

## Microbial Spectrum and Antibiogram of Non-surgical Wounds in Children in a Rural Setting in Nigeria

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### Abstract

The aim of this study was to determine the microbial spectrum and susceptibility pattern of non-surgical wound infections in children in a rural setting in our environment. This study was a cross sectional study of children aged 0 to 15 years in Bakassi, Nigeria. The children were screened for non-surgical wounds using an interviewer administered semi-structured questionnaire. Identified wounds were evaluated clinically for signs of infection and specimens were collected and cultured using standard microbiologic techniques. Susceptibility test was performed on all the isolated Micro-organisms. Data were collected and analysed using SPSS version 20 for windows. Sixty four wound infections out of a total of 115 wounds giving an infection rate of 55.7% were encountered. Of 64 wound cultures, 46.9% (30/64,) yielded mono-microbial growth, while poly-microbial growth of two and three microorganisms were obtained in 46.9% (30/64) and 1.6% (1/64) specimens respectively.

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A total of 92 organisms were isolated belonging to seven different species. *Staphylococcus aureus* (n= 57/92, 62.0%) and *Streptococcus pyogenes* (n = 30/92, 32.6%) were the predominant pathogens isolated. High rate of community acquired Methicillin Resistant *Staphylococcus aureus* (MRSA) (38/57, 66.7%) was observed. The microbial spectrum of non-surgical wounds of children in rural communities is wide. The high rate of antimicrobial resistance particularly MRSA and high predominance of *S. pyogenes* are potential sources of dire consequence in management and long term morbidity.

**Keywords:** Bakassi; Children; Infection; Non-surgical wounds; Susceptibility pattern.

## 1. Introduction

A non-surgical wound is a breach in the skin and underlying tissues due to causes other than a surgical incision. Non-surgical wounds are otherwise called untidy wounds [1]. The possible causes are puncture, crushing, traction or tearing, avulsion, road traffic accidents, vascular injuries or burns [1] as well as chronic vascular ulcers [2]. Two main factors make these wounds very different: High risk of infection due to persistent exposure of the subcutaneous tissues and presence of devitalized tissue in the wound [1, 3]. Consequently, they provide veritable ground for contamination, colonization and growth of a wide range of micro-organisms [4]. The presence of bacteria in the wounds not only causes wound infections but also delayed healing [5, 6]. Furthermore, some of the micro-organisms tend to acquire resistance to numerous antibiotics such as Penicillins and Cephalosporins [7, 8], which are some of the most commonly used antibiotics in the community thereby leading to increased morbidity and mortality.

Previous studies on microbiology and susceptibility patterns of wound infections have largely been on post-surgical wound infections [9, 10] rather than on non-surgical ones. Similarly, most of the studies have been on mixed populations of children and adults [10 -12] and not children alone. Few studies that are available, however, show that there is some variation in the microbiology and susceptibility patterns of non-surgical wound infections and more so in children [13, 14]. Yet none of them was carried out in the rural community, when it is well known that there is a great difference in the response of hospital acquired versus community acquired infections.

The aim of this study therefore was to investigate the microbial spectrum and susceptibility patterns of non-surgical wound infections in children in a rural setting in our environment so as to aid treatment of community acquired infections and so obviate the deleterious consequences of these microorganisms in children while also serving as a stimulus for further research.

## 2. Materials and methods

This was a cross sectional observational study of children aged 0 to 15 years in Bakassi LGA of Cross River State, Nigeria conducted in March, 2016. Bakassi lies south of the Metropolitan city of Calabar. It is a settlement carved out of Akpabuyo Local government area as home of the displaced people of Bakassi Peninsula which was ceded to Cameroon. The Community is typically rural and the main occupation of the people is fishing and farming. Through cluster sampling technique, using the households as the sampling units

while the sampling frame was the resettlement register containing list of the households, 430 households out of 6,017 existing ones were selected by random sampling technique. All children within the age bracket who were members of the selected households and who resided within the study area, whose parents or guardians willingly gave consent to participate were consecutively recruited for the study. Ethical approval for the study was obtained from the Cross River State Health Research / Ethics Committee and informed consent was gotten from their parents / guardians. The researcher and his trained assistants moved from house to house to screen children for non-surgical wounds using a face to face interviewer administered semi-structured questionnaire. The purpose of the study was explained to each responding parent and guardian of the children. They were told that participation was voluntary and they will not suffer any consequences if they chose not to participate.

