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# Antimicrobal susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolatetd from pediatric clinical samples at Webuye District Hospital

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## ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen causing a significant mortality and morbidity. The main objective of the study was to establish antibiotic susceptibility pattern MRSA isolated from pediatrics clinical samples at Webuye District Hospital. A total of 96 clinical samples that include blood, abscess, ear swabs, and urine and wound/pus swabs were collected by simple random techniques. These samples were cultured onto Blood agar and MacConkey agar respectively and incubated at 37$^{o}$C for 24 hours. In the result 83 (86.5%) clinical samples had pure colonies of *S. aureus* which were identified morphologically and biochemically by standard laboratory procedures using Bergey's Manual of Clinical Microbiology. All confirmed positive isolates were screened for MRSA whereby 18 (21.7%) were MRSA and these were subjected to susceptibility testing to common antibiotics by modified Kirby-Bauer disc diffusion method. The susceptibility was interpreted according to National Clinical laboratory Standard guidelines. From the study the prevalence of MRSA was 18 (21.7%) with the highest obtained in the wound/pus swab. Almost all MRSA was resistant to penicillin (92.8%) and cephalexin (96.6%) and amoxicillin (91%). The most effective antibiotics against the of MRSA strain were vancomycin (98.97%), ciprofloxacin (88.4%) and gentamycin (83.06%). Vancomycin was the most effective drug showed the largest inhibition zone.

**Keywords**: *Staphyloccocus aureus*, MRSA, Antimicrobial, Susceptibility

## 1. INTRODUCTION

*Staphylococcus aureus* is a gram positive bacteria and non-moving small round and is non-motile cocci. It is seen under microscope as grape-like clusters and forms a fairly large yellow colony on rich medium. Although more than 20 species of *Staphylococcus areus* described in Berger's manual 2001, [1] only *S. aureus* and *S. epidermidis* are significant in their interaction with human. *S. aureus* colonise mainly the nasal passage, but, it may be found regularly in most other anatomical locales. S.aureus is one of the most considered resistant bacteria that cause nosocomial infection [2,3]

The genus Staphylococcus is in bacteria family Staphylococciceae, *S. aureus* is often haemolytic on Blood agar *S. epidermis* is non-haemolytic [1,4]. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yield principally lactic acid. The bacteria are catalase-positive and oxidase negative *S. aureus* can grow at a temperature range of 15 to 45 °C and at sodium chloride concentration as high as 15.Almost all strains of *S. aureus* produce the enzyme coagulase nearly all strains of *S. epidermis* lack this enzyme, thus, *S. aureus* should always be considered a potential pathogen. *S. aureus* can produce a wide variety of diseases from relatively soft tissue infection (such as furunucrelosis, abcess, impetigo, wounds and burns) to deep seated and life threatening condition arising from systemic infection (crysipelas deep tract infection, osteomyelitis, pneumonia, sepsis, urinary tract infection and endocarditis [1-5]. The heterogeneity of these diseases and the unique ability of *S. aureus* to develop resistance to antibiotic agent reflect its extra-ordinary capacity to adapt to a variety of environment [6].

The gene for methicillin resistance, *mec*A and staphylococcal chromosal cassette *mec* (SCC*mec*) which integrates as specific location in *S. aureus* genome that facilitate resistance [7,8] Methicillin Resistant *Staphyloccocus aureus* (MRSA) is a short form of methicillin resistant *S. aureus*. MRSA is a specific strain of bacterium *S. aureus* which has developed resistance to all penicillin, methicillin and other narrow spectrum to beta-lactamase resistant penicillin antibiotics [9,10]. MRSA is responsible for widespread infection of hospital bound patients in UK and abroad, reported death rate of 34% within 30 days amongst patients infected with MRSA. The strains of *S. aureus* in hospital are usually resistant to variety of different antibiotics. This organism is one of the important pathogens in hospital acquired infection in human [11]. A few strains are resistant to all clinically useful antibiotics except vancomycin and vancomycin resistant strains are increasingly reported. A high level of resistance to vancomycin is related to development of complicated bacteria. MRSA refers to methicillin resistant *S. aureus;* the term is used to describe a number of strains of the bacteria *S. aureus* that are resistant to a number of antibiotics including methicillin [12]. The MRSA are widespread in hospitals. This occurs in people who have been in hospital or other healthcare such as nursing homes and dialysis Centre that involve surgery and other intravenous tubing. Most MRSA infection occurs frequently among patient in health setting [13].

