Journal of Microbiology, Biotechnology and Food Sciences

Oyetunji I. Ajoke et al. 2012 : 1 (6) 1389-1405

REGULAR ARTICLE

PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM HEALTHY COMMUNITY INDIVIDUALS VOLUNTEERS IN JOS SOUTH, NIGERIA

Oyetunji I. Ajoke[1], Ikenna Osemeka Okeke[1], Olumide A. Odeyemi [2, 3], A.E.J. Okwori [1]*

[1]Federal College of Veterinary and Medical Laboratory Technology, Vom Plateau State, Nigeria.

[2]School of Biosciences and Biotechnology, Faculty of Science and Technology, National University of Malaysia.

[3]International Centre for Biomedical Research and Training, Ile Ife, Osun State, Nigeria.

*Corresponding author: ajoke2009@ymail.com

ABSTRACT

This study investigated the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) from the nasal swabs of healthy community individual volunteers in Jos South, Nigeria and its susceptibility pattern to seven other antibiotics. Standard procedures were employed for isolation, screening, and susceptibility testing. The result of this study reveal that 98 (49 %) *S. aureus* were isolated from 200 nasal swab samples collected. The prevalence rate for male and female group was 48 % and 50 % respectively. Sixty two isolates (63.3 %) were found to be methicillin resistant. The MRSA isolated were highly resistant to Ampicillin (88.7 %), Amoxicillin (85.5 %), Tetracycline (80.6 %), Cotrimoxazole (80.6 %) but had low resistance to Erythromycin (35.5%). The MRSA isolated showed high susceptibility to Ofloxacin (98.4 %) and Gentamicin (83.9 %). While 55 (88.7 %) of the MRSA isolated showed multidrug resistance and only 3 (4.8 %) were susceptible to all other tested antibiotics.

Key words: antibiotics susceptibility, screening, nosocomial infection

INTRODUCTION

The initial discovery of antibiotics (Greek anti; against and bios; life) during the 1940s and 1950s gave clinicians weapons against infections that once wiped out entire populations. The discovery and subsequent development of these antimicrobial agents revolutionized medical care worldwide. Therefore, mankind has been so pleased with its ability to conquer diseases. However, the dawn of the antibiotic era was quickly accompanied by the development of numerous problems including the emergence of microbes with resistant strains (multi-drug resistant bacteria), increased number of nosocomial and community-acquired infections; and the increase in healthcare cost arising from the wide spread use of antibiotics (**Prescott *et al.*, 2008; Synder *et al.*, 2000**). Consequently, diseases caused by these pathogens, for example *Staphylococcus aureus* are becoming increasingly more difficult to treat as the causative organism accumulate new antimicrobial resistance determinants (**Henry and Elias, 1995**, **Chambers, 2005; Susan and Robert, 2006**).

This pathogen causes various types of diseases and infections ranging from minor skin to soft tissues infections including immune-compromised patients due to its ability to survive in different growth conditions (**Lowy, 1998; Lowy, 2003**). *S. aureus* is also recognized as one of the most important bacteria pathogen seriously contributing to the problems of hospital and community-acquired infections all over the world (**Leski *et al.*, 1998***). S. aureus* is a common skin and nasopharynx commensal, a frequent causative agent of burns and wound sepsis. It produces pustules, carbuncles, furuncles and impetigo; it is a frequent causal of septicemia, bacteraemia, osteomyelitis and otitis (**Emmerson, 1994**). It is also a common causative agent of infections in hospitals and it is most liable to infect new-born babies, surgical patients, old and malnourished persons and patients with diabetes and chronic diseases (**Tuo *et al.*, 1995**). The control of this disease as well as the high mortality due to *S. aureus* was greatly reduced with the use of penicillin in the early 1940s. However, this success was short-lived as penicillin resistant *Staphylococcus aureus* (PRSA) producing beta-lactamase quickly emerged and 90 percent of hospital-acquired *S. aureus* were penicillin resistant within 10 years (**Susan and Robert, 2006**). The beta-lactamase enzyme destroys the penicillin antibiotic by hydrolyzing the beta-lactam ring and this decreases the usefulness of the penicillin antibiotic. Methicillin, a beta-lactamase-insensitive beta-lactam, provided new treatment options for PRSA infections in the late 1950s, but methicillin-resistant *Staphylococcus aureus* (MRSA) that are cross resistant to all beta-lactams soon emerged, primarily in health care environments (**Susan and Robert, 2006**).

