REVIEW ARTICLE
AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/200981/**21019** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 150-158

GLOBAL TREND OF METHICILLIN-RESISTANT STAPHLOCOCCUS AUREUS AND EMERGING CHALLENGES FOR CONTROL

O. AZEEZ-AKANDE , DEPARTMENT OF MEDICAL MICROBIOLOGY, AND PARASITOLOGY, FACULTY OF MEDICINE, BAYERO UNIVERSITY, P.M.B 3011, KANO – NIGERIA
E-MAIL: akadaze@yahoo.com

ABSTRACT
Background: Following its first recognition in early 1960s, the increasing incidence of nosocomial and community-acquired methicillin resistant *Staphylococcus aureus* (MRSA) infections has become a global problem. The emergence of multiple-drug resistant MRSA strains and dissemination of epidemic antibiotic clones including presence of wide spectrum of virulence and predisposing risk factors complicate diagnosis, chemotherapy and control causing significant morbidity and mortality. Detection of MRSA strains in domestic animals and protozoan has widened the epidemiologic characters of the organism and may influence infection control policies.

Objectives: To review the emergence and epidemiologic spread of resistant strains of MRSA, molecular/genetic basis of resistance in the organism and challenges facing control strategies worldwide. It also aims to suggest intervention strategies so as to checkmate the spread of MRSA infections.
Methods: By reviewing local and international literatures on MRSA infections coupled with practical experience in the field of this endeavour.
Result/Conclusion: MRSA has shown increasing endemic and epidemic spread in the last four decades causing serious medical and socio-economic difficulties. Routine and regular surveillance (uncommon in poor-resourced developing areas of especially sub-Saharan Africa), good hospital practices and personal hygiene, public enlightenment, development of effective therapeutic agents and rational administration of antibiotics based on reliable test results will limit the spread of MRSA infections.
Key words: MRSA, incidence, morbidity, mortality, surveillance, control.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important bacterial pathogen causing nosocomial and community-onset infections which has shown increasing endemic and epidemic spread in the last four decades (1,2) ,while its control has become a serious concern worldwide (3,4). These MRSA associated infections impose a serous burden in terms of medical and socio-economic costs and cause significant morbidity and mortality (5-7).

S. aureus (including MRSA strains) are cluster-forming, facultative aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning Mannitol Salt Agar (MSA) yellow (8). The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, often called enzymes (clumping factor) used for their identification. MRSA strains exhibit resistance to oxacillin or methicillin (1 or 5µg/disk: zone of inhibition < 14mm in diameter used as marker for all β-lactams) and other antimicrobial agents (8,9).

MRSA has been severally shown to cause variety of diseases ranging from mild, superficial dermatological diseases to severe and potentially fatal systemic debilitations (10-11). In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an important and increasing cause of post-surgical wound infections (12,13). some invasive infections such a nosocomial bacteremia and septicemia (sepsis) (14), acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections (STIs),[13,15]. The increasing prevalence of MRSA multiple-drug resistant strains which limits the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide [2].

This paper aims to review the emergence and global epidemiologic spread of MRSA including multiple-resistant strains, molecular/genetic basis of resistance in this organism and the challenges facing the control strategies worldwide. It also aims to suggest ways of overcoming these challenges in order to limit the spread of MRSA and associated health problems.

Emergence and global epidemiological trend of MRSA

Strains of MRSA were first detected in the United Kingdom (UK) in the early 1960s[16] soon after methicillin antibiotic was introduced for clinical use. During the next decade, the existence of MRSA

strains was reported in the United States (US) with prevalence rate of less than 1%.[17] Since then, the endemic and epidemic outbreaks of the organism have been reported worldwide[18-20] but overwhelmingly from developed economies of the world. Presently, hospital of all sizes, other care centers, and increasing number of different population groups at various communities globally are facing the problem of MRSA infections (1,7,21).

Reports emanating from different parts of the world revealed increasing rates in the incidence of MRSA and population at risk. The epidemiological data from north America including Center for Disease Control and Prevention (CDC) in the US showed that the prevalence of MRSA strains in both large and small hospitals located at different regions increased progressively over the years (21-25). Other studies conducted at various health care centers in the country revealed that out of all hospital bacterial isolates, the prevalence of MRSA, cumulatively increased from 6% in 1998 to 50% in 2002.[4] A similar study conducted earlier elsewhere in the country showed a 30% increase (from 20% to 50%) in MRSA prevalence within a two year period (i.e. from 1988 to 1990) (26) .

