Molecular analysis of methicillin resistance and beta-lactamase production by clinical isolates of *Staphylococcus aureus* in Ekpoma, Edo State Nigeria

[1]Ikheloa J [2]Inyang NJ, [2]Adelekun A. [3]Agwu E,

[1] Department of Obstetrics and Gynecology, Irrua Specialist Teaching Hospital, Irrua, Edo State Nigeria.[2]Microbiology Department, Irrua Specialist Teaching Hospital Irrua Edo State, Nigeria. [3]Department of Microbiology, Kampala International University W/C Ishaka Bushenyi, Uganda.

Correspondence: nyohoinyang@yahoo.com**. TeL: +2348033724973**

Citation:

Abstract
Background: *Staphylococcus aureus*-associated infections continues to be a public health challenge in resource-limited developing countries of sub-Saharan Africa. Understanding the genetic basis of resistance of Staphylococcus aureus remains a paramount panacea to associated illnesses.
Objective: To isolate and identify methicillin-resistant and beta-lactamase-producing *Staphylococcus aureus* from a different clinical specimen collected from hospital attendees in Ekpoma, Edo State Nigeria.
Materials and methods: A total of 200 *Staphylococcal* isolates were recovered from different clinical specimens analyzed. Standard Microbiological methods used in isolation and identification of *Staphylococcus aureus* included the use of Prolex™ Staph Latex Kit, cultivation in MRSA Chromagar, and confirmation using species-specific oligonucleotide primers to amplify DNA regions of interest and result read by electrophoresis.
Results. Out of 200 isolates of *Staphylococcus aureus* isolates, 80(40%) strains were positive for the mecA gene, while 120(60%) were beta-lactamase positive. Age groups $26-30$ had 23.0% of *S. aureus* with 25.0% positive for MecA gene and 30.0% positive for β-lactamase production. *S. aureus* from wound swabs had 52% high potential for 52% for β-lactamase production and 42.5% for mecA gene. Female/male urine had 40.9%/20.0% prevalence of *S. aureus*. Methicillin resistance and beta-lactamase production were high (25% and 30% respectively) among isolates from urine samples.

Conclusion. Methicillin-resistant (mecA) genes and beta-lactamase enzymes remain important determinants of resistance of *Staphylococcus aureus* to conventional antibiotics in resources limited settings. Strict use of prescription drugs to avoid abuse, improved personal hygiene, good laboratory practice, screening of all in-patients upon admission into the hospitals, screening of visitors during visits to in-patients, hand washing, and the use of disposable aprons and gloves by staff to reduces skin-to-skin contact and therefore further reduces the risk of transmission are strongly recommended prevention protocols.

Introduction

Staphylococcus aureus is the most common cause of *Staphylococcal* infections and is frequently found in the nose, skin, upper respiratory tract, and intestinal tract of healthy individuals (1). They are halophilic and thrive well at a pH of 7.4 to 7.6 (2). *S. aureus* is primarily a catalase and coagulase enzyme producing organisms which add to their pathogenicity (3). *S. aureus* may occur as a commensal on human skin, nose, and throat (4). The occurrence of *S. aureus* under these different ecological niches may not always indicate infection and therefore may not always require treatment.

 S. aureus can cause a wide range of illnesses from minor skin infections, such as pimples, impetigo, (may also be caused by *Streptococcus pyogenes*), boils, cellulitis, folliculitis, furuncles, carbuncles, scalded skin syndrome, and abscesses; to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, conjunctivitis, especially in neonates and septicemia (5). It is also involved in skin, soft tissue, respiratory, bone, joint, endovascular to wound infections (6).

In the United Kingdom, the most common strains of MRSA are EMRSA15 and EMRSA16. EMRSA16 is identical to MRSA252 which is common in the United States of America. The resistance of both EMRSA15 and EMRSA16 strains to erythromycin and ciprofloxacin in addition to methicillin may explain why they have become so successful in colonizing different organs and systems and why they are present in a different clinical specimen. The use of an intracellular niche to escape antibiotic activity may explain the commonly seen predominance of *S. aureus* in soft tissue infections (7).

Staphylococcal infection that is not antibiotic-resistant can easily be treated. Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943, but by 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant; and by 1960, it had risen to 80%. (8). Onanuga *et al,* (9) reported a 69% MRSA in healthy women in Zaria, while, Wilcox et al, (10) reported the high prevalence of MRSA in wound infections. Prevention and control capabilities focus on recent advances and the use of cutting-edge DNA technologies to answer more difficult questions not possible by the use of phenotypic methods (11).