Community-acquired MRSA infections among individual without healthcare-associated risk factor was first recognized about fifteen years. Since these reports, community-acquired methicillin-resistant *Staphylococcus aureus* have been globally pervasive (**Vandenesch *et al.*, 2003**); and reports of serious and rapidly progressive fatal disease due to virulent community-acquired strains have alarmed healthcare professionals and lay media alike (**Adem *et al* ., 2005; Mongkolrattanothai *et al.*, 2003**). Despite the global incidence of MRSA, yet its spread in developing countries or nations is still under extimated. With increasing travel and movement throughout the world, transmission of multi-resistant super bugs from one country to another became a possibility (**Ang *et al.,* 2004**).

Rationale of the study

Susan and Robert, (2006) reported *S. aureus* as been found both in and out hospital patients. Resistant strains of *Staphylococcus aureus* have hampered attempt to eradicate diseases and infections caused by this pathogen. **Pan *et al.,* (2005) and Lo *et al.,* (2006)** reported that MRSA plays crucial role in diseases acquired from the community. and Increasing reports of CA-MRSA from the United states, Canada, Australia are evidence that there is changing epidemiology of the organism (**Sonal *et al*.,2003**). Due to these changes, it is not important to assess the carriage rate of MRSA in the community amongst healthy individuals who have not been hospitalized nor had antibiotic therapy in the recent past.

AIM AND OBJECTIVES

1. To determine the prevalence of MRSA and MSSA isolated from the nasal swabs of healthy community individuals resident in Jos south.
2. To determine the prevalence of multiple antibiotic resistance pattern of *S. aureus*.
3. To alert laboratory staff and the general public on the virulent nature of CA-MRSA.
4. To recommend appropriate control measures.

MATERIAL AND METHODS

Study Area

The study was conducted in three communities (Vom, Kaduna-vom and Kuru) located in Jos South Local Government Area. The environment is endowed with variety of physical features and the dominant tribe is the Beroms with settlers from Benue, Yorubas and Igbos. Residents are generally industrious and engage in a number of professions.

Ethical Consideration

Before samples were collected, information regarding the study was explained to the community individuals after approval was sought from the head of each community. Oral consent for participation in the study was obtained.

Questionnaire

Questionnaire to obtain relevant information to the study were distributed to the community individuals.

Definition of Community - Associated Samples

Community – associated samples were defined as stated by previous researchers. (Onanuga *et al.,* 2005).

Media

The following media were used in this study: Mannitol Salt Agar – MSA (Oxoid), Oxacillin Agar (Oxoid), Nutrient Agar (Oxoid) and Nutrient Broth (Oxoid). These media were prepared according to manufacturers' instruction and refrigerated prior to usage.

Sampling

Nasal swabs (both anterior nares) were collected randomly from 200 healthy individuals who were classified into two groups namely male and female between 18 - 40 years from three communities in Jos South Local Government Area. Hundred samples from each group were collected using sterile swab sticks in batches and transported immediately to the laboratory. Each swab was properly labelled for easy identification.

Isolation and identification of bacteria

Swab samples were aseptically applied to a small area (the well) of MSA plates whose surfaces have been dried in the incubator shelf at 37 °C for 10 minutes prior to use. Each inoculum was aseptically streaked out from the well to obtain discrete colonies. The plates were then incubated aerobically at 37 °C for 24 hours. The characteristics golden yellow colonies were aseptically isolated subcultured onto nutrient agar slants and further identified using established microbiological methods that include colonial morphology, Gram stain characteristics and biochemical tests (**Cheesbrough, 2004**). Isolates that were Gram-positive cocci in clusters, catalase positive and coagulase positive were considered as *S. aureus* in this study.

Biochemical identification of presumptive MRSA isolates

All the presumptive MRSA isolates were differentiated from other coagulase positive isolates following the methods of **Cheesbrough, (2002)**.

Antibiotics resistance test

A total of eight (8) antibiotics were obtained from a reputable pharmaceutical store in Bukuru, Jos. They are: Cloxacillin (Hovid, Malaysia), Ofloxacin (International Dispensary Asssociation (IDA), Netherlands). Ampicillin (International Dispensary Association (IDA), Netherlands). Erythromycin (Mekophar Chemical Pharmaceutical, GMP, Vietnam). Gentamycin (Green Field Pharmaceutical Limited, China). Tetracycline (Green Field Pharmaceutical Limited, China). Amoxicillin (Troge Medicals, GMBH, Hamburg and Germany). Cotrimoxazole (Petisow Laboratory Limited, Nigeria). Cloxacillin was prepared

according to the method for dilution for antimicrobial susceptibility testing given by the Clinical and Laboratory Standards Institute (CLSI) . The remaining antibiotics were prepared into dics form according to the performance standard for antimicrobial disc susceptibility testing given by CLSI . Thus, the antibiotic discs were used with their concentration as follows: Ofloxacin (5 µg), Ampicillin (10 µg), Erythromycin (15 µg), Gentamycin (10 µg), Tetracycline (30 µg), Amoxicillin (25 µg), Cotrimoxazole (23.75 µg).