The published data from some countries of Europe and Asia presented identical scenario with significant increase in the outbreaks of MRSA infections. For instance, Mangeney and co-workers [27] documented MRSA prevalence of 33%-62% (in relation to *S. aureus* isolates) in their hospital wards in France. The report of De Sousa and colleagues [28] from Portugal though indicated downward trend in the outbreaks of MRSA infections but showed consistently high prevalence of MRSA during the last decade e.g. 65% in 1992; 49% and 47% in 1993 and 1994 respectively.

In contrast, lower prevalence rates of MRSA have been reported in certain parts of Europe. For example, Harbath and co-researchers[29] reported 3% MRSA prevalence in their hospital in Geneva, Switzerland though further evidence showed that the MRSA were not nosocomial strains and could have originated from the community. In a similar study carried out in the UK,[30] the prevalence of MRSA was found to be comparatively low (<10%) but never the less significant in the population group studied. Identical scenario has been observed in Scandinavia and the Netherlands where incidence of MRSA appears relatively low compared to other European countries (31).

In earlier studies carried out in Asia, a remarkable upsurge in MRSA prevalence has been documented in many areas, including Taiwan where an increase in MRSA prevalence from 4.3% in a period covering 1981-1986 was reported (32). In Japan (33) and Republic of Koria (34), MRSA prevalence of up to 54% and 70% have been documented respectively. Both endemic and epidemic outbreaks have continued to rise in that region (35). On the other hand , reports from Africa on MRSA are scanty. Nonetheless, in study conducted in a Khartoum hospital, Sudan, a prevalence of 11% was documented (36). More so, the results of surveillance studies carried out by Kesah and other researchers (37) in some parts of Africa (including Lagos-Nigeria; Cameroon, Kenya and Algeria) and Malta between 1996 and 1997 revealed rates of 21 – 30% MRSA prevalence among the participating countries of the sub-Saharan region (e.g. Nigeria, Cameroon and Kenya) while that of North Africa (e.g. Algeria) and Malta presented lower rates of below 10%. However, results of similar studies carried out at different locations in Nigeria viz: Ilorin, (38) Calabar, (39) Jos, (40) (41) revealed higher MRSA prevalence rates of 34.7%, 36.4%, 43% and 49.1% respectively. More studies are clearly needed particularly in all regions of Africa including Nigeria and other developing poor-resourced areas to determine the current epidemiologic outlook of this increasingly important nosocomial and community-acquired pathogen.

Emergence of MRSA multiple antibiotic clones and public health implications According to Olive and Bean(42), clonally related organisms are mostly members of the same species that share certain characteristics such as virulence factors, biochemical and genetic traits. These traits may aid the pathogenicity, resistance to drugs, or the critical mechanism for survival by the organism. They may also facilitate the general identification of such group of organisms Information from different geographical locations showed that clones, particularly the antibiotic types and those withinherent repertoires of virulence factors are spreading in various care centers, hospitals, among members of different sporting teams and in the communities (7, l1) that hitherto were not considered to be at risk of MRSA infections. Furthermore, MRSA strains have also been detected in domestic animals and birds such as horses, cattle, chickens and dogs as well as associated individuals (43,44). More so, a group of researchers in England has suggested that a protozoan, *Acantha - amoeba polyphaga* which is found virtually everywhere in the environment may aid the spread of MRSA. This is significant because other scientific evidence suggests that pathogens that emerge from amoebas may exhibit broader resistance to antibiotics and may be more virulent with serious epidemiologic and clinical implications (45). Data published by CDC (25) suggested that community - acquired MRSA (Ca - MRSA) is becoming more prevalent. Consequently, the array of virulence factors possessed by Ca-MRSA and broader resistance

to antimicrobial agents being exhibited by the organism as compared to hospital - associated MRSA (Ha -MRSA) is of concern. Subsequently, Ca-MRSA had been implicated in various clinical conditions affecting some persons without any notable risk factors and in many cases the management of such conditions has resulted in poor prognosis (7). In addition, it has been proposed[3, 42, 46] that one clone with homogenous or heterogeneous resistance profile may be present in the same location at any given time thus compounding diagnostic problems (especially in poor - resourced areas) and treatment failures arising from empirical chemotherapy. The situation is even more compounded in patients with critical underlying ailments such as cancer, HIV/AIDS and those undergoing immunosuppressive therapy. Such conditions obviously constitute serious risk factors for MRSA infection and may results in severe complications and fatal consequences, especially if multiple - antibiotic resistant strains are involved.

