Antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions in Lacor Hospital, Uganda

*Kitara LD[1], Anywar AD[1], Acullu D[2], Odongo-Aginya E[3], Aloyo J[4], Fendu M[5]

1. Department of Surgery, Faculty of Medicine, Gulu University, Uganda
2. Department of Biochemistry, Faculty of Medicine, Gulu University, Uganda
3. Department of Radiology, Lacor Hospital.
4. Department of Microbiology and Immunology, Faculty of Medicine, Gulu University,Uganda
5. African Centre for the Treatment and Rehabilitation of Torture victims, Gulu, Uganda

Abstract

Background: *Staphylococcus aureus,* a mainly acquired hospital infection is responsible for many suppurative lesions and has demonstrated the ability of developing resistance to many antimicrobial agents leading to life threatening infections and long hospital stay.

Objective: To determined the prevalence and antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions of the surgical ward and outpatients of Lacor Hospital (Uganda).

Methods: A cross-sectional study was conducted at St. Mary's Hospital Lacor to determine the prevalence and antibiotic susceptibility profiles of *Staphylococcus aureus* in suppurative lesions in both surgical inpatients and outpatients. Using culture techniques on MacConkey and blood agar, *Staphylococcus aureus* was isolated based on the colonial characteristics and confirmed by Catalase and tube Coagulase tests. The antibiotic susceptibility test was done using Kirby-Buer disk diffusion method on 4% Salt Muellar Hinton II agar for the Methicillin and non salted Muellar Hinton II agar for the other antibiotics (NCCLS M100S9).

Results: The prevalence of *Staphylococcus aureus* in 122 patients sampled was 59.4% for the surgical inpatients and 48.3% for outpatients giving an average prevalence of 53.9% for both groups of patients. The average antibiotic susceptibility patterns for the 8 antibiotic tested were: Ampicillin (75.0%), Chloramphenicol (34.4%), Ciprofloxacin (1.6%), Erythromycin (7.8%), Gentamycin (0%), Methicillin (1.6%), Tetracycline (45.3%) and Co-trimoxazole (50.0%). The resistance in surgical inpatients was significantly higher than outpatients (t=1299, $p<0.05$) and Methicillin resistance was confirmed by PCR.

Conclusion: *Staphylococcus aureus* is highly prevalent and more resistant in inpatients. There is a higher risk of acquiring drug resistant *staphylococcus aureus* infection in inpatients of Lacor Hospital with a Methicillin resistance of 0% and 2.6% for out and inpatients respectively.

Key words: *Staphylococcus Aureus*, antibiotic susceptibility, Lacor Hospital, Gulu

African Health Sciences 2011; 11(S1): S34 - S39

Introduction

Staphylococcus aureus has often been encountered and thought to be a Nosocomial infection (hospital acquired) and community acquired infection[1]. In 1881, Alexander Ogston recognized that it is responsible for a number of infections in man and animals especially when there is a breakage of the skin and epithelial linings. It's now known that the organism is a medical hazard. It takes advantage when the inert and active immunity is suppressed[1]. The problem with *Staphylococcus aureus* became more complicated when it was found that it quickly developed resistance and was capable of producing many antibiotic resistant strains. This is very common in hospitals where drug resistant "hospital strains' have caused *S. aureus* infection outbreaks resulting in deaths in surgical units and newborn nurseries[2]. These are the Beta-lactam resistant strains such as MRSA that cause high mortality and morbidity.

Antibiotic resistance leads to prolonged hospital stay and increased costs in terms of treatment. In addition to these, it causes life threatening infections such as in cases of pyomyositis and chronic osteomyelitis. The majority of the MRSA strains world-wide have become resistant to multiple antibiotics including beta-lactams; aminoglycosides, macrolides, lincosamides and more recently floroquinolines[4].

***Corresponding author**
Dr. David Lagoro Kitara
Department of Surgery
Faculty of Medicine
Gulu University
Box 166, Gulu (Uganda);
Phone: +256 772524474
Email:klagoro@gmail.com, klagoro@yahoo.co.uk

Figures from the developing countries, especially from the sub-Saharan Africa, are still scanty and their trends are being continuously monitored and documented. Most hospital acquired infections from the developed world are mainly reports on inpatients only and this is why this study included outpatients to compare the antibiotic susceptibility patterns in the 2 groups (inpatients and outpatients). Control of such hospital acquired infections requires specific surveillance of resistance patterns that can lead to cost control measures[5]. One such method would have been the introduction of a low strain *Staphylococcus aureus* 502A in the nostrils and umbilicus of the new-borns, a method called bacterial interference. However, such control measures have been limited as finances have been used in other visibly deadlier diseases such as HIV/AIDS and malaria, yet *S. aureus* infections are common in surgical wounds and open skin injuries and penetrate into the integuments.