The increasing incidence of infection due *S. aureus* ability to adapt to changing environment and healthcare procedure that leave patients vulnerable to MSRA which is

typically spread in healthcare setting from patient to patient on unclean hand of health care personnel or through improper use or reuse of equipment [14-16]. MRSA is a serious threat to hospitalized patients throughout the world and it has become a public health problem [11]. Resistance to methicillin is mediated via the mec operon part of the staphylococcal cassette chromosome mec (Scc *mec*).Resistance is conferred by the mec A gene which codes for an altered penicillin-binding protein (PBP2a or PBP2b) for binding beta-lactams [17-18]. This allows for resistance to all beta-lactam antibiotics and obviates their use during MRSA infections. As such the glycopeptides, vancomycin is often deployed against MRSA. Aminoglycoside antibiotic such as kanamycin, gentamycin, streptomycin etc., were once effective against staphylococcal infection until strain evolved mechanism to inhibit the aminoglycosides action which occur via protonated amine and or/ a hydroxyl interaction with the ribosomal RNA of the bacteria sub-unit [19]. There are three main mechanisms of amino glycoside resistance which are currently and widely accepted; aminoglycoside modifying enzymes, ribosomal mutation and active efflux of drug out of the bacteria [20]. Originate from the enterococci and codes for an alternate peptidoglycan to which vancomycin will not bind [21]. MRSA infection in both the hospital and community setting are commonly treated with non-beta-lactam antibiotics such as clindamycin (alinocasamine) and co-trimoxale. Resistance to these antibiotics has also led to the use of anew spectrum broad-spectrum anti-gram-positive antibiotic such as linezolid because of its availability as an oral drug. First line treatment for serious invasive infection due to MRSA is currently glycopeptides antibiotic: Vancomycin and telcoplanin [11-22].

*S. aureus* infection has continued to become an important pathogen in hospital and public health facilities causing both nosocomial and community acquired infections. This pathogenic microbe has a remarkable capability of evolving different mechanisms of resistance to most commonly used antibiotics agent and of particular concern is resistance to methicillin with subsequent increased rate of cost of healthcare, and high morbidity and mortality. However, there is a special concern especially in the emergence of MRSA and multi-drug resistant infections in immune compromised patients particularly infants and children who have underdeveloped immune system. This illustrates the extent of the problem and thus aim of the study was to determine antibiotic susceptibility of MRSA isolated from pediatrics clinical samples at Webuye District Hospital.

## 2. METHODOLOGY

The study area was at Webuye District Hospital, the research design used was retrospective on pediatrics patient with the purpose of the study was to describe the susceptibility pattern of methicillin resistant *S. aureus* isolated form clinical samples of children attending Webuye district hospital. Simple random sampling technique was employed in this study. The number of samples examined in this study was being determined using Fisher's formula. The studied samples consists of male and female children's clinical samples of blood, pus, urine, nasal swabs, ear swabs and wound swabs isolated from pediatrics' ward.

The laboratory methodology was according to standard operating procedure of Webuye District Hospital. Blood, pus, nasal swabs, urine ear swabs, and wound specimens were inoculated on Blood agar and MacConkey agar using streaking method of inoculation. Blood

agar was incubated in 5% to 10% jar of carbon dioxide. MacConkey was plated aerobically at 35 to 37 °C for 18 to 24 hours. On day 2 the culture plates were checked for presence of growth and whether it is mixed growth or pure. The suspected isolates of pure colonies were identified morphologically that are circular, pinhead colonies which are convex with entire margin and has golden brown (creamy) or yellow in colour and gram stained where by *S. aureus* are gram positive that appeared in clusters and grape like when viewed under a microscope. The colonies were then subjected to biochemical tests which are: coagulase positive and catalase positive as outlined in Bergey's manual of Determinative Bacteriology ($9^{th}$ edition) [1]. Confirmed *S. aureus* colonies were screened for methicillin resistant *S. aureus* which involves using penicillin binding protein (PBP-2a) agglutination test. This was used to identify methicillin resistant *S. aureus*. Agglutination latex test for the detection of the clumping factor, Protein A and certain polysaccharides found in MRSA. The turbidity of MRSA in saline peptone water was matched with that one of 0.5 McFarland turbidity in a test tube and transferred to Muller Hinton plate and a convened spreading made using a spreader. All the positive isolates for MRSA were subjected to antimicrobial susceptibility pattern by standard disc diffusion as per NCCL standard [23]. The methicillin resistant isolates were transferred to commercial disc. The commercially available disks were placed on the Muller Hinton plate as recommended by manufacturers and incubated at 37 °C overnight incubation [24]. The commercially available disk was having the following antibiotics; ampicillin (10 µg), amoxicillin (30µg), ciprofloxacin (5 µg), cephalexin (30 g), chloramphenicol (30 µg), centrimaxole (25 µg), gentamicin (10 µg) erythromycin (15 µg), penicillin (10 µg), amikacin (30 µg) and tetracyclin (30 µg). The zones of inhibition were measured and recorded after 24 hours of incubation at 37 °C (sensitive >13mm, intermediate 11-12 mm and resistant <10 mm) which is according to CLSI interpretation (2007) [23]. The collected data was entered in excel spread sheets and checked for consistency and accuracy. It was then coded and analysed using descriptive statistical method