Molecular and genetic factors predisposing to antimicrobial resistance in MRSA

It is now recognized that coding for methicillin resistance in MRSA is facilitated by the mec A gene, which is located on the staphylococcal cassette chromosome (SCC), a large mobile genetic element which differs in size and genetic composition among different strains of MRSA (4, 47). Consequently, at least five types of this organism have been classified accordingly [48-50] (Table I shows types I - IV as thus classified).

The mechanism of resistance in this manner involves changes or defects brought about by mutation on mec A gene which result in modification to penicillin - binding protein 2a (PBP - 2a) product. The outcome of the event is that it renders the organism resistant to β-lactams and other antibiotics with the same target site. [4] In addition, other antibiotic resistance genes may be present in the cassette thus conferring on the organism, multiple - resistance to other antibiotics.[49] Apart from the inability of an antibiotic to bind to the target site due to structural defect of such site, other mechanisms that may generally play significant roles in the development of resistance in bacteria such as MRSA include efflux phenomenon (also a product of structural modification of cellular component e.g. cell wall/membrane protein) resulting in continuous pumping of antimicrobial drugs out of the bacterial cell. Others are alteration in the outer-membrane proteins which limit the access of drugs to the cell; resistance can also arise from high level production of β lactamase (51-53).

On the other hand, PBP - mediated resistance in MRSA is suggested to take various forms and may arise from (a) overproduction of a PBP, (b) acquisition of a foreign PBP with low affinity, (c) recombination of susceptible PBP with more resistant varieties or (d) may be due to specific point mutations within PBPs that consequently lower their affinity for β lactams (10,53,54). Consequently, the occurrence of any of these events may lead to changes in antibiotic phenotypic characters of affected *S. aureus* strains. The classification of MRSA strains based on SCCmec present has notably revealed certain epidemiological features of these bacterial strains including possible sources of acquisition and dissemination (nosocomial versus community), nature of drug - resistance (single versus multiple), as well as genome characteristics viz: size of genome and type of ribosome found in individual strain type (Table 1). It is note worthy that type IV MRSA strains originating from the community (Ca-MRSA) were found to possess higher prevalence of certain virulence factors as compared with non methicillin - resistant *S. aureus* (NMRSA) and health care associated MRSA (Ha - MRSA). These factors include enterotoxin, Panton - Valentine leucocidin (PVL) e.g. as observed in USA 300 and 400 strains, toxic shock syndrome toxin 1 (TSST-1), and other superantigens with serious clinical implications.[21,55,56] Consequently, the Ca- MRSA is believed to have inherent potential for greater disease than NMRSA, and broader antibiotic resistance than typical Ha – MRSA (57). The increasing prevalence of Ca-MRSA coupled with associated battery of virulence factors and wide spectrum of resistance to chemotherapeutic agents pose serious challenges for diagnosis, management and general control of MRSA infections.

TABLE 1: CLASSIFICATION OF M RSA BASED ON SCCMEC-TYPE PRESENT (50,51)

Type of SCCmec	Source	Resistance	Genome Size (kb)	Ribotype
I	Hospital	Methicillin	34.3	Conserved
I	Hospital	Multi-drug	53.0	Conserved
III	Hospital	Multi-drug	66.9	Conserved
IV	Community	Methicillin	21- 24	Variable

Risk factors for the acquisition of MRSA

Traditionally, MRSA is associated with health care institutions (11) following its first detection in the UK in the early 1960s (16). However, though Ca - MRSA became prominent during the last decade nevertheless its extent in most communities is uncertain (36,57). In addition, the frequency of Ca - MRSA infections among otherwise healthy persons without typical Ha - MRSA risk factors is increasing with concomitant health problems (58). Reports of Ca - MRSA prevalence indicated high rates among certain population groups such as those living in close contact or proximity to one another such as prisons, barracks and care centres (11,21, 24). The presence of several risk factors (Table 2) , which increasingly predispose the general populace to MRSA infection is raising concern worldwide(2,7, 55).

The above developments therefore constitute a challenge to the researchers in health sector, epidemiologists, policy makers and health care providers at all levels in the developing world to fashion out a coordinated effort at ascertaining the current epidemiologic profile in various communities and groups for necessary intervention programmes. On the other hand Ha - MRSA strains, are usually introduced into the health care institutions by either an infected or colonized health care worker (62).

