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

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Background: *Staphylococcus aureus* associated infections continues to be a public health challenge in resources limited developing countries of sub-Saharan Africa. Understanding the genetic basis of resistance of Staphylococcus aureus remains paramount panacea to associated illnesses. Objective: To isolate and identify methicillin resistant and beta-lactamase producing *Staphylococcus aureus* from 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 analysed. Standard Microbiological methods used in isolation and identification of *Staphylococcus aureus* included 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 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 was high (25% and 30% respectively) among isolates from urine samples. **Conclusion.** Methicillin-resistant (mecA) genes and beta lactamase enzymes remains 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.

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Introduction

Staphylococcus aureus, is the most common cause of *Staphylococcal* infections and are frequently found in the nose, skin, upper respiratory tract and intestinal tract of healthy individuals (1). They are halophilic and thrive well in a pH of 7.4 to 7.6 (2). *S. aureus* is primarily a catalase and coagulase enzyme producing organisms which adds 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 septicaemia (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 different clinical specimen. The use of 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 was 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 focuses on recent advances and use of cutting-edge DNA technologies to answer more difficult questions not possible by use of phenotypic methods (11).

False identification of an isolate can impact on implementation of effective treatment and/or control measures (12). Effective surveillance, prevention and control of any agents of disease depends on early diagnosis. This study was designed to isolate and identify methicillin resistant and beta-lactamase producing *Staphylococcus aureus* from 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 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 acceptable error (5%). When the above formula was used sample size of one hundred and eighty-nine (189.2) was obtained. To make up for sampling error and drop outs, 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 patients. 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 as routine investigation. Further investigations 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 clinical specimen. In addition to the Chromagar MRSA, we also used the Prolex™ Staph Latex Kit to identify *S. aureus* following manufacturer's instructions.

The Prolex™ Staph Latex Kit utilizes polystyrene latex particles which have been sensitized with fibrinogen and IgG. When Staphylococcal colonies which 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 prior to use. The reagent is re-suspended by shaking prior to 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. This 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 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. Nitrocef 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 colour from yellow to red. Bacteria which produce beta-lactamase in significant amounts produce this yellow to red colour 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, Nitrocef disc are removed from the vial and placed on an empty Petri dish or microscopic slide. Prior to 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. gonorrhoeae* 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 in color. 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 Qiaegen 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 votexing and incubated at 56°C for 30 minutes and then for a further 15minutes at 95°C. The set up was centrifuge briefly for few seconds and 200ul of ethanol (90-100%) added and mixed by pause-vortexing for 15 seconds. The set up (1.5ml tubes) were 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 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 in a new 2ml collection tube and discard the filtrate discarded. Centrifuge at full speed for 1minute.

The spin column was placed in a clean 1.5ml tube, opened carefully and 100µl of buffer AE added the spin column. The set up was incubated at room temperature for 1minute and centrifuged at 8000rpm for 5minutes. The 5 minutes incubation of spin column with buffer AE at room temperature before centrifugation increases DNA yield. Filtrate were kept on ice for Polymerase Chain reaction. Twenty microliter 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 millilitres of ethanol was then added 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 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 containing the following specific volumes per reagent: Taq Polymerase - 1u (one international units), 250µm of nNTPs. 10mM of Tris HCL, 1.5mM of MgCl, 4.0µl of template DNA, 8.0µl, 1.5 µl of forward 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 ultra illuminator and result recorded

Result

Out of 200 isolates of *Staphylococcus aureus* recovered from 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. 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 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 in relation to the presence of MecA gene and their β-actamase production. The number of *S. aureus* from the various clinical sites in relation to gender is shown in Table 4.

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 to 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).

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

Out of 200 isolates of *Staphylococcus aureus* recovered from 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 specimen. 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 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. In addition, expression of a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue may help explain its involvement in this sensitive body tissues. 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 apparently 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 expression of any or all of the diseases for which Staphylococcus is an important public health challenge. First is the membrane-damaging alpha-haemolysin, 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, a sphingomyelinase which damages membranes rich in this lipid. The classical test for ß-toxin is lysis of sheep erythrocytes. A lysogenic bacteriophage is known to encode the toxin. 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 the leucocidin, an important factor in necrotizing skin infections and a multi-component protein toxin which act together to damage membranes. Fifth is the coagulase, an extracellular protein which binds to prothrombin in the host to form Staphylothrombin complex used by the bacteria to protect themselves from host phagocytic and immune defences by causing localized clotting. Sixth is the plasminogen activator called staphylokinase with which the bacteria lysis fibrin. Seventh is the lipase, deoxyribonuclease (DNase) which provide nutrients for the bacteria during 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 are 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 opportunistic pathogen of humans, causes 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 release of super antigens into the blood stream (20).

Out of 36% isolates from wound, 42% expressed mecA gene and 52% had beta lactamase activity. Out of 34% from urine, 30% expressed mecA genes while 33% had beta lactamse 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 resist 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 bacteria 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 over a period of 6years while at the same time deaths rate increased by 155% over 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 greater number of MRSA was found outside the hospital settings in San Francisco, changing disease demography due to 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 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 in 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 different clinical specimen in Ekpoma Edo state, Nigeria. Wounds had a high prevalence MRSA of 52.0%. Urine had high percentage in ages 26-30, while females had the higher frequency of isolates of *S. aureus* than the 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.

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