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Methicillin-Resistant Staphylococcus Aureus in Melanesian Children with Haematogenous Osteomyelitis from the Central Highlands of Papua New Guinea

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Abstract

Background: Methicillin-Resistant Staphylococcus aureus (MRSA) has been an important cause of bone infection since the 1940s. Current guidelines recommend targeted antibiotic use for osteomyelitis treatment informed by microbial sensitivity patterns. However, in settings without microbiology facilities, empirical antibiotic use is common. Unrecognized antibiotic resistance potentiates persistence of MRSA with osteomyelitis progression to chronic forms with complications despite antibiotic treatment.

Materials and Methods: A prospective observational study was done to identify common etiological agent (s) in bone infection in Melanesian children (that were admitted to the two surgical and one pediatric wards of the SJNM-KUGH in the Simbu province of Papua New Guinea in 2012 and 2017), observe for presence of antimicrobial resistance, and determine effective antibiotic regimes for treatment of bone pediatric osteomyelitis. Seventy pediatric patients presenting from the community with osteomyelitis were recruited, with bone and non-bone specimens sampled, cultured and isolates tested for resistance to common antibiotics.

Results: Staphylococcus aureus (*S. aureus*) was isolated in 67% (47/70) of collected specimens. Of the 47 isolates, there was 91.5% resistance to penicillin, 85.1% resistance to methicillin, 89.4% resistance to oxacillin, 93.6% resistance to ampicillin and 80.9% resistance to ceftriaxone. *S. aureus* showed 91.5% sensitivity to gentamycin, 93.6% sensitivity to erythromycin, tetracycline and clindamycin, and 95.7% sensitivity to Co-trimoxazole.

Conclusion: MRSA was the leading cause of haematogenous osteomyelitis in Melanesian children. *S.aureus* was isolated mainly from infected long bones of the lower limbs (79%) of children presenting from the community, suggesting a predominantly community-associated MRSA.

Key Words: Children, MRSA, Osteomyelitis, Papua New Guinea.

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1- INTRODUCTION

Staphylococcal resistance to penicillin has been recognized in the mid-1940s soon after its discovery and introduction into treatment regimens (1). Emergence of methicillin-resistant *Staphylococcus aureus* has since been reported over the years, initially in health care facilities and later increasingly in communities (1-8) in the Americas, Europe, Asia and the Pacific (1-6, 9-11). Current treatment guidelines therefore recommend a targeted antibiotic use (oral or parenteral) for bone and soft tissue infections informed by microbial culture and antimicrobial sensitivity pattern of isolates (12-15), with Ciprofloxacin and clindamycin as drugs of choice for the first line treatment of suspected Methicillin-resistant *S. aureus* (MRSA) osteomyelitis over traditional methicillin-based regimes (12).

However, in resource-constrained settings without the capacity to culture and determine microbial sensitivity pattern of isolates, empirical antibiotic use is common with doctors relying on local treatment guidelines and clinical experience to guide antibiotic choice (16-18). In such settings, methicillin (cloxacillin, flucloxacillin), and chloramphenicol are commonly recommended and used widely for a generic treatment of pediatric osteomyelitis, septic arthritis and pyomyositis (19-22).

Unrecognized antibiotic resistance allows for persistence and hypervirulent of *Staphylococcus aureus* or MRSA with progression of osteomyelitis to chronic forms with disabling complications including growth plate arrest, shortening or angular deformity of the limb, pathological fracture, avascular necrosis and septicemia despite antibiotic treatment (23-25). At the Sir Joseph Nombri Memorial-Kundiawa General Hospital in the Central Highlands of Papua New Guinea, a high incidence and prevalence of osteomyelitis with

significant Disability-adjusted-life-years lost (DALYs) lost to disease complications was observed in children treated routinely with flucloxacillin and chloramphenicol between 2009 and 2015 (26). This study was therefore done to: 1) identify the common etiological agent (s) in bone and associated joint or soft tissue infections in Melanesian children, 2) observe for presence of antimicrobial resistance by isolate (s) to commonly used antibiotics for osteomyelitis treatment, and 3) determine an effective, local and age-specific antibiotic regime for treatment of osteomyelitis in children in the region.

2- MATERIALS AND METHODS

2-1. Method

A prospective observational study was conducted in 2012 and 2017 on 70 pediatric patients admitted to the two surgical and one pediatric wards of the SJNM-KUGH in the Simbu province of Papua New Guinea. Study subjects were Melanesian children of both sexes ≤ 13 years of age who presented from the community with a clinical and/or radiological diagnosis of osteomyelitis with no history of a preceding deep-skin or soft tissue infection, trauma or surgery. The study included cases presenting directly from the community with no preceding antibiotic treatment and those in the ward for two days or less on antibiotic treatment; and with a localized pus collection, discharging sinus or associated septic arthritis. A clinical diagnosis of osteomyelitis was made in a child presenting with a painful, swollen limb with limited mobility, fever (temperature $\geq 37.5^{\circ}\text{C}$), and local tenderness with warmth or redness (22, 27-29). A radiological diagnosis, usually in subacute to chronic disease, was made by the presence of periosteal elevation or thickening, osteolytic changes (sequestration) or pathological fractures (22, 28). All surgical registrars and consultants in the two