In Uganda, the resistance of *S. aureus* in patients remains unknown. This is mainly because the laboratory services of the country are scanty, ill-equipped, or poorly financed and this may become a big risk factors for outbreaks such as food poisoning in communities [6] and MRSA outbreaks in hospitals[7], especially in the face of an increasing spectrum of resistance world-wide[8].

A survey conducted in Mulago National Referral Hospital on the surgical inpatients showed an MRSA prevalence of 42%[9]. Mulago Hospital is in an urban setting compared to an upcountry hospital such as Lacor hospital. However, increasing drug availability has led to the possibility of drug abuse and eruption of drug resistance patterns as seen in the Lacor hospital inpatients and outpatients. The prevalence and drug resistance was suspected to be higher among inpatients than outpatients.

The purpose of the susceptibility test was two folds; to guide the choice of the antibiotic treatment for the individual patients and provide surveillance data to monitor resistance trend including the epidemiological data.

Methods

This was a cross-sectional study carried out on pus samples from the purulent lesions such as abscesses, wound infections, pyomyositis and chronic osteomyelitis in Lacor hospital. Samples were obtained from patients of all ages in both inpatients and outpatients from a random sample of cases, after obtaining an informed consent/ assent. Ethical approval was obtained from the Institutional Review Committee of the hospital. Pus samples from closed abscesses were aspirated using a single used disposable syringe after disinfecting the skin with 70% ethyl alcohol soaked in cotton wool swabs. For septic wounds and ulcers, they were first cleaned using aseptic technique, with sterile gauzes and sterile cotton swabs were used to obtain samples from the deeper fresh part of the ulcer/wound. The samples were immediately processed in the laboratory after collection. A loop-full of pus sample from the aspirates or pus swab was gently inoculated on the MacConkey agar (BBL Microbiology systems), prepared according to the manufacturers manual. MacConkey was used to determine other life threatening infections such as enterobacteria, lactose fermenters e.g. Escherichia coli, Klebsiella pneumoniae, enterobacteria species, non-lactose fermenters e.g. salmonella, proteas species, Pseudominas aeroginosa and shiegella species as one of the broader objective of the study to diagnose and help manage the patients' infections and correlate the research findings with previous hospital laboratory findings. It however became difficult to confirm the other isolates individually using other methods since our focus of the research was on *S. aureus*. Ten percet human blood agar prepared from blood agar base, Oxoid and citrate (oxalated human blood) as per the manufacturer's manual was used in the study.

Human blood agar was used instead of sheep blood because of its ready availability and was used to diagnose streptococcus species which were haemolytic. This test was conducted as part of a wider attempt to improve the well-being of the affected patients. However, confirmatory tests were not done to single out what species of streptococcus were cultured.

Swabs were gently rolled on the 2 media to make the primary inoculum. Using a platinum wire loop and the streak looping out technique, the primary inoculum was spread on the agar surface[6]. The inoculated plates were incubated for 24 hours at 35^0C aerobically. Identification of the isolates was done according to the colonial characteristics on the 2 media. Characteristic colonies were picked for analysis by Gram staining Jensen version[6] and confirmed by Biochemical tests[10]. The suspected colonies were subjected to Catalase test[10]. All Catalase positive samples and gram positive cocci growing in clusters (red grapes) from the samples were classified as *Staphylococci.* All tube coagulase positive samples were confirmed as *Staphylococcus aureus.*

The antimicrobial sensitivity testing for *S. aureus* was conducted using a procedure by Kirby-Bauer (1966), modified according to National Council of Clinical Laboratory Standards (NCCLS) disk diffusion standard M100-S9 (1999) as practiced in Lacor hospital, with commonly used antibiotics in the hospital which included the discs with the following antibiotic potency; ampicillin (10ìg), methicillin(5ìg), erythromycin (15ìg), gentamycin (10ìg), ciprofloxacin (5ìg), tetracycline (30ìg), chloramphenicol (30ìg), and co-trimoxazole (25ìg).

Quality control of the procedures was observed by standardization to Macfarland standard for the isolate before spreading on Salted Muellar Hinton II agar. We ensured uniform depth of the Muellar Hinton II agar in all the petri dishes for all disks by placing all the petri dishes on a flat surface and pouring the media gently up to line marked by the manufacturers as the maximum level for the media used. We used a meter ruler for measuring the clearance zones around the antibiotic disks. We used Clinical laboratory standards institute (CLSI) standards to classify the clearance zones as resistant, intermediate and sensitive. In the process of preparation of the media and settings, every batch of the isolates was incubated using the same test conditions to ensure sterility. Incubation conditions for the isolates were made uniform and set at 35^0C for a period of 24 hours for all the dishes. No more than 8 disks were placed on the 150 mm petri dishes.