False identification of an isolate can impact the implementation of effective treatment and/or control measures (12). Effective surveillance, prevention, and control of any agents of the disease depend on early diagnosis.

Objective

This study was designed to isolate and identify methicillin-resistant and beta-lactamase-producing *Staphylococcus aureus* from a different clinical specimen collected from hospital attendees in Ekpoma, Edo State Nigeria

Materials and Methods

The number of isolates sampled was guided by the upper limit required to give a 95% level of confidence at an expected prevalence of about 10% (pilot retrospective study), using the precise prevalence formula: Sample size (N) = Z^2P (100-P)/D^2 where Z is a constant given as (1.96), P is expected prevalence (10%), and D is an acceptable error (5%). When the above formula was used a sample size of one hundred and eighty-nine (189.2) was obtained. To make up for sampling error and dropouts, a

minimum of two hundred (200) patients were sampled (Dean et al. 1995, epidemiological database).

Study Population

A total of 200 *Staphylococcal* isolates were recovered from various clinical specimens. Patients included were those who were attending clinics in Ekpoma for various illnesses including urinary tract infection, chronic soft tissue ulcers, eye, ear, and nasal infections, urethritis, pelvic inflammatory diseases epidemics, and prostatitis. Therefore the following specimens were aseptically collected for the study: urine, wound swabs, eye discharge, ear swabs, sputum, aspirate, semen, endocervical swabs, urethral swab, and high vaginal swab. Informed consent was sought and obtained from each patient. Patients were required to consent by signing or thumb-printing where patients could not read or write. Patients were assured of confidentiality, free of charge, liberty to opt-out should they wish to do so at any point in time.

These specimens were cultured within thirty minutes of collection and incubated aerobically at 37° C for twenty-four hours. (24hrs). Standard aseptic microbiological techniques were employed in the isolation and identification of bacterial isolates from these specimen. Briefly, samples were inoculated on chocolate and MacConkey agar for a routine investigation. Further investigations were done to increase the phenotypic discriminatory power of cholate and MacConkey by the use of Chromagar for methicillin-resistant *Staphylococcus aureus* (MRSA) which is a selective and differential medium for the quantitative direct detection of *Staphylococcus aureus* from a clinical specimen. In addition to the Chromagar MRSA, we also used the Prolex™ Staph Latex Kit to identify *S. aureus* following the manufacturer's instructions.

The Prolex™ Staph Latex Kit utilizes polystyrene latex particles that have been sensitized with fibrinogen and IgG. When Staphylococcal colonies that possess clumping factor and/or protein-A are mixed with the latex reagent, the latex particles agglutinate strongly within 20 seconds. Briefly, the latex reagent is brought to room temperature (22-28°C) for about 10 minutes before use. The reagent is re-suspended by shaking before use. One drop of Staph Test latex reagent is dispensed into separate circles on the test card. With the aid of a mixing stick, two suspect colonies are transferred into a circle from the culture plate. These two liquids (latex reagent and two suspect colonies) are mixed and spread to cover the complete area of the circle and also gently rock the card allowing the mixture to flow slowly over the entire test ring area. After 20 seconds, under normal lighting conditions, the slide was observed for agglutination. If the result is positive, steps two to six are repeated in the same way, using the negative control latex reagent. Visible agglutination indicated positive results and no agglutination means a negative result.

Test for the Production of Beta-Lactamase

Hardy diagnostic Nitrocef disks were employed for the rapid testing of the production of beta-lactamase in *Staphylococcus aureus*. This mechanism yields clinically relevant information earlier than the minimum inhibition concentration or disk diffusion test. Nitrogen disks are impregnated with nitrocefin, which is a chromogenic cephalosporin. As the amide bond in a beta-lactam ring is hydrolyzed by a beta-lactamase, Nitrocefin changes color from yellow to red. Bacteria that produce beta-lactamase in significant amounts produce this yellow to red color change on the Nitrocefin disks™. These beta-lactamases are capable of inactivating "penicillinase-labile-penicillins", such as amoxicillin, ampicillin, penicillin, carbenicillin, mezlocillin, and piperacillin. Using sterile forceps, the Nitrocef disc is removed from the vial and placed on an empty Petri dish or microscopic slide. Before inoculation, the Nitrocef disks™ is allowed to equilibrate to room temperature. With a sterilized loop or application stick a well-isolated colony was removed and spread on the disk surface.