Phenotypic detection of MRSA using the oxacillin agar

Susceptibility of all the isolates, which were *S. aureus* was done by means of the agar screening method on nutrient agar containing 6 µg / mL of Oxacillin (Cloxacillin; 500 mg) and 4 % Sodium chloride. The *S. aureus* isolates were standardized to 0.5 Mcfarland standard. The standardized suspension was spot inoculated aseptically onto the nutrient agar plates. The plates were incubated for exactly 24 hours at 30 °C.

Antibiotics susceptibility testing (AST) to other tested antibiotics

The antibiotics resistance pattern of the isolates was determined against seven commercial antibiotics. A sterile wire loop was used to touch three well isolated colonies of each isolates on agar plate and emulsified in 4 ml of nutrient broth. The broth culture was incubated for few hours until it became slightly turbid and the turbidity of each suspension was then matched to standard turbidity (0.5 Mcfarland standards). A sterile cotton swab was dipped into standardized bacterial test suspension of each isolate and used to evenly inoculate the entire surface of the dried nutrient agar plates. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension before inoculation. After inoculation, the surface of the agar was dried for 5 minutes with the petridish lid in place after which the appropriate antibiotic disks, which have been allowed to attain room temperature about 1 hour before use were aseptically placed evenly on each inoculated plates with sterilized forceps. Each disk was firmly pressed to ensure its contact with the agar surface. Within 30 minutes of applying the disks, the plates were inverted and incubated aerobically at 35 °C for 18 hours. *S. aureus* (Oxford strain) was used as positive control. After 24 hours incubation, zones of inhibition exhibited by each isolate against tested antibiotics were measured and recorded in milimeter. These were then interpreted as

susceptible, intermediate and resistant according to standard specifications of CLSI, designed for antibiotics testings (**CLSI, 2002; Cheesbrough, 2004; Margaret, 1997**).

RESULTS AND DISCUSSION

Out of the two hundred nasal swab samples collected and screened, a total of 98 (49 %) of the isolates were found to be *S. aureus* based on morphology and biochemical tests. The prevalence rate from male and female individuals were 48 (48 %) and 50 (50 %) respectively as shown in Table 1. Table 2 shows the prevalence rate of MRSA and MSSA colonization among healthy individuals in the community. Of the *S. aureus* isolated, 62 (63.3 %) were MRSA while 36 (36.7 %) were MSSA. The result of the antibiotic susceptibility pattern of methicillin-resistant isolate to other antibiotics is shown in Table 3. The result show high resistance to Ampicillin (88.7 %), Amoxicillin (85.5 %), Tetracycline (80.6 %), Cotrimoxazole (80.6 %) but had low resistance to Erythromycin (35.5 %), Gentamicin (12.9%) and Ofloxacin (1.6 %). Also, the antibiotic susceptibility pattern of Methicillin-susceptible isolates to other antibiotics is shown in Table 4.The result also show high resistance to Ampicillin (80.6 %), Tetracycline (72.2 %), Amoxicillin (69.4 %), Contrimoxazole (66.6 %) but low resistance to Erythromycin (25 %), Gentamicin (1 %) and Ofloxacin (1%). The prevalence of multi-drug resistance among MRSA isolates is shown in Table 5. Multidrug resistance was defined in this study as resistance to three or more of the antibiotics tested. Thus 55 (88.7 %) of the MRSA isolates showed multidrug resistance to the antibiotics tested while only 3 (4.84 %) were fully susceptible to all the tested antibiotics. The prevalence of multidrug resistance in the MSSA isolates is shown in Table 6. 26 (72.2 %) of the MSSA isolates showed resistance to at least three 3 tested antibiotics while only 5 (13.9 %) were fully sensitive to all tested antibiotics.

Table 1 Frequency of isolation of *S. aureus* from healthy community individuals in Jos South, Nigeria.