Results

One hundred and twenty two patients' pus samples were taken up for the study, 64 patients from the surgical wards (wards 1 & 2) to represent the inpatient resistant profile while 58 patients' pus samples were from the outpatients, representing the outpatients' resistant profile. The modal age group for both groups was 0-9 years with a total of 40 patients. The age ranges of the patients were from 0 – 90 years. (table 1)

Table 1: Age distribution of the study population

	Inpatients		Outpatients	
Ages(yrs)	**Female**	**Male**	**Female**	**Male**
0-9	11	5	12	12
10-19	3	6	6	3
20-29	5	7	4	6
30-39	10	2	2	4
40-49	3	3	2	2
50-59	2	1	0	0
60-69	2	0	1	1
70-79	1	3	1	0
80-89	0	0	2	0
Total	**37**	**27**	**30**	**28**

Distribution of *Staphylococcus aureus* by sex

There was a high prevalence of *S. aureus* among male (63.0%) inpatients and 50.0% for outpatients, compared to the 56.8% among the females in inpatients and 46.7% in outpatients. However, the overall prevalence of *S. aureus* among inpatients (both females and males) was 59.4% which was far higher than that in outpatients (48.3%).

The prevalence of *Staphylococcus aureus* in the suppurative lesions sampled

The lesions sampled included: septic arthritis, chronic otitis media, osteomyelitis, septic burns, traumatic wounds, subcutaneous abscess, pyomyositis, septic surgical wounds, chronic wounds, tropical ulcers and breast abscesses (table 2).

Table 2: The prevalence of Staphylococcus in the suppurative lesions sampled

	Frequency		Percentages	
Disease	**Inpatients**	**Outpatients**	**Inpatients**	**Outpatients**
Septic arthritis	2/4	0/1	50.0	0.0
Otitis Media	0/0	4/23	0.0	17.4
Cellulitis + abscesses	0/0	2/2	0.0	100.0
Osteomyelitis	5/6	3/4	83.3	75.0
Septic burns/ Traumatic wounds	10/17	10/13	70.5	83.3
S/c abscesses	4/10	8/12	40.0	66.0
Pyomyositis	10/11	1/1	90.1	100.0
Septic surgical wounds	1/11	0/0	9.1	0.0
Chronic wounds/Tropical ulcers	3/4	1/1	75.0	33.3
Breast abscesses	0/2	0/0	0.0	0.0

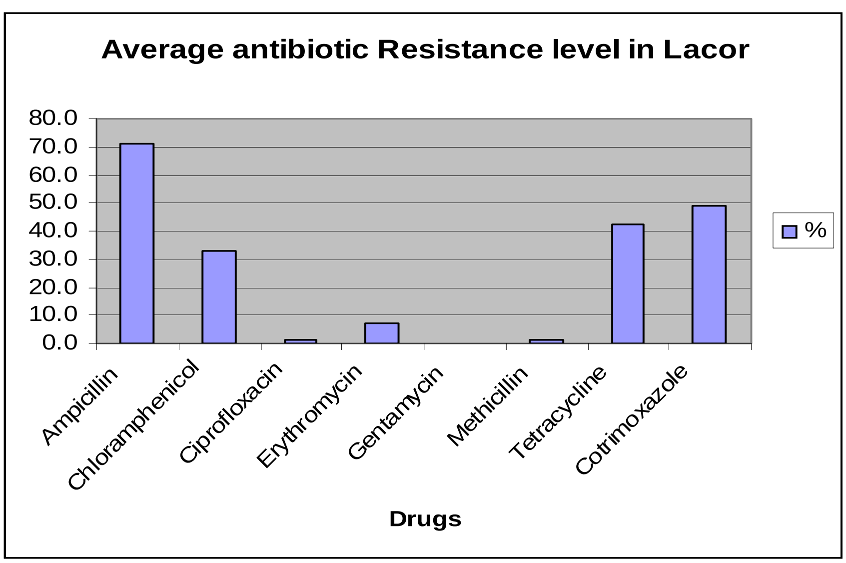
Antibiotic resistance levels for both inpatients and outpatients

Of the 8 antibiotics tested, ampicillin showed the highest overall resistance followed by co-trimoxazole, tetracycline, chloramphenicol and erythromycin respectively. Ciprofloxacin and Methicillin were at the same level while gentamycin was at 0 level of resistance (table 3).