Several risk factors have been suggested for the acquisition of nosocomial MRSA. They include previous or excessive antimicrobial therapy (4), previous hospitalization (1), autoimmune diseases including immunosuppressive therapy as well as surgery and prolonged hospital stay *(>8.4* days) (4). Others are enteral feeding, mechanical ventilation, implantation of prosthetic devices (63) and nasal carriage of MRSA (64).

The increased use of indwelling devices coupled with increasing number of immunocompromised patients such as HIV/AIDS and cancer patients particularly in the developing countries of sub-Saharan Africa and parts of Asia compound the problem of control of MRSA. The existing control strategies have progressively been inundated by the massive increase in antibiotic use in the hospitals worldwide and in the communities (through self medication) especially in developing countries resulting in selective pressure and emergence of highly resistant MRSA strains. In addition, the recognition of several risk factors for MRSA infection and wide spectrum of resistant pattern to antimicrobial drugs by the organism clearly make effective control highly tasking and have been considered a global crisis (5,65).

TABLE 2: RISK FACTORS FOR MRSA (57-60)

Susceptibility patterns of MRSA and antibioticreatment of associated infections.

- **Direct contact with an infected or colonized individual**
- **Crowded and unhygienic living conditions.**
- **Recent long-term antibiotic use (within six months) or history of frequent antibiotic use.**
- **Frequent antibiotic use or abuse (including suboptimal dose) for chronic problems such as otitis media, atopic dermatitis (or eczema) and pharyngitis.**
- **Recent or frequent outpatient visits (including outpatient surgical procedures).**
- **Injection or intravenous drug use and homosexuals.**
- **Shared clothing and/or equipment and other items.**
- **Underlying chronic illness (especially dermatologic diseases) and HIV/AIDS.**
- **Contact with family member or household working in health care facility e.g. Nurses, Doctors, Laboratory scientists e.t.c.**
- **Caretaker for person with unknown history of MRSA infections.**
- **Participation in close contact sports such as wrestling, football e.t.c.**
- **Contact with toiletries or bed linens of an infected individual.**
- **Individuals living in close proximity and with frequent close contact such as prisons, dormitories, army barracks and child care settings.**
- **Regular exposure to clinical specimens without adequate preventive measures (e.g. in hospital wards and laboratories).**

Various reports (4,5,16,30) have described high-level resistance of MRSA to antibiotics apart from methicillin as a common phenomenon. The reports further stated that in the developed economies of North America for instance, up to 90%, 95% and 83% of MRSA infections were resistant to fluoroquinolones, erythromycin and c1indamycin respectively. The organism has also expressed high level resistance to other antimicrobial agents like gentamycin (75-93%), ketolides (82-98%) and trimethoprim/sulfamethoxazole (16-65%) (Table 3). However, rates of resistance to some antibiotics, such as inducible c1indamycin was lower among methicillin-susceptible *S. aureus* than MRSA in some locations (2,6). Certain drugs also were less effective against MRSA infections due to some factors and characteristic nature of such drugs. For example, vancomycin used to treat MRSA systemic infections was found to be less effective in the treatment of ventilator-associated pneumonia (VAP) due to its poor lung penetration unlike such

drugs as linezolid with effective penetration of the body including tissue, muscle, fat and bone (57). On the other hand, antibiotic susceptibility pattern of MRSA is not uniform or clear cut in the developing countries and thus varies from one geographical location to another depending on various factors (36,37-42).

In view of this scenario the following factors are imperative for consideration in the selection of antibiotics for the treatment of MRSA infection. They include: (a) susceptibility of the organism to antibiotics (b) the type of infection (c) drug characteristics such as activity (i.e. bactericidal versus bacteriostatic) (d) mechanism of action (e) blood level/tissue penetration (f) toxicity (g) cost and availability (h) early initiation of appropriate antimicrobial chemotherapy is also essential for favourable prognosis and outcome (4,11).

Antidote for increasing MRSA acquisition dissemination and spread

In the developed countries of North America e.g. US and Canada, and parts of Europe, considerable attention has been given to infection control programme which has led to significant reduction in the incidence of many infectious diseases.(1,2) However, the situation in many developing regions of the world appears gloomy due to inadequate or poor implementation of policy on infection control due to lack of political will, inadequate resources including shortage or ill-equipped manpower, poor motivation of health care workers and researchers. Other militating factors include extreme poverty and ignorance on the part of the general populace. However, in order to bring the present situation under control, some holistic measures are imperative for strict implementation at local and national hospital levels, communities and corporate establishments involving health care workers, researchers, epidemiologists and drug anufacturers. These include:- (a) regular surveillance of endemic and epidemic outbreaks of MRSA especially in high risk centres, (b) screening before employment and periodic test of health care workers and identification of carrier status plus adequate treatment to eliminate potential source of infection,

