Isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) from AIDS Patients Attending State Specialist Hospital, Yola and Federal Medical Centre, Yola, Adamawa State, Nigeria.

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Abstract: One hundred and eleven (111) urine samples were collected from AIDS patients receiving treatment and counselling in State Specialist Hospital, Yola (SSHY) and Federal Medical Centre, Yola (FMCY). A number of organisms namely *Escherichia coli*, species of *Staphylococcus, Proteus, Pseudomonas, Klebsiella* and *Streptococcus* were isolated from urine samples of patients attending these two hospitals. Out of 54 isolates, 21 (39%) were identified as species of *Staphylococcus*, 12 out of these 21 isolates were identified as isolate of *S. aureus*. The prevalence of *S. aureus* among male patients was 17% whereas the respective value for the female patients was 6%. The age of the patients positive for *S. aureus* were in the range of 18-45 years. Antibiotics sensitivity tests reveals that fusidic acid has the highest percentage efficacy (66%) followed by novobiocin (58%) and methicillin (50%). So, AIDS patients may be responsible for hospital associated and community associated infection due to MRSA. [Report and Opinion- 2009; 1(6):103-107]. (ISSN: 1553-9873).

Key words*: MRSA, AIDS, methicillin, Staphylococcus sp.*

1.INTRODUCTION:

Staphylococcus aureus is the most important human *staphylococcal* pathogen and causes boils, abscesses wound infections, Pneumonia, toxic shock syndrome and other diseases. Pyomyositis is a purulent infection of skeletal muscle and is usually caused by *S. aureus.* Recently, Pyomyositis associated with HIV infections has been reported with increasing frequency in patients with or without AIDS. A recent report in Uganda stressed the importance of HIV infections as an underlying condition in patients with Pyomyositis. *Staphylococcus aureus* is the most commonly implicated organism, accounting for 90% of the cases in HIV-positive patients, 65% of the cases in HIV-seronegative patients ($P<0.01$), and more than 90% of the recovered organisms in the tropics. Other bacteria were found less frequently in HIV-positive patients and included *Streptococcus* group *Streptococcus spp, Salmonella enteridis, Salmonella spp, E.coli, Citrobacter freudii, Morganella morganii* and *Pseudomonas aeruginosa*. (Ansaloni *et al*, 1996).

Recently strains of multiple drug resistant *S. aureus* have appeared and proven very difficult to treat (Prescott, 2005). During the late 1950s and early 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial or hospital acquired pathogen and has become the head leading cause of nosocomial infection during the last 2 decades (Nimmo and Phyford, 2003). Since then penicillinase resistant semi synthetic penicillin as proved to be successful antimicrobial agents in the treatment of staphylococcus infections. Unfortunately, MRSA (methicillin resistant *Staphylococcus aureus*) strains isolated are on increasing resistant to multiple non-β-lactam containing antimicrobial drugs. Recent report of vancomycin-resistant *S.aureus* fore shows an area of chemotherapy in which effective bactericidal drugs to treat infections with this organism may not be readily available, (Cookson, 2002).

MRSA is a problematic pathogen in human medicine and appears to be emerging in the world. Historically, hospital associated MRSA infections have predominated in humans and contributed to significant illness and death. Recently a shift in the epidemiology of MRSA infections have been documented, where by community associated methicillin-resistant *S.aureus* (CA-MRSA) infections have become more common. CA-MRSA may arise from the hospital origin clone that are carried into the community and then transmitted between the communities and then transmitted between or from de novo development of resistance through cquisition of resistance factor (mec A) by methicillin sensitive strains of *S. aureus*

2. MATERIALS AND METHODS:

2.1 *SELECTION OF HOSPITALS*

The hospitals selected for this study were State Specialist Hospital, Yola (SSHY) and Federal Medical Center, Yola (FMCY). The patients diagnosed with AIDS in Adamawa state are generally referred to these two hospitals for treatment and counseling purpose. They collected their antiviral drugs at reduced cost from FMCY. The terminal ill patients are generally advised to get admission in either of these two hospitals and they are generally referred to Microbiology laboratories for routine microbiological analysis.

2.2 *PERIOD OF STUDY*

The period of collection of sample was between the month of April, 2007 and May, 2007.