**Collection of swab specimens and culture:** Following debridement of the infected wound, a sterile piece of gauze moistened with normal saline was used to vigorously wipe the wound to remove surface contaminants. Antiseptic solutions were not applied on the wounds. Using sterile technique, a cotton-tipped swab applicator was applied via Levine and Colleagues swabbing method [15] at the center of the wound with adequate pressure and rotated over a 1cm by 1cm area to express wound tissue fluid which moistened the cotton tipped swab applicator. The swabs were immediately inoculated in the field on 5% Sheep Blood agar, Chocolate agar, MacConkey agar and Saboraud Dextrose agar (SDA). All plates were returned to OMS Diagnostic and Research Center – a private laboratory facility, on the same day. The blood agar and Chocolate agar plates were incubated in Candle extinction jar at 35°C overnight, while the MacConkey and Saboraud Dextrose agar plates were incubated in ambient air at 35°C overnight. All plates with no growth were re-incubated for another 24 hours before discarding. Anaerobic culture could not be done due to the challenges of biopsy collection as swabs are not recommended for anaerobic culture.

**Identification of isolates:** All Isolates were identified based on their colonial morphology, Gram stain and biochemical reactions as described by Winn and his colleagues [16]. Isolates appearing as gram positive cocci were evaluated with the catalase test, slide coagulase test and a tube coagulase on all slide coagulase negative isolates. Catalase positive isolates were also sub-cultured on mannitol salt agar (prepared in house from Blood agar base, 1% Mannitol, 7.5% Nacl and Methyl red indicator). All gram positive cocci in clusters that were coagulase positive and mannitol fermenting were identified as *Staphylococcus aureus* (*S. aureus*). Catalase negative gram positive cocci isolates were sero-grouped using oxoid Streptococcal grouping kit (Oxoid, Basinstoke,UK). The gram negative isolates were identified using oxoid Microbact 12E identification system (Oxoid Basinstoke, UK). This system involved the generation of biochemical reactions based octal codes which were then entered into the identification computer software which identified the microorganisms stating the percentage probability.

**Susceptibility testing:** The Modified Kirby-Bauer disk diffusion susceptibility testing method was performed according to the Clinical Laboratory Standard Institute (CLSI) guidelines [17]. Mueller Hinton agar was used for all isolates except *Streptococcus pyogenes* (*S. pyogenes*) for which Mueller Hinton agar with 5% sheep blood was used. All inocula were standardized to 0.5 McFarland turbidity equivalents. Oxoid antimicrobial susceptibility discs (Oxoid Basingstoke,UK) were used. All zone diameters were measured with a ruler and interpreted as susceptible, intermediate or resistant as defined in the CLSI document. Tigacycline susceptibility

was performed using Tigacycline M.I.C.Evaluator E-test (Oxoid, Basingstoke, UK). Tygacycline minimum inhibitory concentration (MIC)  $\leq 0.5\mu\text{g}$  were considered susceptible. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as controls for all susceptibility tests.

**Identification of Methicillin-Resistant *S. aureus* (MRSA):** All *S. aureus* isolates were evaluated for MecA mediated oxacillin or methicillin resistance using cefoxitin 30 $\mu\text{g}$  surrogate method as described in the CLSI document. A 0.5 Mcfarland turbidity equivalent of the test isolate was prepared and inoculated on Muller Hinton agar using a cotton wool swab. A cefoxitin 30 $\mu\text{g}$  disc was placed and the plates incubated at 35°C for 16 to 24 hours. All isolates with cefoxitin zone sizes  $\leq 21\text{mm}$  were interpreted as MecA positive and considered as MRSA [17]. Previously identified local MRSA isolates (ASU11) were used as positive controls while *S. aureus* ATCC 25923 was used as negative controls.