TABLE 3: RESISTANCE RATES OF MRSA STRAINS TO ANTIBIOTICS (4,15,32)

Antimicrobial agent	Rate of resistance (%)
* Erythromycin	90 – 95
* Gentamycin	75 – 93
* Fluoroquinolones	30 – 90*
* Clindamycin	75 – 83
* Ketolides	82 – 98
* Tetracycline	18 – 82
* Trimethoprim/sulfamethoxazole	16 – 65
* Quinupristin/dalfopristin	4 – 31
* Fusidic acid	5 – 10
* Vancomycin	0 – 5
* Oxazolidinones (e.g. Linezolid)	0 – 1
* Tigecycline	0
* Daptomycin	0

*-variable in developing countries and may be lower in some cases

(c) screening of high risk patients and other individuals, (d) routine laboratory diagnosis (which is lacking in most developing areas) and prompt identification of MRSA and determination of antibiotic susceptibility profile while (e) empirical treatment of MRSA infection should be based on prior determination of local resistance patterns. Other measures involve (f) the enforcement of good hospital practices, (g) provision and adequate implementation of educational programs on hospital, community and personal hygiene, and (h) development of effective chemotherapy to replace those drugs to which the organism has developed resistance. More so, rational administration of antibiotics based on rapid and reliable laboratory test results will go along way in reducing the cases of treatment failures, and selective pressure leading to the proliferation of MRSA resistant strains.

CONCLUSION

The increasing prevalence of MRSA infections in the hospitals, other care centres and lately in the community has become a worldwide phenomenon. The wide spread dissemination of multiple - drug resistant strains and antibiotic clones of the bacterium facilitated by inherent or acquired molecular/genetic element is worrying as it complicates diagnosis and chemotherapy. More so, the presence of wide array of virulence and potential risk and spreading factors compounds morbidity and control measures. There is need for adequate policy framework on infection control that will reflect the current realities on the epidemiologic characters of MRSA as well as strict implementation of such control program to checkmate the spread of MRSA infections.

REFERENCES

1 Kuehnert, M.J., Hill, H.A., Kupronis, B.A Methicillin-resistant *Staphylococcus aureus* hospitalization, United States, *Emerg. Infect. Dis.* 2005; 11 (6): 868 - 872.

2 Frazee, B.W., Lynn, J., Charlebois, E.D., Lambert, L, Lowery, D., Perdreau-Remington, F. High prevalence of methicillin - resistant *Staphyloccus aureus* in emergency department skin and soft tissue infection. *Ann. Emerg. Med.* 2005; 45(3): 311 - 320.

3 Pere - Roth, E., Claverie - Martin, F., Villar, J., Mendez - Alvarez, S. Multiplex PCR for simultaneous identification *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J. *Clin. Microbiol.* 2001; 39(11): 4037 - 4041.

4 Stevens, D.L Optimizing outcomes in methicillin_resistant *Staphylococcus aureus* infections: focus on nosocomial pneumonia and SSTI, highlights from a satellite symposium at the 11th annual international congress on infectious disease (ICID), Cancun, Mexico, 2004; PP 1 - 8. ·

5 Maranan, M.C., Moreira, B., Boyle - Vavra, S., Daum, R.S Antimicrobial resistance in Staphylococci: epidemiology, molecular mechanisms and clinical relevance *Inf. Dis. Clin. N. Am.* 1997; 11:813 - 849.

6. Carbon, C. Costs of treating infections caused by methicillin resistant staphylococci and vancomycin-resistant enterococci. J. *Antimicrob. Chemother.* 1999; 44:31 - 36.

7. Bratu, S., Eramo, A., Kopec, R., Coughlin, E., Ghitan, M., Yost R., Chanpnick, E.K., Landman, D; Wuale, J. Community - associated methicillin - resistant *Staphylococcus aureus* in hospital nursery and maternity units. *Emerg. Infect. Dis* 2005; 11(6): 808 - 813.

8. Bannerman, T. L. Staphylococcus, Micrococcus and other catalase-positive cocci that grow aerobically In: *Manual of Clinical Microbiology* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. pfaller and R. H. Yolken eds. 8th edition, 2003 ASM Press Washington DC Pp 384 - 404.