SOURCE	NUMBER SAMPLED	NUMBER OF SAMPLES COLLECTED	*S. aureus*	
			Number	Percentage(%)
Male	100	100	48	48
Female	100	100	50	50
TOTAL	200	200	98	49

Table 2 Percentage prevalence of MRSA and MSSA in the community

SOURCE	MRSA		MSSA	
	Number	Percentage (%)	Number	Percentage (%)
Male	28	28.6	20	20.4
Female	34	34.7	16	16.3
Total	62	63.3	36	36.7

Legend: MRSA – Methicillin resistant *Staphylococcus aureus.*

MSSA – Methicillin susceptible *Staphylococcus aureus.*

Table 3 Antibiotic susceptibility pattern of 62 MRSA isolated from the nasal swab samples of healthy community individuals in Jos South, Nigeria.

ANTIBIOTICS	RESISTANCE		INTERMIDIATE		SUSCEPTIBLE	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Ampicillin	55	88.7	0	0	7	11.3
Amoxicillin	53	85.5	0	0	9	14.5
Tetracycline	50	80.6	0	0	12	19.4
Cotrimoxazole	50	80.6	0	0	12	19.4
Erythromycin	22	35.5	4	6.4	36	58.1
Gentamicin	8	12.9	2	32	52	84.0
Ofloxacin	1	1.6	0	0	61	98.4

Table 4 Antibiotic susceptibility pattern of 36 MSSA isolated from the nasal swab samples of healthy community individuals in Jos South, Nigeria.

ANTIBIOTICS	RESISTANCE		INTERMIDIATE		SUSCEPTIBLE	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Ampicillin	29	80.6	0	0	7	19.4
Amoxicillin	25	69.4	0	0	11	31.0
Tetracycline	26	72.2	0	0	10	27.8
Cotrimoxazole	24	66.6	1	2.8	11	31.0
Erythromycin	9	25.0	0	0	27	75.0
Gentamicin	1	2.8	7	19.4	28	77.8
Ofloxacin	1	2.8	1	2.8	34	94.4

Table 5 Percentage revalence of multiple drug resistance among 62 MRSA isolates

Parameter	Number	Percentage (%)
Complete antibiotic sensitivity	3	4.84
Resistance to 1 antibiotic	1	16
Resistant to 2 antibiotics	3	4.84
Resistant to 3 antibiotics	15	24.2
Resistant to 4 antibiotics	18	29.2
Resistant to 5 antibiotics	17	27.4
Resistant to 6 antibiotics	5	8.1
Resistant to 7 antibiotics	0	0

Table 6 Percentage prevalence of multiple drug resistance among 36 MSSA isolates

Parameter	Number	Percentage (%)
Complete antibiotic sensitivity	5	13.9
Resistance to 1 antibiotic	1	2.8
Resistant to 2 antibiotics	4	11.0
Resistant to 3 antibiotics	10	27.8
Resistant to 4 antibiotics	8	22.2
Resistant to 5 antibiotics	7	19.4
Resistant to 6 antibiotics	1	2.8
Resistant to 7 antibiotics	0	0

Methicillin-resistant *S. aureus* (MRSA) has been proven to be one of the most worldwide spread nosocomial pathogen of the 20th century (**Nimmo *et al.*, 2000**). New strains of community-associated (CA)-MRSA that cause infections in healthy people have also been detected worldwide (**Vandenesch *et al.*, 2003**), and its increasingly developing resistance to many antibiotics (**Lowy, 2003**). An overall prevalence rate of 98 (49 %) of *S. aureus* was obtained from the nasal swab samples of healthy individuals in this study. The rate of *S. aureus* colonization in this study were 48 (48 %) and 50 (50 %) for male and female healthy individuals respectively. This finding is in agreement with the reports of **Nester *et al.* (2001**) that 20 % of healthy adults have continually positive nasal cultures for a year or more while over 60 % will be colonized at some time during a given year. Also, the colonization rate may range from 10 % or more than 40 % in a normal adult population (**Kloos,1998**). In addition, samples were obtained when the environment was dry and contaminated with various potential sources especially dust particles and consequently inhaled since the nostrils serves as an air passage. In significant difference (P = 0.7) was observed in colonization rate of *S. aureus* in the male and female group indicating that sex is not a remarkable factor in colonization and there is no activity or way of life of any of the groups that predisposes them to *S. aureus*.