**Inducible Macrolide LincosamideStreptogramin B (IMLS<sub>B</sub>):** All *S. aureus* isolates that were resistant to Erythromycin but susceptible to Clindamycin were evaluated for Inducible Macrolide LincosamideStreptogramin B (IMLS<sub>B</sub>) also known as inducible clindamycin test using the D-test as described in the CLSI document. A clindamycin 3 $\mu\text{g}$  disc was placed 15mm edge to edge adjacent to an Erythromycin 15 $\mu\text{g}$  disc. Isolates showing a blunting of the susceptibility zone of clindamycin on the side adjacent to the Erythromycin (D-zone) were considered to be IMLS<sub>B</sub> positive. *S. aureus* ATCC 25923 was used as IMLSB negative control while a local IMLS<sub>B</sub> isolated previously isolated in our laboratory was used as IMLS<sub>B</sub> positive control.

### 3. Data analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp. NY, USA) and Computer Programme for Epidemiologic Analysis (CPEA). Descriptive statistics (Frequency, proportions, means, medians and standard deviation) were used to summarize variables. Categorical data were presented as % (n/no. of wounds).

### 4. Results

A total of 1010 children were screened out of which 115 children were identified with non-surgical wounds. Sixty four specimens were collected from 63 of the children with wounds showing clinical signs of infection. The age range of the children was 3-15 years with a median age of 10.40 $\pm$ 3.11. There were 44 males and 19 females giving a male:female ratio (M: F ratio) of 2.3:1.

The lower limbs were most frequently affected accounting for 78.0% (50/64) of the wounds with clinical signs of infection while 19.0% (12/64) were located on the upper limbs. None were observed on the trunk and perineum.

Out of the 64 specimens collected, 46.9% (30/64) yielded a single organism, and another 46.9% (30/64) yielded 2 organisms, while 1.6% (1/64) yielded 3 isolates. No microbial growth occurred in 4.7% (3/64) of the specimens (Table 1).

**Table 1:** Combination of isolated pathogens per swab culture (n= 64\*)

Pathogen	Frequency	%
<i>S. aureus</i>	27	42.1
<i>S. aureus, S. pyogenes</i>	27	42.1
<i>S. aureus, P. mirabilis</i>	1	1.6
<i>S. aureus, K. ozaenae</i>	1	1.6
<i>S. aureus, S. pyogenes, K. oxytoca</i>	1	1.6
<i>S. pyogenes, E. coli</i>	1	1.6
<i>S. pyogenes</i>	2	3.1
<i>P. aeruginosa</i>	1	1.6

\*There were no growth of micro-organisms in 3 swabs

**Table 2:** Antibigram of Gram negative organisms isolated from non - surgical wounds

Organism	Ceftriazone	Cefuroxime	Gentamicin	Amikacin	S <sup>n</sup>	S <sup>n</sup>	amox-clav	Meropenem	Cefepime	Ampicillin	Colistin	Cefoxitin	Imipenem
<i>P. aeruginosa</i>	NT	NT	R	S	S <sup>n</sup>	S <sup>n</sup>	NT	S	R	NT	S	NT	S
<i>K. ozaenae</i>	S	R	S	S	S	I	R	S	S	S	NT	R	S
<i>P. mirabilis</i>	S	R	S	S	S	I	R	S	R	S	NT	S	S
<i>E. coli</i>	S	R	S	S	S	S	S	I	S	R	NT	I	S
<i>K. oxytoca</i>	S	R	S	S	S	S	R	S	S	R	NT	R	S

Key

S= Susceptible

I= Intermediate

R= Resistant

NT= Not tested

Amox-clav= Amoxicillin Clavulanate

A total of 92 organisms were isolated belonging to 7 different species. *S aureus* and *S pyogenes* were the most predominant organisms accounting for 62.0% (57/92) and 32.6% (30/92) of the isolates respectively. *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella ozaenae* were isolated in 1.1% (1/92) of the isolates each. No fungi was isolated from any of the wounds.