9. Duguid, J. P. Staphylococcus: Cluster-forming Gram-positive Cocci In: *Mackie and McCartneyPractical Medical Microbiology* J. G. Collee, J. P. Duguid, A. G. Fraser, B. P. Marmion eds. 13 edition, 1989 Churchill Livingstone London, Pp 303 - 316.

10. Chambers, H.F. Methicillin - resistant in staphylococci: Molecular and biochemical basis and clinical implications. *Clin. Microbiol. Rev.* 1997; 10: 781 - 791.

11. Moran, GJ., Amii, R.N., Abrahamian, F.M., Talan, D.A Methicillin - resistant *Staphylococcus aureus* in community - acquired skin infections. *Emerg. Infect. Dis* 2005; 11 (11): 928 - 930.

12. Gottileb, G.S., Fowler, V.G. Jr., Kong, L.K. *et al. Staphylococcus aureus* bacteremia in the surgical patients: a prospective analysis of 73 postoperrative patients who developed *Staphylococcus aureus* bacteremia at a tertiary care facility. *J. Am. Coll. Surg.* 2000; 190:50 - 57.

13. Graffunder. E.M., Venezia, R.A Risk factors associated with nosocomial methicillin - resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J. Antimicrob. Chemother.* 2002; 49:999 - 1005.

14. Mylotte, J.M., Tayara, A. *Staphylococcus aureus* bacteremia: predictors of 30 - day mortality in a large cohort. *Clin. Infect. Dis.* 2000; 31: 1170 - 1174.

15. Rello, J., Diaz, E. Pneumonia in the intensive care unit. *Crt. Care Med.* 2003; 31: 2544 - 2551.

16. Jevons, M.P. "Celbanin" - resistant staphylococci. *Sr. Med J.* 1961; 1: 124 - 125.

17. Peacock, J.E. Jr., Marsik, FJ., Wenzel, R.P Methicillin - resistant *Staphylococcus aureus:* introduction and spread within a hospital. *Ann. Intern. Med* 1980; 93: 526 - 532.

18. Chambers, H.F. The changing epidemiology of *Staphylococcus aureus? Emerg. Infect. Dis.* 2001; 7: 178 - 182.

19. Ayliffe, A.G. The progressive intercontinental spread of methicillin - resistant *Staphylococcus aureus. Clin. Infect. Dis.* 1997; 24:574 - 579.

20. McDougal, L.K., Steward, C.D., Killgore, G.E. *et al.* Pulse field gel electrophoresis typing of oxacillin - resistant *Staphylococcus aureus* isolates from the United States establishing a national database. J. *Clin. Microbiol.* 2003; 41:5113 - 5120.

21. Mulvey, M.R., MacDouglall, L., Cholin, B. *et al.* Community associated methicillin - resistant *Staphylococcus aureus,* Canada. *Emerg. Infect. Dis.* 2005; 11(6):844 - 850.

22. Horan, T., Culver, D., Jarvis, W. Pathogens causing nosocomial infections: preliminary data from the national nosocomial infections surveillance system. *Antimicrob. Newsl.* 1988; 5: 56 67.

23. Hughes, J.M. Setting priorities: nationwide nosocomial infection, prevention and control programs in the U.S.A *Eur.* J. *Clin. Microbiol.* 1987;6:348 - n351.

24. Centers for Disease Control. Public health dispatch outbreaks of community - associated methicillin - resistant *Staphylococcus aureus* skin

infections - Los Angels County California. Morbid. *Mortal. Weekly Rep.* 2003; 1: 52 - 88.

25. Centers for Disease Control. M RSA August 2008. http:jwww.cdcjgov.ncidodjhipjARESISTjmrsajfag.htm. retrieved September 22, 2008.

26. Mangeney, N., Bakkaouch, A, Pons, J.L Methicillin - resistant *Staphylococcus aureus* isolates from trauma patients. J. *Clin. Microbiol.* 1998; 36 (2): 414 - 420.

27. Mangeney, N., Bakkauch, A, Pons, J.L., Dupeyon, C., Niel, P., Leluan, G. methicillin - resistant *Staphylococcus aureus* subtyping: interest of combined antibiotyping and esterase electrophoretic typing. J. *Appl. Bacteriol.* 1995; 79: 347 - 351.

28. De sousa, M.A Sanches, 1.S., Ferro, M.L. *et al.* Intercontinental spread of a multidrug - resistant methicillin - resistant *Staphylococcus aureus* clones. J. *Clin. Microbiol.* 1998; 36: 2590 - 2596.