A positive beta-lactamase result is recorded when the Nitrocef Disk™ changes in color from its original yellow to orange or red. Most positive bacterial strains will produce a color change within 5 minutes. Some staphylococci, however, may take up to 60 minutes for a positive result. A positive beta-lactamase result predicts the following: Resistance to penicillin, ampicillin, and amoxicillin among *Haemophilus* spp., *N. gonorrhea,* and *M. catarrhalis.* Resistance to penicillin, as well as acylamino-, carboxy-, and uriedo-penicillins among staphylococci and enterococci. A negative beta-lactamase result is recorded when the Nitrocef Disk™ remains yellow. A negative result does not rule out resistance due to other mechanisms.

Molecular assay

DNA materials were extracted from overnight pure cultures of *Staphylococcus aureus* using the Qiagen Extraction kit containing the following reagents: tissue lysis buffer, AL: Lysis buffer contains guanidine hydrochloride (AL), Elution buffer (AL), AW1: Wash buffer 1. AE: Elution buffer and proteinase K solution.

To extract DNA from Gram-positive bacteria, bacterial pellets were harvested into1.5ml Eppendorf tubes and suspend in 180ul of buffer ATL, mixed by vortexing, and incubated for at least 30 minutes at 37°C. Twenty microliters of proteinase k and 200ul of buffer AL were also added and mixed by vortexing and incubated at 56°C for 30 minutes and then for a further 15minutes at 95°C. The setup was centrifuge briefly for few seconds and 200ul of ethanol (90-100%) added and mixed by pause-vortexing for 15 seconds. The setup (1.5ml tubes) was centrifuged to remove drops from the lids. The mixture was carefully applied on a spin column, without wetting the rim and the spin column was placed in a clean 2ml collection tube and centrifuge at 8000rpm for 1minute, and the tube containing the filtrate was discarded. The spin-column was carefully opened and 500ul of buffer AW1 was added without wetting the rim. The cap was centrifuged at 8000rpm for one minute.

The spin column was transferred in a clean 2ml collection tube and the previous filtrate was completely discarded. The spin-column was carefully opened and 500ul of the buffer AW2 was added without wetting the rim. The cap was closed and centrifuged at 14000rpm for 3minutes. The spine column was transferred into a new 2ml collection tube and discard the filtrate was discarded. Centrifuge at full speed for one 1minute.

The spin column was placed in a clean 1.5ml tube, opened carefully and 100µl of buffer AE added to the spin column. The setup was incubated at room temperature for 1minute and centrifuged at 8000rpm for 5minutes. The 5 minutes incubation of the spin column with buffer AE at room temperature before centrifugation increases DNA yield. The filtrate was kept on ice for the Polymerase Chain reaction. Twenty microliters of proteinase k and add 200ul of buffer A were mixed by vortexing and incubated at 56°C for 30 minutes and then for a further 15minutes at 95°C and centrifuged briefly for few seconds. Two hundred milliliters of ethanol was then added to two hundred 200ul of ethanol (90-100%), mixed by pause-vortexing for 15 seconds. The 1.5ml tubes were briefly centrifuged to remove drops from the lids.

The following primers were used for characterization of a methicillin-resistant gene (mecA) from clinical isolates of Staphylococcus aureus: Primers for mecA gene mecA gene forward primer 5[1] CAA GAT ATG AAG TGG TAA ATG GT 3[1] mechA gene reverse reaction 5[1] TTT ACG ACT TGT TGC ATA CCA TA 3[1]. A master mix containing a final volume of 20µl of the following reagents were prepared to contain the following specific volumes per reagent: Taq Polymerase - 1u (one international unit), 250µm of dNTPs. 10mM of Tris HCL, 1.5mM of MgCl, 4.0µl of template DNA, 8.0µl, 1.5 µl of forwarding primers, and reverse primers. The thermal cycler used for this experiment was the Gradient 96 well Peltier Thermal Cycler (Biodirect MJ PTC-200 RESEARCH). The cycling condition for this experiment was 94 °C for 5minutes to denature DNA and render the DNA single-

stranded. This is followed by another 94°C for 1minute to completely render DNA strands single-stranded. For the annealing to take place, cycling conditions must be changed to 50 °C for 1minute and 72°C for 2 minutes for change elongation to take place for 29cyles and then it is allowed for extra 10 minutes for complete chain elongation. The amplicons were then visualized on a 2% ethidium bromide-stained agarose gel electrophoresis using an ultra illuminator and the result recorded