Among the *S. aureus* isolates 66.7% (38/57) were MRSAs (Figure 1). All *S.aureus* isolates were susceptible to Imipenem and Tigacycline. A high rate of susceptibility of 96.5% (55/57) was also observed for Amikacin, Gentamicin, and Levofloxacin while 93% (53/57) were susceptible to Ciprofloxacin. A high rate of resistance to the Penicillins was observed. All *S pyogenes* isolates were susceptible to levofloxacin (Figure 2) while 96.7% (29/30) were susceptible to Cefepime and Ceftriazone. These isolates were least susceptible to Erythromycin and Penicillin with 60.0% (18/30) and 86.7% (26/30) susceptible to this antimicrobial agents. All Gram negative isolates were susceptible to Imipenem, Levofloxacin and Amikacin. However, all but the *E. coli* isolate were resistant to Amoxicillin-clavulanate (Table 2).

The antibiotic susceptibility pattern of *S. aureus* revealed that almost all isolated strains of the organism were resistant to Penicillin (n= 50/57, 87.7%) and Ampicillin (n= 52/57, 91.2%). The pathogen, however, was highly susceptible to Gentamicin (n= 55/57, 96.5%), Amikacin (n= 55/57, 96.5%), Cefprofloxacin (n= 53/57, 93.0%), levofloxacin (n= 55/57, 96.5%) and above all, Imipenem (n= 57/57, 100%) (Figure 1). Thirty eight (66.7%) of the 57 *S. aureus* strains were MRSA while 19 (33.3%) of them were Methicillin Susceptible *S. Aureus* (MSSA). The MRSA was susceptible to tigacycline (Figure 3) but ceftioxin resistant.

On the other hand Figure 2 shows that *S. pyogenes* was far more susceptible to ceftriazone (n = 29/30, 96.7%), cefepime (n= 29/30, 96.7%) and most especially levofloxacin (n= 30/30, 100%) than to penicillin (n= 26/30, 86.7%), erythromycin (n = 18/30, 60%) and clindamycin (n= 27/30, 90.0%).