The difference in the MRSA colonization in the male and female group was also not significant (P = 0.5). MRSA isolated from individuals in this study is community-associated. Some studies have suggested that recent antimicrobial drug use play a role in CA-MRSA colonization (**Bagget *et al.*, 2004; Ellis *et al.*, 2004**). This study does not confirm this hypothesis since none of the individuals has had recent antimicrobial therapy, healthcare contacts and strains were resistant to beta-lactam antibiotics. However, this findings support the reports by **Onanuga *et al.* (2005)** who observed 69 % MRSA colonization rate from healthy women who were not on any antibiotics and had not been admitted in the hospital in the last one year before sampling. **Lina *et al.*, (1999)** reported that a common stable marker gene in CA-MRSA is the Panton-Valentine Leukocidin gene , and CA-MRSA isolates are distinct strains emerging *de novo* from community-associated methicillin susceptible isolates (**Susan and Robert, 2007**). Therefore, possession of MRSA isolated in this study of Panton-Valentine Leukocidin gene is to be investigated. In this study, the percentage resistant to oxacillin (methicillin) is of great concern and has been widely reported internationally (**Fridkin, 2001; Hiramatsu *et al.*, 1997**), and in Nigeria communities (**Ikeh, 2003; Onanuga *et al.*, 2005; Olayinka *et al.*, 2005**).

The MRSA isolated in this study showed high resistance to Ampicillin (88.7 %) and Amoxycillin (85.5 %), which support the findings that MRSA strains are equally resistant to all beta-lactam antibiotics (**Weems, 2001; Gross- Schulman *et al.,* 1998**). The high level of resistance observed in ampicillin is also in agreement with the reports by **Onanuga *et al.*(2005**) who observed a resistivity of 91.7 % and 100 % to ampicillin respectively in their two different findings. The MRSA isolated also showed a high resistance to tetracycline (80.6 %) and Cotrimoxazole (80.6%) but had low resistance to Erythromycin (35.5 %). The resistance observed in tetracycline and cotrimoxazole is similar to the reports of **Ogmibu and Omu (1986)** and **Uwaezuoke and Aririatu (2004)** who observed a resistivity of 71.4 % and 87.5 % respectively to tetracycline and **Sonal *et al.* (2003)** who observed a resistivity of 88 % to cotrimoxazole. In addition, resistance observed toward tetracycline and cotrimoxazole may be attributed to the capability of the organism to exhibit remarkable versatility in its behavior towards antibiotics and that most isolates of *S. aureus* are resistant to commonly prescribed antibiotics (**Grassi, 1998; Olayinka *et al.,* 1995**).

The low resistance observed by the MRSA isolated in this study towards Erythromycin is also similar to the reports of **Hantash *et al.* (2008)** who observed a resistivity of 38 % to Erythromycin. In this study, a high susceptibility to Ofloxacin, a Fluoroquinolone and Gentamycin in the MRSA isolated were recorded. The high sensitivity to Ofloxacin (98.4 %) and Gentamycin (83.9 %) supports some previous reports (**Ehinmindu, 2003; Fridkin *et al.,* 2005; Ikeh, 2003;Onanuga *et al.,* 2005; ; Nordmann and Nass, 2005; Olayinka *et al.,* 2004; Uba and Umar, 2004**). In 2001, **Polyzou *et al.*,** reported that high susceptibility to Ofloxacin and Gentamycin may be as a result of conferring genes being absent in these MRSA strains. Also, gentamycin, an aminoglycoside though very cheap, its high susceptibility may be due to the complexity of the aminoglycoside and the route of administration while the fluroquinolones are newer drugs with mode of action centre on inhibition of the DNA replication which stops the multiplication of bacterial cells and relatively expensive therefore are more likely not available for abuse (**Onanuga *et al.,*2005)**. In addition, susceptibility of the MRSA isolated to Ofloxacin and Gentamycin supports the findings of **Onanuga *et al.* (2005**) that these non-beta-lactam antibiotics may provide an opportunity for the recommendation of these drugs for the treatment of CA-MRSA infections. Furthermore, a high level of multiple drug resistance was observed in both MRSA and MSSA isolated from healthy community individuals in the study. 94 % of the MRSA and 83.3 % of the MSSA isolated were resistant to at least two antibiotics tested and only 4.8 % of the MRSA and 13.9 % of the MSSA isolated were susceptible to all the antibiotics tested in this

study. This observation confirms the postulation that healthy members of the community are the highest reservoir of antimicrobial resistant bacteria (**Laminkanra *et al.*, 1996).** The high prevalence rate of multi-drug resistant MRSA from the nasal swabs of healthy individuals in the community should be of great and immerse concern to the health professionals and all members of the society because transmission of infections caused by these strains is readily established by close contact (**Xander *et al.*, 2006**). This study however involved a small number of isolates, larger community based studies are needed to confirm the true prevalence and that transmission is occurring more frequently in community setting.