2.3 *SPECIMEN COLLECTION*

In the clinic, the counseling unit, the wards and hospital laboratories, urine samples were collected from the patients after explaining the aim and objectives of the research to them. Only the patients who were willing to take part in the research selected for this work. Sterile screw-caped wide – naked specimen bottles were used to collect urine samples with the help of the staff of clinic, counseling unit, the wards and the hospital laboratories. All the samples were properly labeled for each sample, age and sex were recorded. The samples after collection were transported to the microbiological laboratory of SSHY within 2-3 hours of collection using a cooler packed with ice blocks.

2.4 *ISOLATION OF ORGANISMS*

A loopful of each specimen was inoculated onto MacConkey agar using streak plate method. Then the plates were incubated at 37^0C for 24 hours. The discrete colonies were isolated and further report01subcultured using MacConkey agar and were kept at 4^0C for further research work (Benson, 2002). Same procedure was followed for isolation of discrete colonies using CLED agar and mannitol salt agar from the collected urine samples.

2.5 *IDENTIFICATION OF ISOLATES*

The following procedures were used in order to identify the isolated colonies on MacConkey agar, CLED agar and Mannitol salt agar.

2.6 *MICROSCOPIC CHARACTERISTIC OF ISOLATED COLONIES*

The colour and size of the colonies were recorded for identification purpose (Cheesbrough, 2002).

2.7 *GRAM STAINING*

Gram staining was done according to the method stated in Cheesbrough, 2002.

Twenty one isolates which were considered to be *S. aureus* isolates were selected for further studies.

2.8 *COAGULASE TEST*

A loopful of growth for each isolate was inoculated into 5ml of peptone water which was then incubated at 37^OC until turbidity reached 0.5 Mcferland standards. Then test tube procedure and slide test method were employed using this broth culture (Benson, 2002).

2.9 *CATALASE TEST*

A drop of hydrogen peroxide (3%) was placed on a microscopic slide and a bit of growth of the test organisam was placed and then emulsified. Effervescence caused by liberation of oxygen as gas bubbles indicates the presence of catalase in culture under test (Benson, 2002).

2.10 *SUGAR UTILIZATION TEST*

The sugars used were glucose, lactose and sucrose for the twenty one isolates obtained. A loopful of growth for each isolate was inoculated into 5ml of peptone water which was then incubated at 37^0C for 24hours. 1ml of 1% sterile sugar solution was added to a tube containing Durham tube (inverted) and to it 1ml of cell suspension as prepared above was added. Observations were made for any color change and gas production after 24hours.

2.11 *NOBOVIOCIN TESTING*

The isolates were tested against Novobiocin (5µg/ml) in order to observe their sensitivity or resistance against this antibiotic. Streak plate method was used for this purpose (Benson, 2002).

2.12 *SENSITIVITY TEST*

Twelve *S.aureus* isolates were obtained after biochemical tests. These were used for this purpose. A speck of growth of each isolate was inoculated into sterile peptone water incubated for 24 h and 1ml of the growth using sterile spreader was evenly spread on the surface of nutrient agar plate. Sensitivity discs containing conventional antibiotics manufactured by Mastering Laboratories Ltd. placed on surface of nutrient agar plate and incubated at 37^oC for 24 h. The antibiogram was then read and recorded (diameter of zone of inhibition). In this study, zone of inhibition less than 3mm was considered as resistant (Cheesbrough, 2002)

Table 1: Results of Gram staining, biochemical test and Novobiocin test

Isolate No.	SC	TC	Catalase	Sugar	GS	Novobiocin	Novobiocin
A_1	-	-	-	+	+	S	-
A_2	+	+	+	+	+	"	+
A_3	+	+	+	+	+	"	+
A_4	-	-	-	+	+	"	-
A_5	-	-	-	+	+	"	-
A_6	+	+	+	+	+	"	+
A_7	+	+	+	+	+	"	+
A_8	-	-	-	+	+	"	-
A_9	+	+	+	+	+	"	+
A_{10}	+	+	+	+	+	"	+
A_{11}	-	-	-	+	+	"	-
A_{12}	+	+	+	+	+	"	+
A_{13}	-	-	-	+	+	"	-
A_{14}	+	+	+	+	+	"	+
A_{15}	-	-	-	+	+	"	-
A_{16}	+	+	+	+	+	"	+
A_{17}	+	+	+	+	+	"	+
A_{18}	-	-	-	+	+	"	-
A_{19}	+	+	+	+	+	"	+
A_{20}	-	-	-	+	+	"	-
A_{21}	+	+	+	+	+	"	+

KEY: + = Positive, - = Negative, S C = Slide Coagulase, T C= Tube Coagulase,
G S = Gram staining, S = Sensitivity

Table 2: Showing prevalence of organisms among studied groups in relation to age and gender from different locations.