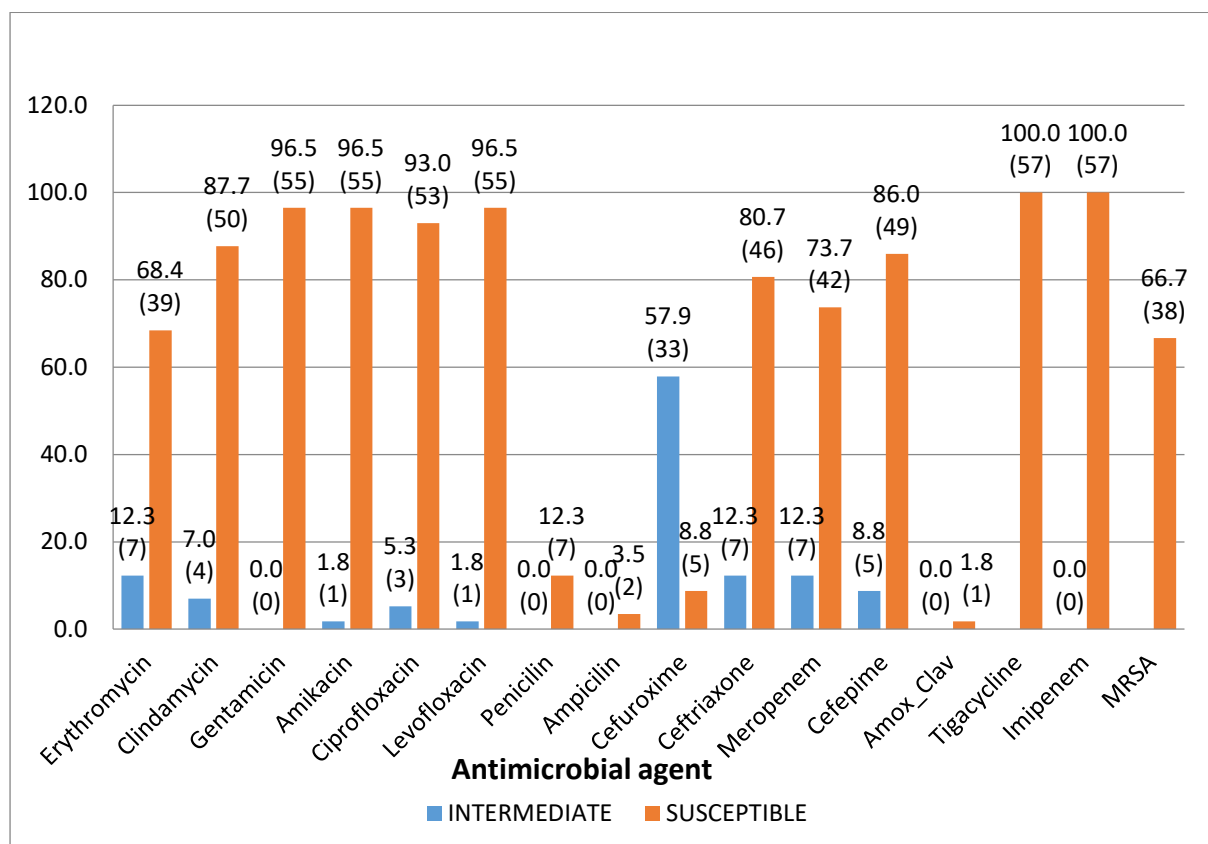
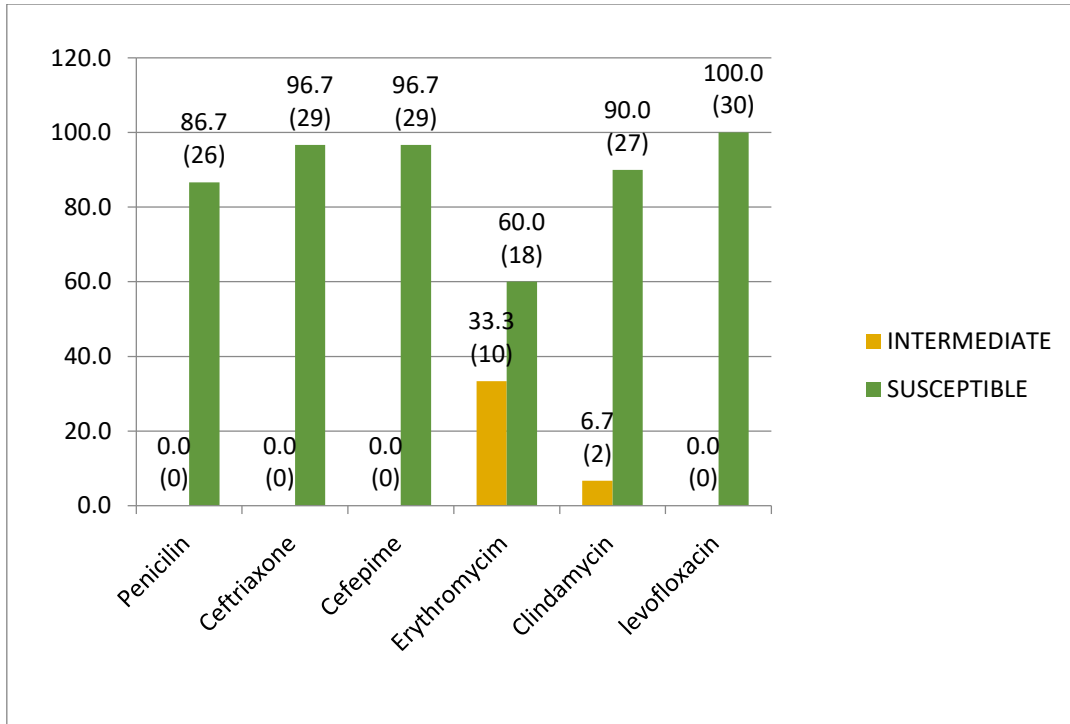
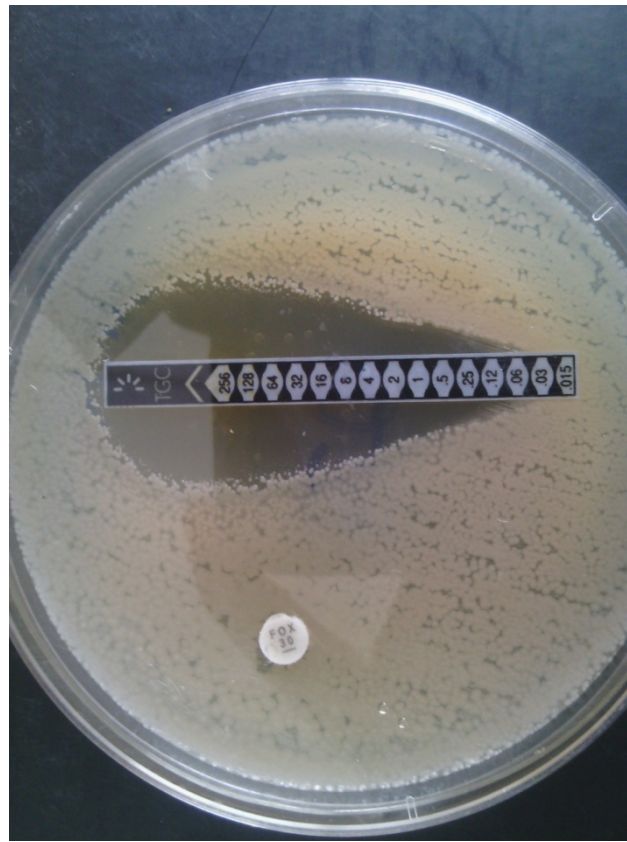