Result

Out of 200 isolates of *Staphylococcus aureus* recovered from clinical specimens collected from various clinical sites, 80(40%) strains were positive for the mecA gene, which codes for methicillin resistance; while 120(60%) were beta-lactamase positive. Age groups 26 – 30 had 23.0% of *S. aureus* with 25.0% positive for MecA gene and 30.0% positive for β-

lactamase production. *S. aureus* from wound swabs had a very high percentage of 52.0 for β-lactamase production and 42.5% for the MecA gene. The urine specimen from females had 40.9% and 20.0% for males of *S. aureus*. Urine had the highest percentage prevalence in ages 26-30 for methicillin resistance and beta-lactamase production with 25% and 30% respectively. Table 1 shows the frequency of *S. aureus* in relationship to the presence of MecA gene and β-lactamase production. Table 2 shows the number of *S. aureus* isolated between males and females of different age groups. Table 3 shows the number of *S. aureus* from the various clinical sites about the presence of MecA gene and their β-lactamase production. The number of *S. aureus* from the various clinical sites about gender is shown in Table 4.

Table 1&2: The frequency of *S. aureus* showing its β-lactamase activity in relation to the presence of MecA gene among 200 participants

Ages (yrs)	No of *S. au* (%)	MecA +ve (%)	β-lact +ve	Males	Females
1 – 5	4 (2.0)	1 (1.0)	2 (2.0)	2 (3.0)	2 (2.0)
6-10	8 (4.0)	2 (3.0)	3 (3.0)	3 (4.0)	5 (4.0)
11-15	12 (6.0)	5 (6.0)	5 (4.0)	6 (9.0)	6 (5.0)
16-20	18 (9.0)	6 (8.0)	9 (10.0)	8 (12.0)	10 (8.0)
21- 25	36 (18.0)	17(23.0)	20 (17.0)	12 (18.0)	24 (18.0)
26-30	46 (23.0)	20(25.0)	30 (30.0)	6 (9.0)	40 (30.0)
30-35	24 (12.0)	10(13.0)	14 (12.0)	11 (16.0)	13 (10.0)
36-40	20 (10.0)	4 (5.0)	10 (8.0)	8 (12.0)	12 (9.0)
41-45	20 (10.0)	8 (10.0)	12 (10.0)	10 (15.0)	10 (8.0)
≥46	12 (6.0)	6 (8.0)	6 (5.0)	2 (3.0)	10 (8.0)
Total	200 (100)	80 (100)	120 (100)	68 (100)	132 (100)

β-lact =β-lactamase

Table 3&4: Showing the number of *S. aureus* from the various 200 clinical sites in relation to gender and the presence of Mec A gene, and their β-lactamase activity.

Clinical sites	No of S. aureus	Mec A +ve n (%)	β-lact +ve n (%)	Males n (%)	Females n (%)
Wound swabs	72(36.0)	34 (42.5)	62 (52.0)	25 (36.8)	47 (35.6)
Urine	68(34.0)	24 (30.0)	40 (33.0)	14 (20.6)	54 (40.9)
HVS	10 (5.0)	2 (2.5)	5 (4.0)	0 (0.0)	10 (7.6)
Sputum	5 (2.5)	2 (2.5)	2 (2.0)	2 (2.9)	3 (2.3)
Semen	3 (1.5)	2 (2.5)	3 (3.0)	3 (4.04)	0 (0.0)

Aspirate	10 (5.0)	4 (5.0)	2 (2.0)	6 (8.8)	4 (4.5)
Eye swabs	8 (4.0)	3 (3.75)	1 (1.0)	2 (2.9)	6 (3.0)
Ear swabs	6 (3.0)	3 (3.75)	1 (1.0)	2 (2.9)	4 (4.5)
Urethral swabs	12 (7.0)	6 (7.5)	4 (3.0)	14(20.6)	0 (0.0)
ECS	4 (2.0)	0 (0.0)	0 (0.0)	0(0.0)	4 (4.5)
Total	200(100)	80 (100)	120(100)	68(100)	132 (100)