Table 3: The resistance level for both inpatients and outpatient isolates

	Outpatients		Inpatients	
Antibiotics	**Resistance**	**%**	**Resistance**	**%**
Ampicillin	17/28	60.4	31/38	81.5
Chloramphenicol	28/28	28.6	14/38	36.8
Ciprofloxacin	0/28	0.0	1/38	2.6
Erythromycin	1/28	3.6	4/38	10.5
Gentamycin	0/28	0.0	0/38	0.0
Methicillin	0/28	0.0	1/38	2.6
Tetracycline	9/28	32.1	20/38	53.0
Co-trimoxazole	15/28	53.6	17/38	44.7

Graph 1: Average antibiotic resistance for inpatients and outpatients



Relative Risks

The relative risk (RR) of exposure to antibiotic resistant strains of *S. aureus* in inpatients and outpatients was 1.370.

Discussion

There is a higher prevalence of *Staphylococcus aureus* among inpatient isolates(59.4%) than outpatients (48.3%) and more cases were found among males (63.0%) than in females (56.8%). *Staphylococcus aureus* is recognized as one of the most important bacterial pathogen that seriously contribute to the problem of nosocomial and community acquired infections[6,12]. The combined prevalence in inpatient and outpatients isolates was higher than that of the normal career rates of 30-40% for the healthy non-hospitalized individuals[1].

The high prevalence among inpatients is expected due to the long hospital stay, ward conditions such as bed making, changing of clothes, sneezing, nose picking and other personal habits like poor hygiene, which pollute every patient in the wards[6]. Transmission of MRSA occurs primarily from colonized or infected patients or staff to other patients or vice versa[13]. Among the resistant pathogens, MRSA is of great concern because of its particular importance in causing various clinical conditions[13]. Therefore, the risk of acquiring *S aureus* infection is increased in the wards in the presence of other hospitalized 'shedders' who may be possibly infected with the antibiotic resistant strains[17]. This chance is further increased in the terminally ill and immuno-suppressed patients (HIV/AIDS patients and those on immunosuppressive drugs)[17].

Methicillin resistant staphylococcus aureus (MRSA) is a major nosocomial pathogen causing serious morbidity and mortality in immuno-suppressed patients[17]. The use of broad spectrum antibiotics in treating infections also increases the risk of acquiring MRSA and other resistant bacteria[16,17].

On the pattern of resistance, inpatient isolates showed a greater level of resistance on all antibiotics except co-trimoxazole (Trimothoprin + Sulfamethoxazole), where outpatients' isolates had a resistance of 53.6% compared to 44.7% for inpatients. The major challenge of controlling hospital acquired infections has been due to the emergence of multiple antibiotic resistant strains among the MRSA isolates[14].

Gentamycin recorded no resistant isolates in both departments. This drug which was proposed to be an alternative treatment for MRSA infection, was found to be better at treating MRSA infection in this part of the country. This is perhaps due to different colonial expansion and drug pressure in this community[14].

The resistance to beta-lactamase ampicillin was highest of all antibiotics at 81.5% in inpatients and 60.7% for outpatients. We attributed this to the presence of beta-lactamase producing *S. aureus* in hospital environment and 'selection pressure' due to the use of the beta-lactam antibiotics for the treatment, offering advantage for the selection colonization to more resistant beta-lactamase strains.

Knowledge of the pattern of antibiotic resistance among isolates is very important both clinically and epidemiologically. The results of antimicrobial resistance patterns are of great concern due to these predominant bacterial isolates which are highly resistant to commonly available antimicrobial agents[14]. The MRSA accounted for 2.6% of the surgical isolates and 1/66 of the isolates had a resistant profile to tetracycline, chloramphenicol, ampicillin and erythromycin. Intermediate to ciprofloxacin and only sensitive to gentamycin. This can be explained by the close relationship between the Methicillin resistant gene with other resistances such as ample production beta-lactamase responsible for resistance to ampicillin and other non-penicillinase-resistant penicillin (PRP) since resistance to penicillin is due to beta-lactamase. A high rate of MRSA isolates (88.2%) from the clinical specimens which showed multiple drug resistance with a substantial increase in the number of hospital acquired infections due to MRSA has been reported in many countries [14,15]. The resistance to different antibiotics among MRSA strains is very high[14]. This resistance has also been encountered in the methicillin resistance strains evidenced by the resistance to erythromycin and which could mean a possible resistance to all the MLS antibiotics.

Another alternative to gentamycin treatment of MRSA infections would be clindamycin. However, due to cross resistance with erythromycin for the patients who have ever been exposed to one drug, resistance was found to be high (82.4%)[14] in this study.