Figure 1: Susceptibility profile of *S aureus* isolates from non-surgical wounds (n=57)



**Figure 2:** Susceptibility profile of *S. pyogenes* isolated from non - surgical wounds (n=30)



**Figure 3:** Tigacycline susceptible Methicillin Resistant *S. aureus* (Cefoxitin resistant) isolate

## 5. Discussion

This study investigated the microbial spectrum and antibiogram of non-surgical wound infections in children in a rural setting in our environment. Despite the numerous studies on microbiology and susceptibility of surgical wound infections world-wide [18, 19] only few studies on the non - surgical type are available [8, 9]. These studies posited that there is variation in the pattern and antibiogram of the bacterial isolates of the non-surgical variety and more so in children [13, 14]. This dearth of data and the reported variability in the spectra and sensitivity patterns informed the need for this present study.

The results of the study showed that non-surgical wound infections in children were commoner in males than females similar to the observation in other studies [13, 14]. In addition, the finding that majority of the wounds were located in the lower limbs was in keeping with the predominant male population which was often more actively involved in physical contact activities with their attendant high risk of injury..

The pattern of microbial yield whether pure mono-microbial or poly microbial was similar to findings in earlier series [13, 19]. The high poly-microbial yield in this series may be accounted for by the duration of time that the wounds may have been present on the children. It is an established fact that initially, wounds have predominantly Gram positive organisms, but over time, wounds of long duration, particularly when deeper structures are involved, tend to have several different pathogenic species within the wound bed, even including anaerobic flora [20, 21]. However, anaerobic culture could not be done due to the challenges of biopsy collection as swabs are not recommended for anaerobic culture.