β-lact =β-lactamase

Discussion

Although most *Staphylococcus* species are common inhabitants of the skin and mucous membranes, certain species have been found frequently as etiological agents of a variety of human infections. Superficial supportive infections caused by *S. aureus* are the most common human staphylococcal infections. Food poisoning and toxic shock syndrome also have been attributed to infection with *S. aureus*. Preventive medical practice is fast becoming the norm all over the world, hence, this study highlighted the possible risk factors that could result in MRSA infections and therefore, suggested ways of reducing and eliminating the disease. *Staphylococcus aureus*, though a normal flora of the skin can result in life-threatening conditions such as osteomyelitis and endocarditis. However, the increased incidence/prevalence of Staphylococcal infection together with its high ability to resist methicillin may predispose sufferers/carriers to life-threatening disease conditions (13-17).

Out of 200 isolates of *Staphylococcus aureus* recovered from a clinical specimen collected from various clinical sites, 80(40%) strains were positive for mecA gene, which codes for methicillin resistance; while 120(60%) were beta-lactamase positive Table 1.
The pathogenicity of *S. aureus* has never been in doubt especially when they are involved in deep-sited infections. However, a quick review of the pathogenicity of this bacteria will explain the diversity of their isolation from different clinical specimens. The abundance of pathogenic principles and the ability for *Staphylococcus aureus* to express them under different conditions indicates that they will either be directly involved in different illnesses

or are a potential reservoir for life-threatening infections (18). It has been well documented that *S. aureus* expresses many potential virulence factors such as Surface proteins that promote colonization of host tissues; invasions such as leukocidin, kinases, hyaluronidase that promote bacterial spread in tissues; surface factors that inhibit phagocytic engulfment (capsule, Protein A);
biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); immunological disguises (Protein A, coagulase, clotting factor); membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin; exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET); inherent and acquired resistance to antimicrobial agents. These can highlight why the bacteria was highly prevalent in urine, aspirates, and High vaginal swab (HVS) samples
The isolation of Staph from 2.0% endo-cervical 1.5% swabs and semen may be explained by its ability to express surface

proteins that promote attachment to host proteins such as laminin and fibronectin that form the extracellular matrix of epithelial and endothelial surfaces. Besides, the expression of a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue may help explain its involvement in these sensitive body tissue. Although we did not investigate its particular role either as a career or a pathogen, it should be noted that the invasion of host tissues by staphylococci involves the production of a huge array of extracellular proteins, some of which may occur also as cell-associated proteins well expressed by the

bacteria. Further studies should outline the exact role of the following proteins in deep tissue infections. Such studies should also show conditions conducive for the expression of any or all of the diseases for which Staphylococcus is an important public health challenge.

First is the membrane-damaging alpha-hemolysin, which acts by osmotic lysis (19). It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak. Second is the ß-Toxin, sphingomyelinase which damages membranes rich in this lipid. The classical test for ß-toxin is the lysis of sheep erythrocytes. A lysogenic bacteriophage is known to encode the toxin. The third is the delta toxin which is a very small peptide toxin produced by most strains of *S. aureus* and *S. epidermidis* (16). Fourth is leucocidin, an important factor in necrotizing skin infections, and a multi-component protein toxin that acts together to damage membranes. Fifth is the coagulase, an extracellular protein that binds to prothrombin in the host to form the Staphylothrombin complex used by the bacteria to protect themselves from host phagocytic and immune defenses by causing localized clotting. Sixth is the plasminogen activator called staphylokinase with which the bacteria lysis fibrin. Seventh is the lipase, deoxyribonuclease (DNase) which provides nutrients for the bacteria during the infection process. Eight is the fatty acid modifying enzyme (FAME) which may be important in abscesses formation, where it could modify anti-bacterial lipids and prolong bacterial survival (16).

The urine specimen from females had 40.9% and 20.0% for males of *S. aureus*. Urine had the highest percentage prevalence in ages 26-30 for methicillin resistance and beta-lactamase production with 25% and 30% respectively. MRSA are frequent causes of healthcare-associated bloodstream and catheter-related infections, hence they are incriminated in many nosocomial infections. MRSA is also an emerging cause of community-associated infections, especially

skin and soft tissue infections and necrotizing pneumonia probably explaining why we observed up to 30% prevalence in this study. *S. aureus* can infect other tissues when normal barriers have been breached, this leads to furuncles (boils) and carbuncles (a collection of furuncles). In infants *S. aureus* infection can cause a severe disease Staphylococcal Scalded Skin Syndrome (SSSS) (17). Prosthetic joints put a person at particular risk for septic arthritis, and staphylococcal endocarditis (infection of the heart valves), and pneumonia, which may be rapidly spread (18). They act as an opportunistic pathogen of humans, cause a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome (19). *S. aureus* causes food poisoning by releasing entero-toxins into food, and toxic shock syndrome by the release of superantigens into the bloodstream (20).