CONCLUSION AND RECOMMENDATION

The study has established a high prevalence of multi-drug resistance MRSA among healthy community individuals without any healthcare risk factor. The MRSA isolated showed multiple drug resistance to the beta-lactams and commonly prescribed antibiotics tested-cotrimoxazole and tetracycline. The society is presently characterized with inappropriate prescription, unethical dispensing and indiscriminate use of antibiotics. Drugs are made available to the public at large in the country commonly known as over-the-counter availability of antimicrobial drugs which allows people to practice self medication. This increases the rate at which most antibiotics are losing the battle in the treatment of infections. Antibiotics are also sometimes prescribed without determining bacterial sensitivity to antibiotics. Furthermore, many physicians administer antibacterial drugs to patients with cold or other viral diseases and most patients frequently stop taking their medications when syndromes go away but the infection is not completely gone. All these encourage the emergence of resistant strains. It can be concluded therefore, that there is an urgent need to re-assess policies on antibiotics use within and outside the hospital environment. Therefore, control of multiple drug resistance will provide a major challenge to both the healthcare community and the society in general.

The following recommendations are essential in the containment of resistance to antimicrobial agents:

1. Formulation and implementation of a national drug policy by the government to ensure rational use of antibiotics.
2. Effective education and training of the public about the appropriateness and limitations of antibiotics to ensure that they are utilized wisely and also the need to adopt a high personal hygiene.

3. Proper prescription of antibiotics and implementation of an agreed control infection policies.
4. Laboratories should screen patients properly to give an early warning of the presence of a resistant organism and allow the assessment of barrier and infection control techniques.
5. The use of antibiotics in livestock and animal feeds should be adequately controlled. This may aid transmission of resistant strains from animals or livestock to humans and further complicating the epidemiology of this organism.

REFERENCES

ADEM, P.V - MONTGOMERY, C.P - HUSAIN, A. N. 2005. *Staphylococcus aureus* sepsis and the Waterhouse-Friderichen syndrome in children. In *New England Journal of Medicine,* vol. 353, 2005, p. 1245-1251.

ANG, J.Y - EZIKE, E - ASMAR, B.I. 2004. Antimicrobial resistance. In *Indian Journal of Pediatrics*, vol. 71, no. 3, 2004, p. 229-239.

BAGGET, H.C - HENNESY, T.W - RUDOLPH, K - BRUDEN, D - REOSONOVER, A - PARKINSON, A. 2004. Community onset of MRSA associated with antibiotic use and the cytotoxin Panton-Valentine Leukocidin during a furunculosis outbreak in rural Alaska. In *Journal of Infectious Disease,* vol. 189, 2004, p. 1556-1573.

CHAMBERS, H.F. 2005. Community-associated MRSA-resistance and virulence converge. In *New England Journal of Medicine,* vol. 352, 2005, p. 1486-1487.

CHEESBROUGH, M. 2004. District laboratory practice in tropical countries part 2, Cambridge University Press, United Kingdom, 2004, pp. 434.

CLINICAL LABORATORY STANDARDS INSTITUTE. (CLSI). 2002. Performance standard for antimicrobial disc susceptibility test. Twelfth informational supplement M100 – S12., Wayne, PA. USA.

CLINICAL LABORATORY STANDARDS INSTITUTE. (CLSI) 2000. Methods for dilution of antimicrobial susceptibility testing for bacteria that grows aerobically. 5th edition., Approved standard MT – A5. Clinical laboratory Standards Institute, Wayne, PA. USA.

EHINMIDU, J.O. 2003. Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. In *Tropical Journal of Pharmaceutical Research,* vol. 2, 2003, p. 223-228.

ELLIS, M.W - HOSPENTHAL, D.R - DOOLEY, D.P - GRAY, P.J - MURRAY, C.K. 2004. Natural history of CA-MRSA colonization and infection in soldiers. In *Clinical Infectious Disease,* vol. 39, 2004, p. 971-979.

EMMERSON, M. 1994. Nosocomial staphylococcoal outbreak. In *Scandanvian Journal of Infectiou Disease,* vol. 93, 1994, p. 47-54.

