36%)

TABLE 3: Distribution of staphylococcus species in males with uti and males with uti and infertility

Organisms	Cohorts with UTI (n=220) Mean + sd=36.66±17.00	Cohorts with UTI and infertility (n=52) Mean + sd=10.40±10.86
Staphylococcus aureus	105 (47.73%)	31 (14.09%)
Staphylococcus saprophyticus	32 (14.55%)	5 (2.27%)
Staphylococcus epidermidis	2 (0.91%)	0 (0.00%)

Table 3 below shows that *Staphylococcus aureus* is the most isolated pathogen among the isolated staphylococcus species obtained from the semen cultures of males with UTI and males with UTI having additional complaint of infertility. At $P < 0.001$, the mean and standard deviation for enrolled cohorts with UTI was 36.66±17.00. While the mean and standard deviation forenrolled cohorts with UTI and infertility problem was 10.40±10.86.

Apendix I highlights the in vitro susceptibility pattern of the 105 isolated *Staphylococcus aureus* strains from semen samples, including the 31 (strain 31 – 62) isolated from males with complaints of infertility. Strain 40 (Ajumali's mnemonic code: 0000000) was completely resistant to all the test antibiotics, while Strain 26 (Ajumali's mnemonic code: 0000010) was only sensitive to Cephalexin.

DISCUSSION

Staphylococcus aureus was the most isolated microorganism from males with infertility problems. In this study, 31 (14.09%) *Staphylococcus aureus* strains were isolated from males with infertility problems who had a concomitant urinary tract infection, while a total of 105 (47.73%). *Staphylococcus aureus* strains were isolated from the cohorts.

It is imperative to note that a highly resistant strain of *Staphylococcus aureus* (Ajumali's mnemonic type 40 with code: 0000000), was isolated from a patient with infertility problems. This *Staphylococcus aureus* strain 40 was resistant to all 21 test antibiotics. There is also a statistical correlation between isolated *Staphylococcus aureus* strains and infertility in male cohorts (X^2=10.83; $P<0.001$). In the course of the study, the prevalence of male factor infertility was observed to be 47.73%; this is consistent with 46% observed in Kano, Nigeria (Onwudiegwu and Bako, 1993)

It is pertinent to note that some investigators are of the opinion that the presence of significant bacteria population in semen samples are not enough to attribute them to casual factors in male infertility (Comhaire et al., 2008). However, in the absence of any other major known cause(s) of infertility, a strong association between these pathogenic microbes and male infertility is not out of place.

Infection of the semen by microorganisms, including *Staphylococcus aureus* usually end up decreasing the motility of sperms alongside the leukocytospermia. Invariably, the quality and viability of such infected sperm cells decreases (Momoh et al., 2009).

Emokpae et al., 2009 report that seminal fluid infection contributed to the reduction of sperm density, asthenospermia and teratospermia (with greater than 50% abnormal sperm cells). Interestingly, *Staphylococcus aureus* as a causative organism accounted for 68.2% of the seminal fluid infection.

CONCLUSION

The various microorganisms likely to cause semen infections (seminal fluid infections) include auto-agglutinating and non-agglutinating strains of *Staphylococcus*. *Staphylococcus aureus* is a frequently isolated organism from semen samples. In the management of male factor infertility, proper treatment of *Staphylococcus aureus* and all isolated *Staphylococcus* species as well as other isolated microorganisms is advocated. Giamarellou *et al.,* (2009) reported that

following long term treatment of chronic prostatitis caused by *Escherichia coli* and Staphylococci, in males with infertility, using co-trimoxazole, doxycycline and erythromycin over 6 – 8 months duration, spermatograms were normalized or improved in 70% of the patients, while some patients were able to impregnate their wives. Hence, long term treatment with proper antimicrobials subsequently cures or improves male infertility. Suffice to add that the results of an antibiogram is essential to initiate an appropriate antimicrobial therapy.

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Appendix I: The antibiogram types of *staphylococcus aureus* isolated from semen samples.

ANTIBIOTICS	AMOXICILLIN	AMPLICILLIN	CLOXACILLIN	AUGMENTIN	AMPICLOX	FLUCLOXACILLIN	GENTAMYCIN	STREPTOMYCIN	NEOMTCIN	CO-TRIMOXAZOLE	CHLORAMPHENICOL	TETRACYCLINE	LINCOCIN	ERYTHROMYCIN	AZITHROMYCIN	CEPHALEXIN	RIFAMPIN	CEFUROXIME	OFLOXACIN	NORELOXCIN	CIPROFLOXACIN	MNEMONIC CODING
Mg/disc	10	10	5	10	10	10	10	10	10	25	10	10	5	10	5	10	10	10	5	5	5	
Numeric No Isolated Bacterial Strains	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	**1**	**2**	**4**	
1	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	0202010
2	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	0240040
3	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	4200005
4	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	0001100
5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0200020
6	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	1002041
7	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	0120010
8	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	0204014
9	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	2200010
10	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	4200010
11	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	0004040
12	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	0200400
13	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	4400101
14	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0200020
15	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	0004040
16	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	0010040
17	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	0005010
18	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0200010
19	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	1040040
20	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	0020600
21	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0400104
22	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	2004012
23	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	0002040

Appendix I Cont

24	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	0014000
25	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	0001020
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0000010
27	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	0201014
28	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	0440014
29	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	1000140
30	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	4000010
31	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	2040014
32	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	0202200
33	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	0024000
34	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	0040040
35	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1202004
36	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	0202020
37	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	4000200
38	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	0001140
39	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	0014401
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0000000
41	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	2001124
42	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	1004040
43	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	1140040
44	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	4011200
45	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	0202010
46	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	0020001
47	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	0402020
48	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	5000040
49	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0104000
50	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	0040020
51	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	0000240
52	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	2021004
53	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0100010
54	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	1200120
55	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	2000404
56	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	0102040
57	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	0014004
58	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	0001001
59	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	5000020

Appendix I Cont

60	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	0022040
61	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	0200001
62	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	0040202
63	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	0010101
64	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0400040
65	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	0004401
66	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	1020020
67	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	1100101
68	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0010020
69	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	0040020
70	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0400010
71	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	0400200
72	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	0100040
73	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	0002003
74	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	4000120
75	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	0004010
76	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	1020040
77	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1400400
78	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	0012010
79	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	2001004
80	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	0200420
81	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	0140002
82	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	0010400
83	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	0002020
84	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	0200404
85	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	0001202
86	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	0440010
87	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	0014004
88	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	0021040
89	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	0200026
90	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1000400
91	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	0101004
92	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	4000240
93	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	2014001
94	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+	0020124
95	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	4000010

Appendix I Cont

96	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	0202002
97	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	0100220
98	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	0404040
99	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	1040400
100	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	2000010
101	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	0040402
102	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	0012040
103	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	0000640
104	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	0200441
105	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	0040064