## 3. RESULTS

A total of 96 clinical samples were collected from pediatric ward. Out of these, 83 were confirmed positive isolates for *S. aureus* and analyzed for antibiotic study. This was done when clinical isolates cultured on Blood agar had shown beta hemolysis with creamy to yellow colonies, on the MacConkey the colonies appeared to be circular, pinhead and convex with entire margin with golden cream or yellow in color as shown in the Plate 1. Gram staining was then done on the described colonies, whereby the microscopy study revealed gram positive cocci appearing as grape-like (clusters) these appeared purple under oil immersion objective (X100) of the bright field compound microscope in Figure 1. Also biochemical tests included catalase and coagulase tests whereby the colonies were catalase positive which was used to distinguish between *Streptococcus* and *Stapylococcus*. *Staphylococcus* produces bubbles in hydrogen peroxide on a slide and absence of bubbles concludes *Staphylococcus* are absent. The colonies were subjected to coagulase test that is used to identify *Staphylococcus aureus* from other species of Staphylococcus. *S. aureus* is coagulase positive as performed on the tube test using EDTA Figure 2 and 3. The confirmed *S. aureus* isolates screened for methicillin resistance.

**Plate 1.** *Staphylococcus* culture growing on MacConkey agar.

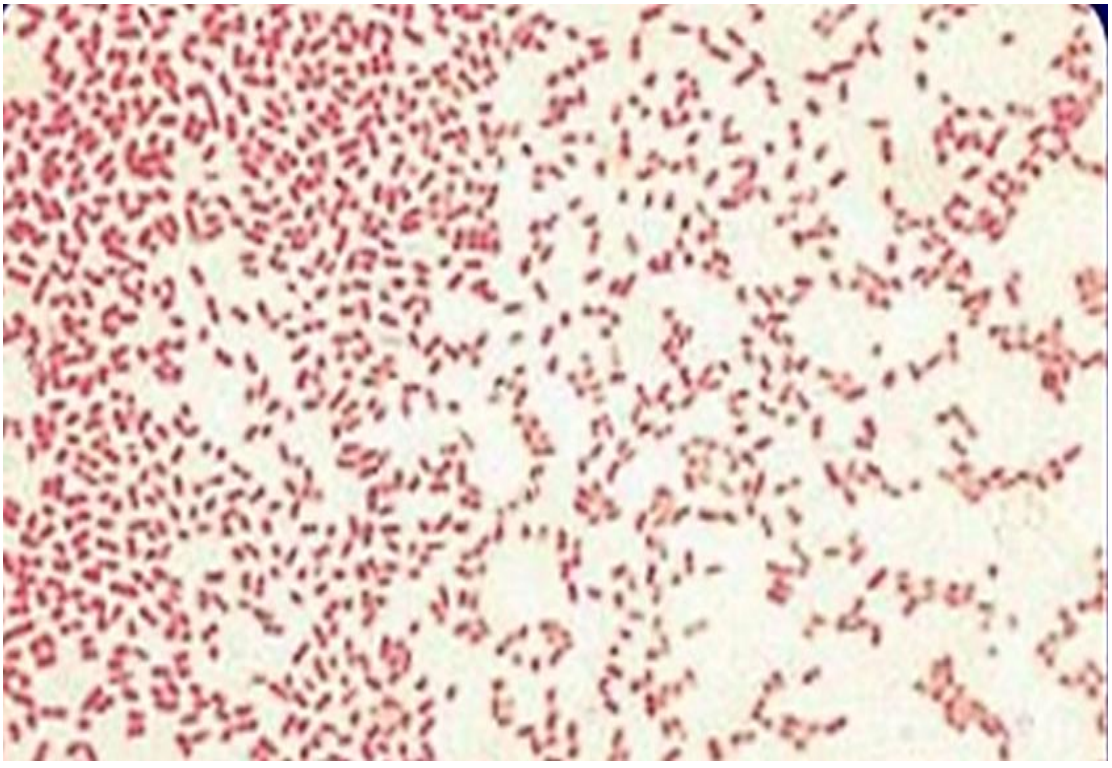

**Figure 1.** Gram positive slide

**Figure 2.** Coagulase positive tube.

**Figure 3.** Catalase positive test.

Out of 83 isolates confirmed positive *S. aureus*, 18 (21.69%) were found to be Methicillin resistant while the remaining, 65 (78.31%) were sensitive to Methicillin as shown in the Figure 4.

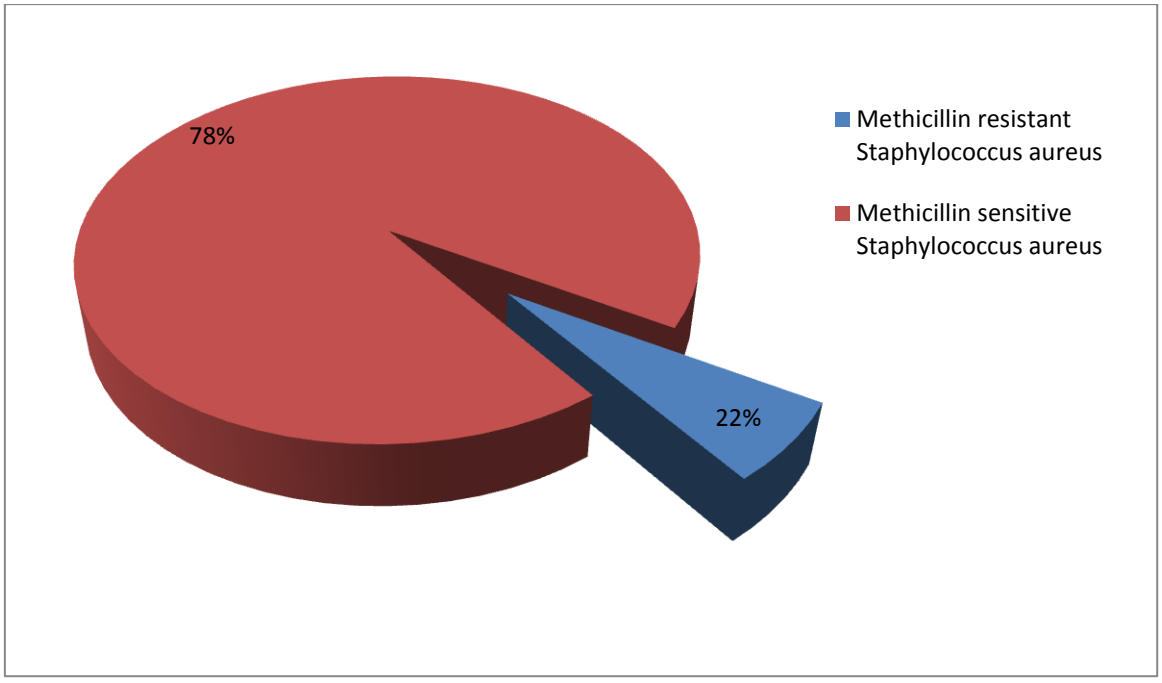


**Figure 4.** Percentage of methicillin sensitive and methicillin resistant Staphylococcus aureus

Majority of the MRSA isolates were recovered from wound/pus swab and the least was from ear swab and other clinical samples are as shown in the Table 1.