FRIDKIN, S.K. 2001. Vancomycin-intermediate and resistant *Staphylococcus aureus:* What the infectious disease specialist need to know. In *Clinical Infectious Disease,* vol. 32, 2001. p. 108-115.

GRASSI, G.G. 1998. Infections by Gram-positive bacteria: An overview. In *Journal of Antimicrobials and Chemotherapy,* vol. 21, 1998, p. 1-7.

GROSS-SCHULMAN, S - DASSEY, D - MASCOLA, L - ANAYA,C. 1998. CA-MRSA disease in three communities. In *New England Journal of Medicine,* vol. 352, 1998, p. 1436 - 1444.

HANTASH, A.E - MUNA, J - ABDUL, J.T. 2008. Nasal carriage of methicillin-resistant *Staphylococcus aureus* in individuals exposed and not exposed to hospital environments. In *European Journal of Scientific Research,* vol. 22, no. 4, 2008, p. 570-574.

HENRY, S.F - ELIAS, A.M.1995. Pathogens resistant to antimicrobial agents. In *Infect. Dis. Clin. North American,* vol. 9, no. 3, 1995, p. 497-528.

HIRAMATSU, K - HANAKI, H - INO, T - OGUN, T - TENOVER, F.C. 1997. MRSA clinical strains with reduced vancomycin susceptibility. In *Journal of Antimicrobials and Chemotherapy,* vol. 40, 1997, p. 135-136.

IKEH, E.I. 2003. Methicillin resistant *Staphylococcus aureus* (MRSA) at the Jos University Teaching Hospital. In *African Journal of Clinical and Experimental Microbiology,* vol. 4, 2003, p. 52-62.

KLOOS, W.E. 1998. *Staphylococcus*: In Collier, L., Ballows, A. and Sussman, M(ed),Topley and Wilson's Microbiology and Microbial infections. 9th edition, volume 2, Oxford University Press, New York, pp. 577-632.

LAMIKANRA, A - AKO-NAI, A.K - OGUNNIYI, D.A. 1996. Transferable antibiotic resistance in *Escherichia coli* isolated from healthy Nigerian school children. In *International Journal of Antimicrobial Agents,* vol. 7, 1996, p. 59-64.

LESKI, J - OLIVEIRA, D - TRZUNSKI, K. 1998. Clonal distribution of MRSA in Poland. In *Journal of clinical microbiology,* vol. 36, 1998, p. 3532-3535.

LINA, G - PIEMONT, Y - *ET AL*. 1999. Involvement of Panton- Valentine Leukocidin producing *Staphylococcus aureus* in primary skin infections and pneumonia. In *Clinical Infectious Disease,* vol.12, 1999, p. 1267-1270.

LO, W - LIN, W.T - TSENG, M.H - WANG, S.R - CHU, M.L - WANG, C.C. 2006. Commmunity acquired methicillin-resistant *Staphylococcus aureus* in children, Taiwan. In *Emerging Infection Disease*, vol. 12, 2006, p. 1267-1270.

LOWY, F.D.1998. *Staphylococcus aureus* infections. In *New England Journal of Medicine,* vol. 339, 1998, p. 520-532.

LOWY, F.D.2003. Antimicrobial resistance: The example of *Staphylococcus aureus*. In *Journal of Clinical Investigations,* vol. 111, 2003, p. 1265-1267.

Margaret, B. 1997. Customized Microbiology Laboratory Exercises. 2nd edition, McGraw-Hill Companies, Inc, New York, pp. 251.

MONGKOLRATTANOTHAI, K - BOYLE, S - KAHANA, M. D - *ET AL*. 2003. Severe *Staphylococcus aureus* infections caused by clonally related community acquired methicillin susceptible and methicillin resistant isolates. In *Clinical Infectious Disease*, vol. 37, 2003, p. 1050 – 1058.

NESTER, E. W - DENISE, G. A - EVANS ROBERTS JNR. C - NANCY, N. P - MARTHA, T. N. 2001. Microbiology : A Human Perspective. 3rd edition., McGraw-Hill Companies, Inc, New York, pp. 820.

NIMMO, G.R - SCHOONEVELDT, J.O - O'KANE, G - MCCALL, B - VICKERY, A. 2000. Community acquisition of gentamicin sensitive methicillin-resistant *Staphylococcus aureus* in South East Queensland, Australia. In *Journal of Clinical Microbiology,* vol. 38, 2000, p. 3926-3931.