The finding that *S. aureus* was the predominant pathogen isolated from the wound cultures was consistent with reports of similar studies within and outside the country [12 -14, 19]. But contrary to the findings from series reporting microbiology of non-surgical wounds, our study found *S. Pyogenes* as the second most frequently isolated pathogen from the wound cultures as against *Pseudomonas aeruginosa* and *Escherichia coli* reported by Okesola and his colleagues [13] and Noroozi and his colleagues [14] respectively. The predominantly children population in our study may have accounted for this observation, since *S. pyogenes* may have been spread by respiratory secretions and formites [22]. The incidence of both respiratory and skin *S. pyogenes* infections is said to peak in childhood [22]. Asymptomatic carriage may therefore account for the wound infections. These *S. pyogenes* infections bear a potential for acute glomerulonephritis and rheumatic heart disease in these rural children [23].

As demonstrated by this study, *S aureus* showed substantial susceptibility to gentamicin, a readily available and affordable drug similar to the findings in the Owerri series [24] but contrary to the observation by Iregbu and his colleagues [19]. Gentamicin being an injectable drug was less likely to be abused, hence the use of the antimicrobial for empirical therapy and surgical prophylaxis [5]. The susceptibility of *S. aureus* to ciprofloxacin, levofloxacin and imipenem was also remarkably high compared to other antibiotics in line with the finding by Sani and his colleagues [26]. With the uncontrolled use of antibiotics, these antimicrobials are also endangered, as it takes an average of 7-10 years for microorganisms to develop resistance to a new drug [27].



On the other hand, the resistance of *S. aureus* to Penicillin, Ampicillin and Cefuroxime as found in this study agreed with those of others [24, 26]. As observed in this study, the high frequency of isolation of MRSA compared with that of other studies [28]. It was reported that community associated MRSA infections were on the increase [29, 30]. These community related MRSA infections despite being more susceptible to antibiotics than hospital acquired strains, are more virulent and invasive due to the possession of the Panton-Valentine-Leucocidin (PVL) gene [31]. A more expanded study will however be required to ascertain the true prevalence of community associated MRSA in our setting.

The findings of the study showed that *S. pyogenes* was generally more susceptible to Ceftriazone, Cefepime and Levofloxacin than to Penicillin, Erythromycin and Clindamycin, similar to other studies [24, 26]. Universally, *S. pyogenes* was known to be Penicillin sensitive, while macrolides such as Erythromycin were commonly employed as first line drugs against *S. pyogenes* in patients with B-lactam allergies [32]. The resistance of *S. pyogenes* to erythromycin found in this study was similar to the observation by Carlo and his colleagues [33].

## **6. Conclusion**

The microbial spectrum and antibiogram of non - surgical wound infections in children differed from that of the surgical variety and more so in rural setting. There is a high rate of *S. pyogenes* which are beta-haemolytic streptococcus group A (BHSA) commonly associated with rheumatic heart disease and acute glomerulonephritis. High rates of antibiotic resistance to commonly used first line drugs were observed with a high rate of community acquired MRSA which may complicate the management of these wounds.

## **7. Recommendation**

We recommend that microbial culture and sensitivity should guide wound treatment due to the diverse nature of the potential isolates. Appropriate and prompt treatment of wounds in children will eliminate infection by *S. pyogenes* and reduce the risk of rheumatic heart disease and acute glomerulonephritis.

## **8. Limitations**

Only indigenous Bakassi children resident in the camp were examined. Those living elsewhere as well as non-indigenous ones in the community were not covered. One very major limitation of the study was funds.

## **9. Consent**

Written informed consent was obtained from the patients' legal guardians for publication of this manuscript.

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survey had been concluded

### Competing interests

The authors declare that they have no competing interests.

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