Out of 36% isolates from the wound, 42% expressed the mecA gene and 52% had beta-lactamase activity. Out of 34% from urine, 30% expressed mecA genes while 33% had beta-lactamase activity, Table 3&4. This is not a surprise because *Staphylococcal* resistance to penicillin is known to be mediated by penicillinase (a form of β-lactamase) production. Again, the mechanism of resistance to methicillin is known to be by the mecA gene, which codes for an altered Penicillin-Binding Protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillin's, cephalosporins, and carbapenems). This allows for resistance to all β-lactam antibiotics and avoids their clinical use during Methicillin-Resistant *S. aureus* (MRSA) infections. As such the glycopeptide, vancomycin is often deployed against MRSA (21). It would be nice to state that aminoglycosides such as kanamycin, gentamicin, and streptomycin, are resisted through protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S Ribosome (22). Staphylococcus resists aminoglycoside by three main mechanisms: first is the production of aminoglycoside modifying enzymes such as "Aminoglycoside adenylyltransferase 4' IA" which renders the antibiotics ineffective (23).

Second is by ribosomal mutations a molecular deletion or insertion of nucleotides which changes the reading frame of the bacteria DNA making the antibiotics unable to recognize certain targets to act. The third is by use of active efflux of the drug out of the bacterial cytoplasm.

Isolation of beta-lactamase Methicillin-Resistant *S. aureus* (MRSA) may also be referred to as multiple-resistant *Staphylococcus aureus* or Oxacillin-Resistant *Staphylococcus aureus* (ORSA) because the resist the penicillins and the cephalosporins. A 2007 CDCP report estimated that the number of MRSA infections treated in hospitals recorded a 220% increment throughout 6years while at the same time death rate increased by 155% over the same 6years period (24*)*. CDC also reported that MRSA was associated with 18,650 hospital stay-related deaths in the United States in 2005 (25-26) these figures suggest that MRSA infections are responsible for more deaths in the U.S. each year than Acquired immune deficiency syndrome; AIDS (27*)*.

Our report of 42% isolation of mecA genes among resistant Staphylococcus in Ekpoma is similar to an old Nigerian report stated that 43% Staphylococcus isolates in Jos University teaching hospital with 81% of the MRSA isolates from hospital's in-patients, while 19% were from out-patients and also a different from 69% report by Onanuga *et al,* (28) from health women in Zaria, Nigeria (29). This result is however different from a population-based study of the incidence of MRSA infections in San Francisco during 2004-5 which showed that 85% of these infections occurred outside of the health care setting (30*)*. Our report is higher than 31% report from surgical site infections in Uganda, (31).

While it is not clear why a greater number of MRSA was found outside the hospital settings in San Francisco, changing disease demography due to the emergence of complex socio-demographic and cultural dynamics of disease transmission including but not limited to abuse of home-based care and rise in the incidences of injected drug users, may highlight the difference observed between

Ekpoma and San Francisco (32). Constant use of antibiotics by nurses caring for the wound may explain why the prevalence of MRSA was lower in the Uganda report compared to our present report of 42% prevalence of MRSA in wounds. It will be nice to mention here that absence of coordinated surveillance on MRSA in Africa with emphasis on countries like Uganda and Nigeria with no clear law restricting the purchase and use of antibiotics and where most antibiotics are still purchased and used without prescription from qualified healthcare providers, use of a sub-lethal dose of drugs bought across the counter remain a major risk factor for resistance.

Although alcohol-based rubs are somewhat effective, a more effective strategy is to wash hands with an anti-microbial cleanser with persistent killing action, such as Chlorhexidine (33). Poor hygiene habits remain the principal barrier to significant reductions in the spread of MRSA (34).