NORDMANN, P - NASS, T. 2005. Transmission of methicillin- resistant *Staphylococcus aureus* to a microbiologist. In *New England J Medicine,* vol. 352, 2005, p. 1489-1490.

O'BRIEN, F. G - PEARMAN, J. W - GRACY, M - RITLEY, T. V - GRUBB, W. B. 1999. Community stain of methicillin – resistant *Staphylococcus aureus* involved in a hospital outbreak. In *Journal of Clinical Microbiology,* vol. 37, 1999, p. 2858 – 2862.

OGBIMU, A. O - OMU, A. E. 1986. Nasal carriage rates of *Staphylococcus aureus* among hospital individuals. In *Nigerian Journal of Microbiology,* vol. 6, no. 12, 1996, p. 41 – 46.

OLAYINKA, B. O - OLAYINKA, A. T - ONAOLAPO, J. A - OLURINOLA, P. F. 2005. Pattern of resistance to vancomycin and other antimicrobial agents in a university teaching hospital. In *African Journal of Clinical and Experimental Microbiology,* vol. 6, 2005, p. 46-52.

ONANUGA, A - OYI, A. R - ONAOLAPO, A. J. 2005. Prevalence and susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. In *African Journal of Biotechnology,* vol. 4, no. 11, 2005, p. 1321- 1324.

PAN, E. S - DIEP, B.A - CHARLEBOIS, E.D - AVERSWALD, C - CARLETON, H.A - SENSABAUGH, G.F - ET AL. 2005. Population dynamics of nasal strains of methicillin-resistant *Staphylococcus aureus* and their relation to community-associated disease activity. In *Journal of Infectious Disease,* vol. 192, 2005, p. 811-818.

POLYZOU, A - SLAVAKIS, A - POURNARAS, S - ET AL. 2001. Predominance of a MRSA clone susceptible to erythromycin and several other non-beta lactam antibiotics in a Greek hospital. In *Journal of Antimicrobial agents and Chemotherapy,* vol. 48, 2001, p. 231-234.

PRESCOTT, L.M - JOHN, P.H - DONALD, A.K. 2008, Microbiology,7th edition, McGraw-Hill Companies, Inc, New York, pp. 992.

SONAL, S - KAVITA, S - VIBHA, T. 2003. MRSA prevalence in a community in the east Delhi area. In *Japanese Journal of Infectious Disease,* vol. 56, 2003, p. 54-56.

SUSAN, B - ROBERT, S.D. 2006. Community-associated methicillin resistant. In *Journal of Applied Science Environmental Management,* vol. 8, no. 1, 2004, p. 60 - 65.

SYNDER, J.W - MCDONALD, L.C - VANENK, R. 2000. Common bacteria whose susceptibility to antimicrobials is no longer predictable. In *Lebanon Medical Journal,* vol. 12, 2000, p. 124-127.

TOU, P - MONTOBBIO, G - CALLARINO,R - TUMOLO, M. - CALERO, M.G - MASSONE, M.A. 1995. Nosocomial Infection caused by multi-resistant *Staphylococci* in a neonatal and pediatric intensive care unit. In *Pediatric Med. Clin.*, vol. 17, no. 2, 1995, p. 117-122.

UBA, A - UMAR, U. 2003. Incidence and antibiotic susceptibility patterns of *Staphylococcus aureus* from clinical specimens in Bauchi, Nigeria. Book of abstracts, In 26th Annual Conference of Nigeria Society of Microbiology. University of Uyo, Akwa-Ibom state, Nigeria.

UWAEZOUKE, J.C - ARIRIATU, L.E. 2004. A survey of antibiotic resistant *Staphylococcus aureus* strains from clinical sources in Owerri, Nigeria. In *Journal of Applied Science Environmental Management,* vol. 8, no. 1, 2004, p. 67-69.

VANDENESCH, F - NAIMI, T - ENRIGHT, M.C - LINA, G - NIMMO, G.R - HEFFERNAN, H - LIASSAINE, N - BES, M - GREENLAND, T - REVERDY, M.E - ETIENNE, J. 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*

carrying Panton-Valentine Leukocidin genes: Worldwide emergence. In *Emerging Infectious Disease,* vol. 9, 2003, p. 978-984.

XANDER, W.H - ANS, M.C - ERIC VAN, K - LIESBETH, V - MARGA, G - EMILE, S - WIM, J.B.W. 2006. MRSA in Dutch soccer team. In *Emerging Infectious Disease,* vol. 12, 2006, p. 1584-1586.