**Table 1.** Isolation rates of MSSA and MRSA from different clinical samples**.**

| **Specimen** | ***S.aureus*** **(n = 83)** | **MSSA (n = 65)** | **%MSSA** | **MRSA (n = 18)** | **%MRSA** |
|---|---|---|---|---|---|
| Pus/Wound swab | 46 | 37 | **80.44** | **9** | **19.56** |
| Urine | 13 | 10 | 76.92 | 4 | 23.07 |
| Blood | 9 | 6 | 56.64 | 3 | 43.33 |
| Ear Swab | 7 | 4 | 71.42 | 2 | 28.57 |
| Nasal Swab | 8 | 8 | 100 | 0 | 0.00 |

The mentioned antibiotics were tested for susceptibility using the commercial disc as shown in Plate 2. Methicillin resistant were amoxicillin, ampicillin, cephalexin, chloromphenicol, erythromycin and penicillin

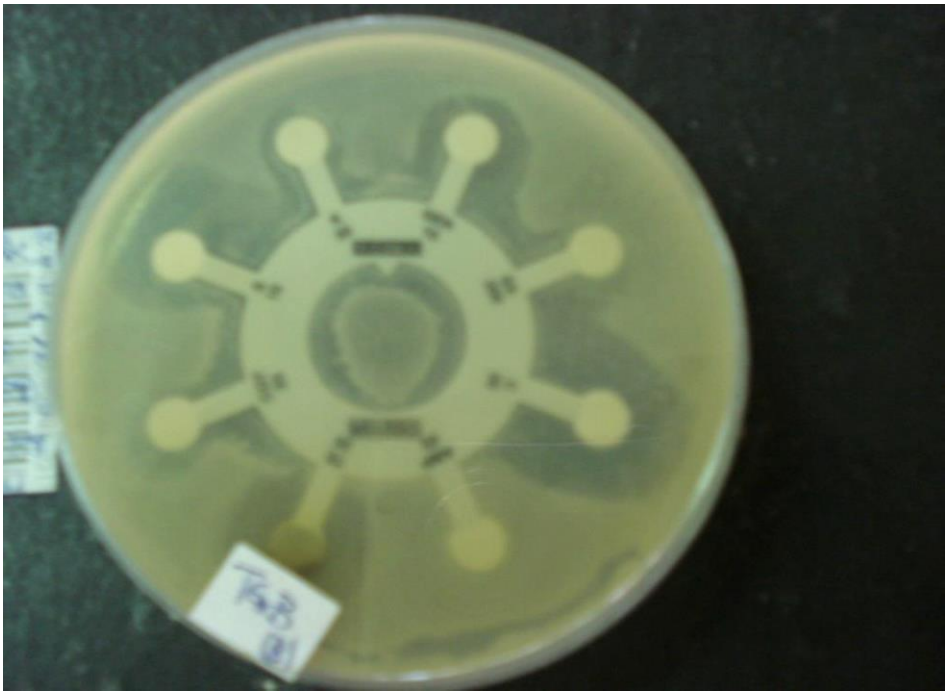

**Plate 2.** Commercial disk showing antibiotic susceptibility of methicillin resistant S.aureus to other antibiotic

The highest percentage of the isolated MRSA were susceptible to vancomycin (98.97%) followed by ciprofloxacin at (88.41%) and tetracycline at (85.42%). All the MRSA isolates were resistant to cephalexin (96.55), penicillin (92.78%) and ampicillin (90.72%). The susceptibility rates of other antibiotic agents were variables as indicated in the Table 2.

**Table 2.** Methicillin Resistant *S.aureus* to other antibiotics.

| Antibiotic agent | Sensitivity | Resistant |
|---|---|---|
| Ampicillin | 32 (31.96) | 68 (68.04) |
| Amoxicillin | 9 (9.29) | 91 (90.72) |
| Ciproflaxicin | 88 (88.41) | 12 (11.29) |
| Cephelaxin | 3 (3.45) | 97 (96.55) |
| Chloramphennicol | 24 (23.71) | 76 (76.29) |

| | | |
|---|---|---|
| Centrimaxole | 67 (67.01) | 33 (32.99) |
| Gentamycin | 83 (83.06) | 17 (16.94) |
| Erythromycin | 12 (12.37) | 88 (87.63) |
| Penicillin | 7 (7.22) | 93 (92.78) |
| Amikacin | 67 (67.01) | 33 (32.99) |
| Tetracycline | 85 (85.42) | 15 (14.58) |
| Vancomycin | 99 (98.97) | 1 (1.03) |

## 4. DISCUSSION

This study focused on the antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* whereby 83 (86.5%) of the total isolates was confirmed to have *S.aureus* and the prevalence of Methicillin resistant *Staphylococcus aureus* was found to be at (18) 21.7% in the pediatric ward at Webuye District Hospital and this can be as a result of high prescription of patient in paedriatic ward with methicillin. Similarly there was high prevalence of multidrug resistance (MDR) *S.aureus* from pediatrics samples that might have been brought up by in effective disinfection and improper sterilization of personal protective equipment. This resistance of MRSA has been a major nosocomial pathogen encountered in hospital by medical profession due to prolonged hospital stay, weak or the under developed immunity among the pediatric patients and also small ratio of nurses to patients. This prevalence rate was within the range as in pan-European data on Methicillin resistant *Staphylococcus aureus* (MRSA); with least in New Zealand which had 2% and the largest percentage was 70% in Korea and Japan [26].