Worldwide, an estimated 2 billion people carry some form of *S. aureus*; of these, up to 53 million (2.7% of carriers) are thought to carry MRSA. In the United States, 95 million carry *S. aureus* in their noses; of these, 2.5 million (2.6% of carriers) carry MRSA (34). MRSA has also been found in some public school systems(34). A 2007 study found that 4.6% of patients in U.S. health care facilities were infected or colonized with MRSA (35). A 2008 study in Canada found MRSA in 10% of tested pork chops and ground pork and a U.S. study in the same year found MRSA in the noses of 70% of the tested farm pigs and 45% of the tested pig farm workers (35).

Conclusion

Methicillin-resistant (mecA) genes and beta-lactamase production were identified among *Staphylococcus aureus* isolates recovered from a different clinical specimen in Ekpoma Edo State, Nigeria. Wounds had a high prevalence of MRSA of 52.0%. Urine had a high percentage in ages 26-30, while females had a higher frequency of isolates of *S. aureus* than males. Strict use of prescription drugs to avoid abuse, improved personal hygiene, good laboratory practice, screening of all in-patients

upon admission into the hospitals, screening of visitors during visits to in-patients, hand washing, and the use of disposable aprons and gloves by staff to reduces skin-to-skin contact and therefore further reduces the risk of transmission are strongly recommended prevention protocols.

REFERENCES

1. Ogston A. "On Abscesses". Classics in Infectious Diseases". *Rev Infect Dis* 1984, **6** (1): 122–28.

2. Kollef MH, Micek ST. Methicillin-resistant *Staphylococcus aureus*: a new community-acquired pathogen? Curr opin infect dis.2006; 19[2]; 161-168.

3. Ryan K J, Ray CG (editors). *Sherris Medical Microbiology* (4ᵗʰ ed.), McGraw Hill. 2004.

4. Matthews K. R, Roberson J, Gillespie B. E, Luther D. A, Oliver S. P (1997*). "Identification and Differentiation of Coagulase-Negative Staphylococcus aureus by Polymerase Chain Reaction*". *Journal of Food Protection* *60* *(6): 686–8*, http://www.ingentaconnect.com.

5. Whitt DD, Salyers AA. "*14*". *Bacterial Pathogenesis: A Molecular Approach* (2nd edition ed.) USA: ASM Press.1998.

6. Jawetz Melnick & Adelberg's Medical Microbiology. 24ᵗʰ Edition. Mc Graw Hill Lange.

7. Agwu E, Ihongbe JC, Inyang NJ. Prevalence of Quinolone susceptible *Pseudomonas aeruginosa* and *Staphylococcus aureus* in delayed-healing diabetic foot ulcers in Ekpoma Nigeria. *Wounds* 2010; 4: 100-105.

8. Chambers HF. "*The Changing Epidemiology of Staphylococcus aureus?*". Emerg *Infect Dis* 2001; *7* *(2): 178–82.*, www.cdc.gov/ncidod/eid/vol7no2/chambers.

9. Thompson RL, Cabazudo I, Wenzel RP. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann intewn wed. 1982; 97(3): 309-317.

10. Wilcox MH, Hall J, Pike H, et al. Use of perioperative mupirocin to prevent methicillin-resistant Staphylococcus aureus (MRSA) orthopedic surgical site infections. *J Hosp Infect 2003*; *54:196–201.*

11. 11. Johnson AP, Pearson A, Duckworth G. "Surveillance and epidemiology of MRSA bacteremia in the UK". *J Antimicrob Chemother* 2005; **56** (3): 455–62.

12. Cheesbrough, M. District Laboratory Practice in Tropical Countries. Part 2 (434). Cambridge University Press. 2000

13. Agwu E, Ihongbe JC, Ezeonwumelu JOC, Moazzam ML. Baseline burden and antimicrobial susceptibility of pathogenic bacteria recovered from oral lesions of HIV/AIDS patients in South-Western Uganda. Oral Science International, (in press) March 2015. DOI: 10.1016/S1348-8643(15)00018-.

14. Ojulong J, Mwambu T, Joloba M, **Agwu E,** Bwanga F, Najjuka C & Kaddu-Mulindwa D. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) among isolates from surgical site infections in Mulago hospital- Kampala, Uganda. *The Internet Journal of Infectious Diseases* 2009 Volume 7 Number 2

15. Agwu E, Ihongbe JC, Okogun GRA, Ezeonwumelu JOC, Igbinovia O. *Chromobacterium violation* associated with recurrent vaginal discharge among apparently healthy females in Ekpoma Nigeria. *OJHAS*, **2007**, 1: 2.