29. Harbath, S., Francois, P., Schrenzel, J. *et aI.,* Community - associated methicillin -resistant *Staphylococcus aureus* Switzerland. *Emerg. Infect. Dis.* 2005 2005; 11(6).

30. Grundmann, H., Tami, A, Hori, S., Halwani, M., Slack, R. Nottingham *Staphylococcus aureus* population study: prevalence of MRSA among elderly people in the community. *BMJ. 2002;* 324: 1365 - 1366.

31. Murchnas, S., Kaufmann, 1.E., Deplano, A., Ryck, R., Sruelens, M., Zinn, C.E. Harmonization of pulsed - field gel electrophoresis protocols for epidemiological typing of strains of methicillin - resistant *Staphylococcus aureus.* a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J. Clin. Microbiol. 2003:* 41(4): 1574 - 1585.

32. Hsueh, P., Chen, M.L, Sun, C.C., W.H., Pan, HJ., Yang, L.S., Chang, S.C. Antimicrobial drug resistance in pathogens causing nosocomial infections at a university hospital in Taiwan 1981 - *1999. Emerg. Infect. Dis,* 2002; 8(1): 63 - 68.

33. Lotus, D.K., Imamura, T., Tukamine, F. Current status of antimicrobial susceptibility in MRSA isolates typed by coagulase and phage typing in Okinawa, *Acta. Med. Okayama.* 1995; 49: 81- 89.

34. Woojoo, K. Seunchill, P. Bacterial resistance to antimicrobial agents: an overview from Korea. *Yon Med. J.* 1999; 39(6): 488 494.

35. Euichong, K., Hyunjin, J., Myoung Don, O. *et al.* Epidemiological typing of methicillin-resistant *Staphylococcus aureus* out break isolates by pulsed-field gel electrophoresis and antibiogram *Trop. Dis. Bull.* 2008 (10): 1023-1028

36. Musa, H.A., Shears, P., Khagali, A. First report of methicillin resistant *Staphylococcus aureus* from hospitalized patients in Sudan. *J. Hosp. Infect.* 1999; 42(1): 74 -'- 75.

37. Kesah, c., Rdjeb, B., Odugbemi, T. *et al.* Prevalance of methicillin - resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clin Microbiol. Infect.* 2003; 9(2) 153 - 156.

38. Taiwo, S.S., Onile B.A., Akanbi, A.A. Methicillin - resistant *Staphylococcus aureus* (MRSA) isolates in Nigeria. *Afr. J. Clin. Exptl. Microbiol.* 2004; 5(2): 189 - 197.

39. Azeez, O.A., Utsalo, SJ., Epoke, J. Distribution and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates in a University Teaching Hospital in Nigeria. *Sahel Med.* J. 2008, 11(4): 142-147.

40. Ikeh, E.I. Methicillin - Resistant *Staphylococcus aureus* (MRSA) at Jos teaching hospital. *Afr,* J. *C/in. Expl. Microbiol.* 2003; 4(1): 52 - 55.

41. Olayinka, B.O., Olayinka, A.T., Onaolapo, J.A., Olurinola, P.F.
Pattern of resistance to vancomycin and other antimicrobial agents in staphylococcal isolates in a university teaching hospital *Afr,* J. *C/in Exptl. Microbiol.* 2005; 6(1): 21 - 27.

42. Olive, D.M Bean, P. Principles and applications of methods for DNA - based typing of microbial organisms: Minireview. 1. *Clin. Microbiol.* 1999; 37(6): 1661 - 1669.

43. Weese J.S., Archambault, M., Willey, B.M *et al.* Methicillin resistant *Staphylococcus aureus* in horses and horse personnel, 2000 - 2002. *Emerg. Infect. Dis,* 2005; 11(3): 430 - 435.

44. Cuny, C, Kuemmerie, J., Stannek, C, Willey, B., Strommenger, B., Witte, W. Emmergence of MRSA infections in horses in a veterinary hospital: strain characterization and comparison with MRSA from humans *Euro Surveil* 2006: 11(1): 44 - 47

45. Birchard, K. Amoeba aids spread of MRSA bacterium.
http://www.medicalpost.comjmpcontentjarticle.jsp. Retrieved August 16, 2008.

46. Oren, H., Leejene, T., Pnachyr, Y., *et al,* Dissemination of two methiocillin - resistant *Staphylococcus aureus* clones exhibiting negative staphylase reactions in intensive care units. J. *Clin. Microbiol.* 2007; 37(3): 504 - 509.