The highest prevalence of MRSA were encountered on pus/wound swabs, this is probably due to it being located on the cutaneous layer of the skin thus is easily contaminated by the pathogen (MRSA) via direct contact with the skin or contaminated environment since the wounds/pus sheds a high number of this organism which may be carried asymptomatically by pediatric patients for several months. These findings are also in agreement with the result in Lebanese hospitals by Tokajian et al [27] which reported the prevelance of MRSA was 72%, and went further to show that the 18% of MRSA strains where resistant to 10-18 antibiotics. A high MRSA prevalence was also reported in several types' of clinical infection at Alghaithy et al (61% in Saudi Arabia [28], Rijial et al (56.1% in Pokhara) [29], Młynarczyk et al. (40% in Warszawie) [30].

The MRSA isolates showed resistance to almost all the common antibiotics tested which are: cephalexin (97%), penicillin (93%), amoxicillin (91%) and erythromycin (88%) respectively and this pose a great risk to other patient and medical staff as it can result to emergence of more resistant strain of MRSA which is difficult to treat. MRSA are often termed as multi-drug resistant, the microorganism pump the drug out by efflux pump inactivating it. A study in Tehran [31-32] reported that from the 90 MRSA isolates approximately half of them displayed resistance to one or more antibiotic agents including penicillin, cephalosporin and aminoglycosides.

MRSA showed resistance to beta-lactam antibiotics like penicillin and cephalexin (92.8%) and (96.6%) respectively since the microorganisms might have a single genetic element that confers resistance to beta-lactam antibiotics. This is probably due to the antibiotics being cheap and easily accessed in pharmaceutical shops and sold over the counter without the doctor's prescription, also the production of the beta-lactamase enzyme by MRSA to these drugs rendering it ineffective. This correlates with the earlier findings [33]

In the study multi-drug resistant MRSA had developed in resistance to ampicillin (90.7%), penicillin (92.8%), cephalexin (96.6%), erythromycin (87.6%) and chloromphenicol (76.3%).

This result is due to the weak immune system of pediatric patients and common prescription of these antibiotics in chemists, dispensary, clinics and hospitals. This leads to development of a mechanism of resistance by MRSA that inactivate the drug, other form are the resistant plasmid that carry gene that enable the survival of organism against antibiotic. This observation is in agreement with [34-35], in his study he reported that MRSA were generally multi-drug resistant with increasing resistance to chloromphenicol and fusidic acid.

Vancomycin is the most effective antibiotic agent which showed (98.97%) susceptibility to MRSA and thus used as drug of choice in the treatment of MRSA infection in pediatrics' due the fact that the microorganisms have not yet developed the mechanism for resistance. Additionally the drug is well prescribed to the patient by clinician thus avoiding under dose or overdose of the antibiotic. This result is similar with the previous studies as Alborzi 2000, [36] in Shiraz Iran reported that 100% of isolates were sensitive to vancomycin and recently in Qassim Saudi Arabia. However, vancomycin is not commonly prescribed drug due to the higher price of antibiotic and unavailability to many parts of the country thus making other efficient antibiotics being used.

## 5. CONCLUSIONS

MRSA strains in infected children in paedriatic ward at Webuye District Hospital showed an important public health problem. The most effective antibiotics for treatment of MRSA strain was vancomycin (98.97%), ciprofloxacin (88.41%) and gentamycin (83.06%) at Webuye District Hospital. Vancomycin is the most effective drug which can be administered since it had the largest zones of inhibition as compared to other antibiotics which is a clear indication that it has low bacteria resistance in this study. There should be active screening and compliance with recommended control practice to play important role in the control of MRSA. There is need of surveillance of MRSA and its microbial profile. The hospital control policy and guidelines that already exist should be strictly followed so as enable the clinicians to deliver better and proper health care to the patient in the pediatric ward Webuye District Hospital.

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