Furthermore, sensitivity of methicillin resistant strains to gentamycin could mean that there is a possibility of sensitivity to all aminoglycosides such as streptomycin, neomycin and kanamycin. However, this is not totally certain as each of the aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation e.g. streptomycin and impermeability of membranes[11]. The relative risk of acquiring antibiotic resistant S *aureus* in the surgical ward was as high as 1.299. This is attributable to ward conditions which favour the spread of nosocomial infections[6].

Conclusion

The prevalence of *Staphylococcus aureus* was higher among inpatients (59.4%) as compared to outpatients (48.3%). Inpatient isolates were more resistant to antibiotics than outpatient isolates. The prevalence of S*taphylococcus aureus* in males in both departments was higher than among females.

Recommendation

More effective drugs such as ciprofloxacin, gentamycin, methicillin and erythromycin should be reserved and used for infection whose susceptibility profile is not yet known in Lacor Hospital and the surrounding areas but should also not be used solely, because rapid resistance may develop.

Continuous surveillance using susceptibility tests should be carried out as antibiotic susceptibility profile varies from time to time, and control measures should be put in place in hospitals.

Further research should be undertaken to ascertain why the prevalence of *Staphylococcus aureus* was higher in males than females in both inpatients and outpatients of St. Mary's Hospital Lacor.

Acknowledgement

We thank Lacor Hospital administration and staff for the support they gave us to enable us conduct and complete this study successfully.

References

1. Taussig JM. Processes in Pathology and Microbiology, 2nd Edition: *Blackwell scientific publications;* 1984, 480:518-530.
2. Meyers HF, Ernest J, Allan G. A review of Medical Pharmacology.1970; 49:467-474 and 50:475-581. *Lange Medical Publishers (1970).*
3. Duckworth GT, Lothan JLE, Williams JD. MRSA; A report of an outbreak in London teaching Hospital. *J Hospit infect;* 1988:11:1-11.
4. Cookson BD, Phillips I. Epidemic MRSA. *J Antimicrob chemother.1998*;21: supplement c 57-65.
5. Wenzel RP, Neltteman MD, Jones RN, Karakawa WW. MRSA implications for the 1990s and effective control measures. *Am J Med. 1991;91(supplement 3B)*:2215-2275.
6. Collins CH, Patricia M, Grange JM. Collins and Lyne's microbiological Methods, 6th Edition, *Butterworth-Geubnmann Publishers*: 1991:24:299-304.
7. Shanson DC. Antibiotic Resistant Staph aureus. *J Hospit infect.*1981; 2:11-16.
8. Boyce JM. Increasing prevalence of MRSA in the USA. *J infect control and Hospital epidemiol.*1990; 11:639-642.
9. Baliruno D. Prevalence and antimicrobial sensitivity patterns of Staph aureus in pus sample isolates of patients attending Mulago Hospital surgical ward. *A special project report of Makerere University faculty of Veterinary Medicine,* 2001; 4:18-21.
10. Cheelsborough M. Medical Laboratory Manual for the tropical countries.1984; 24 (2):58-69 and 36:225-227.
11. AL. Masaudi SB, Day MJ, Russel AD Antimicrobial resistance and gene transfer in Staph aureus. *J Applied Bacteriol.*1991; 70:279-290.
12. Jan MB, Turnidge JD, Sentry A. High prevalence of oxacillin-resistant Staphylococcus aureus isolates for hospitalized patients in Asia-Pacific and South Africa. Results from the sentry antimicrobial surveillance program, 1998-1999. *J antimicrob agents Chemotherap.*2002; 46:879-887.
13. (Stephani & Varaldo, 2003; Kloos and Schleiffer, 1986), Stephani S, Varado PE (2003). Epidemiology of Methicillin Resistant Staphylococcus aureus in Europe. *J Clinic Microbiol infect.* 2003; 9:1179-1186.
14. Ojulong J, Mwambu TP, Jalooba M, Bwanga F, Kaddu-Mulindwa DH. Relative prevalence Methicillin-Resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzania J Health Research.* 2009; 11(3):149-153.
15. Leclercq R. Staphylococcus resistant to antibiotic therapy. *Annales Francaises d'anaestheise e_t de Reanimation.* 2002; 21:375-83.
16. Vidhani N, Mehndiratta PL, Mathur MD. Study of the Methicillin Resistant Staphylococcus aureus (MRSA) from high risk patients. *Indian Med J Microbiol.*2001; 19:13-16.
17. Sheff B. VRE and MRSA: Putting bad bags out of business. 1998. Nursing findarticles.com.