47. Euichong, K. Hyunjin, J., MyongDon, 0., Hoanjong, 1., Hyamgsoon, 0., Kangwon, CO. Epidemiological typing of methicillin - resistant

Staphylococcus aureus outbreak isolates by pulsed - field gel electrophoresis and antibiogram. *Yon. Med.* J. 1998; 39(6): 587 - 594.

48. Daum, R.S., Ito, T., Hiramatsu, K. *et al.* A novel methicillin resistance cassette in community acquired methicillin - resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds J. *Infect Dis* 2002; 186: 1344 - 1347.

49. Ito, T., Katayama, Y.I., Asada, K. *et al.* Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin - resistant *Staphylococcus aureus Antimicrob. Agents Chemother.* 2001: 45: 1323 - 1336.

50. Ito, T., X.S., Takeuchi, F., *et al.* Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase cere. *Antimicrob. Agents Chemother.* 2004; 48: 2637 - 2651.

51. Rasheed, J.K., Tenover, F.e. Detection and characterization of antimicrobial resistance genes in bacteria in: *Manual of Clinical Microbiology.* Eds. P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken. 8th edtn. 2003 ASM press, Washington DC PP. 1197 - 1212.

52. Rice, L.B., Sahm, D., Bonomo, R.A. Mechanisms of resistance to antimicrobial agents in: *Manual of Clinical Microbiology eds.* P.R. Murray, EJ. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken 8th Edtn. 2003, ASM press, Washington De. PP 1674 - 1101.

53. Hackbarth, CJ. Kacagoz, T. Kacagoz, S., Chambers, H.F. Point mutations in *Staphylococcus aureus* PBP 2 gene affect penicillin binding kinetics and are associated with resistance. *Antimicrob. Agents. Chemother.* 1995; 30: 103 - 106.

54. Vandenesch, F. Naimi, T. Enright, M.C *et al.* Community acquired methicillin - resistant *Staphylococcus aureus* carrying panton - valentine leucocidin genes: worldwide emergence. *Emerg. Infect. Dis.* 2008; 9: 978 - 984.

55. Fey, P.O., Said - Salim, B., Rupp. M.E. *et al.* Comparative molecular analysis of community - or hospital acquired methicillin resistant *Staphylococcus aureus. Antimicrob. Agents Chemother.* 2003; 47: 196 - 203.

56. Ochoa, TJ., Mohr, J., Wanger, A., Murphy, J.R., Haresi, G.P. Community - associated methicillin - resistant *Staphylococcus aureus* in pediatric patients. *Emerg. Infect. Dis* 2005; 11(6): 966 – 968.

57. Said - Salim, B., Mathema; B., Kreiswirth, B.M. Community acquired methicillin - resistant *Staphylococcus aureus.* an emerging pathogen. *Infect Contr. Hosp. Epidemiol.* 2003; 24(6): 451 - 455.

58. Centers for Disease Control. MRSA. *http.!/www.cdc/gov.ncidod/hip/ARESIST/mrsa/fag.htm* retrieved August 10, 2008.

59. Salgado, C.D., Farr. B.M., Calfee, D.P. Community - associated methicillin - resistant *Staphylococcus aureus.* a meta - analysis of prevalence and risk factors. *Clin. Infect. Dis.* 2003; 36: 131 139.

60. Basco, W.T. Jr. Community acquired MRSA: evolving pathogens. *http./ /www.edscape.com/viewarticlej507869*retrieved February 28, 2008.

61. Fang, H., Hedin, G. Rapid screening and identification of methicillin - resistant *Staphylococcus aureus* from clinical samples by selective - broth and real - time PCR assay. J. *Clin. Microbiol.* 2003; 41(7): 2894 - 2899.

62. Haddad, S.H., Arabi, Y.M. Memish, Z.A, AI-Shimemeri, AA. Nosocomial

infective endocarditis in critically ill patients: a report of three cases and review of the literature. *Intern.* 1. *Infect. Dis.* 2004; 8: 210 - 216.

63. Becker, K., Friedrich, AW., Lubritz, G., Weilert, M., Peters, G., Von Eiff, C. Pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. J. *C/in. Microbiol.* 2003; 41(4): 1434-1439.

64. Matinez, J.L., F. Mutation Frequencies in antibiotic resistance.
Antimicrob. Agents Chemother. 2000; 44: 1771 - 1777.

65. Lovering, A. M., Zhang, J., Bannister, G. C. *et al.,* Penetration of Iinezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement J. *Antimicrob. Chemother.* 2002; 50: 73 - 77.