16. Curran JP, Al-Salihi FL (1980). "Neonatal Staphylococcal Scalded Skin Syndrome: Massive Outbreak Due To An Unusual Phage Type". Pediatrics 1980, **66** (2): 285–90. http://www.cirp.org.

17. Kluytmans J, van Belkum A, Verbrugh H (1997). "N*asal Carriage of Staphylococcus aureus: Epidemiology, Underlying Mechanisms, and Associated Risks*". *Clin. Microbiol. Rev.* *10* *(3): 505–20.*, http://cmr.asm.org.

18. Ihongbe JC, Agwu E, Inyang NJ, Pneumococcal pneumonia complicates the presentation of pulmonary Tb & pseudomembranous candidiasis, predictive of unknown HIV infection in Ekpoma Nigeria: *Internet J Micro.* 2008; 5 (2)

19. *Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V*. *Functional Insights from the Structure of the 30S Ribosomal Subunit and its Interactions with Antibiotics. Nature* 2000, *407 (6802): 340–8.*

20. Sakon J, Liao HH, Kanikula AM, Benning MM, Rayment I, Holden HM. "*Molecular structure of kanamycin nucleotidyltransferase determined to 3.0-A resolution*". Biochemistry 1993, **32** (45): 11977–*84.*

21. Okuma K, Iwakawa K, Turnidge J, *et al* (2002). "Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community". *J Clin Microbiol* **40** (11): 4289–94.

22. Klein E, Smith DL, Laxminarayan R (2007). "*Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus*, United States, 1999–2005". *Emerg Infect Dis* **13** (12): 1840–6.

23. Klevens SA. "*Invasive Methicillin-Resistant Staph aureus infections in the United States*". *JAMA*. Retrieved on 2007-10-31.

24. Centers for Disease Control and Prevention (2007), *MRSA: Methicillin-resistant Staphylococcus aureus in Healthcare Settings*

25. Stein R. "*Drug-resistant staph germ's toll is higher than thought*". Washington *Post*. Retrieved on 2007-10-19.

26. Adcock P. M; Pastor P. Medley F. MRSA in two child care centres. J. infect dis;1998 178; 577-580.

27. UK Office for National Statistics Online, "*MRSA Deaths continue to rise in 2005*" Wikipedia, the free Encyclopedia, 2007.

28. Muto HM; Jalvis WR, Boyca JM, Farr, BM, "SHEA guideline for preventing nosocomial transmission of multidrug-Resistant strains of *Staphylococcus aureus* and enterococcus". Infect control, Hosp. Epidemiol. 24 (5): 362-86.

29. Blot SI, Vandewoude KH, Hoste EA, Colardyn FA. "*Outcome and attributable mortality in critically Ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant Staphylococcus aureus*". *Arch Intern Med 2002, 162 (19): 2229–35.* http://archinte.ama-assn.org

30. Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, Lapetina E, Gemmen E. "*The Burden of Staphylococcus aureus Infections on Hospitals in the United States: An Analysis of the 2000 and 2001 Nationwide Inpatient Sample Database*". *Arch Intern Med* 2005, **165**: 1756–1761.

31. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. "*The Impact of Methicillin Resistance in Staphylococcus aureus Bacteremia on Patient Outcomes: Mortality, Length of Stay, and Hospital Charges. Infection Control and Hospital Epidemiology* 2005, **26**: 166–174.

32. Hardy KJ, Hawkey PM, Gao F, Oppenheim BA. "Methicillin-Resistant Staphylococcus aureus in the Critically ill". *British Journal of Anaesthesia* 2004, **92**: 121–30.

33. Birmingham MC, Rayner CR, Meagher AK, Flavin SM, Batts DH, Schentag JJ. "*Linezolid for the Treatment of Multidrug-Resistant, Gram-Positive Infections*: Experience from a Compassionate-Use Program". Clin. Infect. Dis. 2003,**36** (2): 159–68.

34. Bisaga A, Paquette K, Sabatini L, Lovell EO. "A prevalence study of methicillin-resistant Staphylococcus aureus colonization in emergency department health care workers". *Ann Emerg Med* 2008, **52** (5): 525–8.

35. Association for Professional in infection control and Epidemiology. "National prevalence study of Methicillin-Resistant Staphylococcus aureus (MRSA) in US. Healthcare facilities". June 25, 2007 (http://www.apic.org)