Location	Age	Total no. of sample collected	Total (%) of *Staphylococcus* sp.	Total (%) of *S. aureus*	Total (%) of other organisms
FW	20 – 52	12	1 (4.76)	-	6(14.3)
MW	23 – 44	9	3 (14.28)	2 (16.7)	3 (7.1)
CUF	19 – 34	5	-	-	3 (7.1)
CUM	24 – 37	8	1 (4.76)	1 (8.33)	4 (9.2
HLF	5 – 6	6	1 (4.76)	-	2(\4.8)
FMCF	7 – 37	36	8 (42.85)	4 (33.33)	3 (3.1)
FMCM	4 – 45	38	5 (23.80)	4 (33.33)	11(26.1)
HLM	26 – 29	3	1 (4.76)	1 (8.33)	)12 (2.4)

KEY:-
FW - female warder
CUM -counseling unit male
HLF -hospital laboratory female, sshy
MW - melwe warder
CUF -counseling unit female
HLM -hospital laboratory male, sshy
FMCF -federal medical center female
FMCM- federal medical center male

Table 3: Showing percentage Efficiency of various antibiotics

Antibiotics	No. of sensitive *S.aureus*	% Efficacies
Chloramphenical	2	16
Erythromycin	2	16
Fusidic acid	8	66
Methicillin	6	50
Novobiocin	7	58
Penicillin G	-	0
Streptomycin	1	8
Tetracycline	-	0

KEY:-Negative

3. RESULTS AND DISCUSSION

Out of one hundred and eleven urine samples analyzed, sixty one were collected from female patients, forty three were collected from male patients and seventy seven were collected from children suffering from AIDS. Fifty four (54) colonies were isolated from CLED agar, Mannitol salt agar and MacConkey salt plates and these were properly labeled. Twenty one (21) isolates showed cultural characteristics of *Staphylococcus sp.* whereas other isolates were*E.coli* and species of *Proteus*, *Pseudomonas*, *Klebsiella* and *Streptococcus*. Results of Gram Staining experiments reveal that all the twenty one isolates (A1-A21) were gram positive (Table 1). Results of biochemical characteristics of isolates reveals that out of 21 isolates 12 were *S.aureus (Table 1)*, all the isolates were sensitive to Novobiocin (Table **1).**

Al-Tawfiq et al (2000) reported that pyomyositis, a purulent infection of skeletal muscles and usually caused by *S.aureus*, associated with HIV infections with increasing frequency in patients with or without AIDS. Also Herchline, 2007 observed that the major infections related to AIDS patients include urinary tract, skin infections, pneumonia, salmonella *septicemia, pyomyositis and mycobacterium* tuberculosis. The organisms isolated from the patients were responsible for some of these infections.

Schwartzman *et al.* (1991) reported that *S.aureus* is most commonly implicated organism in HIV positive patients and other bacteria present in HIV positive patients include *Streptococcus* group *C, Streptocossus spp, S.enteritidis, Salmonella spp, Escherichia coli, Citrobacter ferundi, Morganella morganii* and *Pseudomonas aeroginos*a.

Table 2 shows the prevalence of organisms among the studied group in relation to age and gender. The prevalence of *S.aureus* among the male patients was 17% whereas the prevalence among female patients was 6%. It may be due to the fact that male patients often still have multiple sex partners contrary to female patients with exception of female sex workers and are at risk of having different types of infection. Out of 111 samples only 12 positive for *S.aureus* (10.8%). The ages of the patients' positive for S.aureus were in the range of 18-45years. The patients of this age group are engaged in sexual activities and most of the times suffer from UTI, skin infection and pneumonia (Madigan et al, 2000).

The *Staphylococcus aureus* isolates were selected for sensitivity testing against some conventional used antibiotics. The percentage efficacy of various antibiotics are shown in Table 3.Out of those 12 *S.aureus* isolates, only 6 were methicilline resistant. The result for determination of percentage efficacy of various conventional antibiotics show that fusidic acid has the highest antimicrobial activity with percentage efficacy of 66% followed by novobiosin (58%) and methicillin (50%). The results obtained from this study revealedthat AIDS patient may be responsible for hospital and community acquired infection caused by MRSA.

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