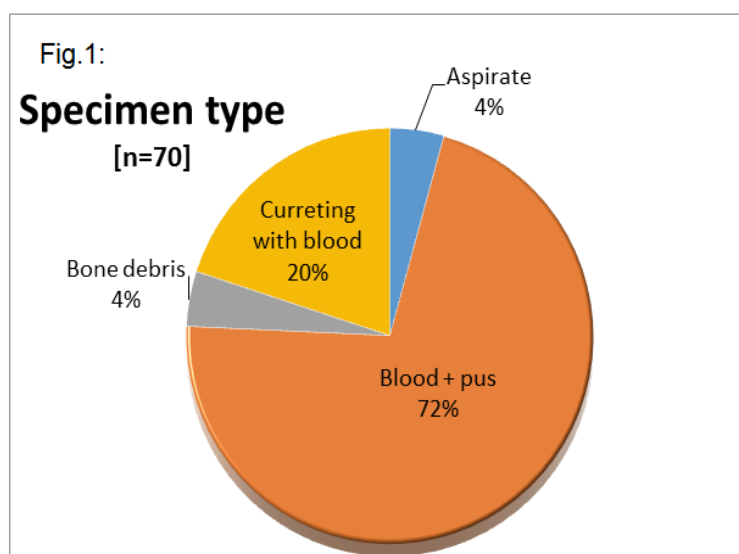
surgical and one pediatric wards of the hospital participated in identifying study subjects while two designated surgical registrars enrolled them and collected samples from infection sites for microbial culture and antimicrobial-sensitivity analysis. Pus and blood from subperiosteal, intracortical, intramedullary or surrounding soft tissue collections, bone curetting, and associated-joint aspirates were sampled for analyses during therapeutic surgical procedures including incision and drainage, bone curettage, sequestrectomy and bone drilling. Pus swabs or blood collected from infection sites were stored and transported in Aimes transport media or culture bottles, respectively. Specimen collected were packed into a cool box and transported by road to the PNGIMR microbiology laboratory 200 kilometers (2 hours' drive) from the hospital. If not delivered on the same day as collection, specimen in Aimes transport media were stored at 2 to 8 °C in refrigerator whilst those in culture bottles were stored in room temperature (37°C). On arrival, specimen were streaked onto blood agar, chocolate agar and

MacConkey agar plates for incubation. After 24 hours of aerobic incubation at 37°C, any growth colonies present were identified using standard bacteriological methods. All isolates were initially identified by Gram stain, catalase and oxidase. *Staphylococcus aureus* was confirmed by DNase and coagulase tests. Enterobacteriaceae (*Proteus* spp., *Escherichia coli* and *Klebsiella* spp.) and *Pseudomonas* were confirmed by colony morphology, lactose fermentation, motility, IMViC test, triple sugar iron agar reaction, and urease production. Clinically significant isolates were tested for antimicrobial susceptibility using disk diffusion methods according to CLSI (2012a) guidelines. Results were entered into an excel spread sheet and analyzed using tables and graphs.

2-2. Ethics Approval

Ethics consideration and approval was granted by the Papua New Guinea Institute of Medical Research (PNGIMR) for this study.

3- RESULT



	Number
Aspirate	3
Blood + pus	50
Bone debris	3
Curretting with blood	14
Total	70

Table-1 and **Fig.1** showing types of specimen sampled from infected bones.

Table-2: Sampling Site

	Number
Left hip	3
Left femur	9
Left knee	2
Left tibia/fibula	19
Left ankle	3
Left elbow	1
Right hip	3
Right femur	12
Right knee	2
Right tibia/fibula	15
Right arm	1
Total	70

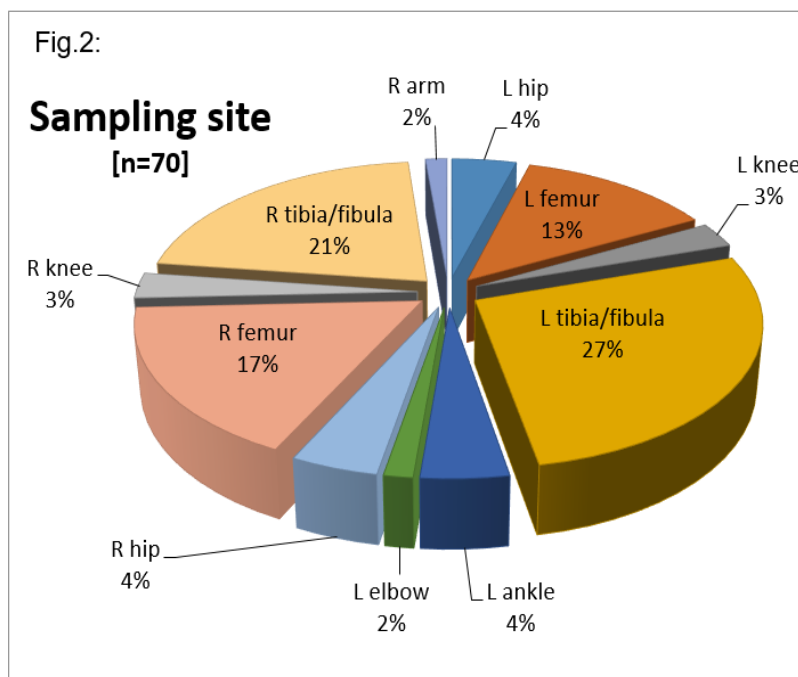


Table-2 and **Fig.2** show bones and joints that were collected from specimens.

Table-3: Culture Isolate

	Number
Escherichia coli	2
Proteus sp.	3
Pseudomonas aureginosa	1
Streptococcus pyogenes	3
Staphylococcus aureus	47
Klebsiella sp.	1
No growth	13
Total	70

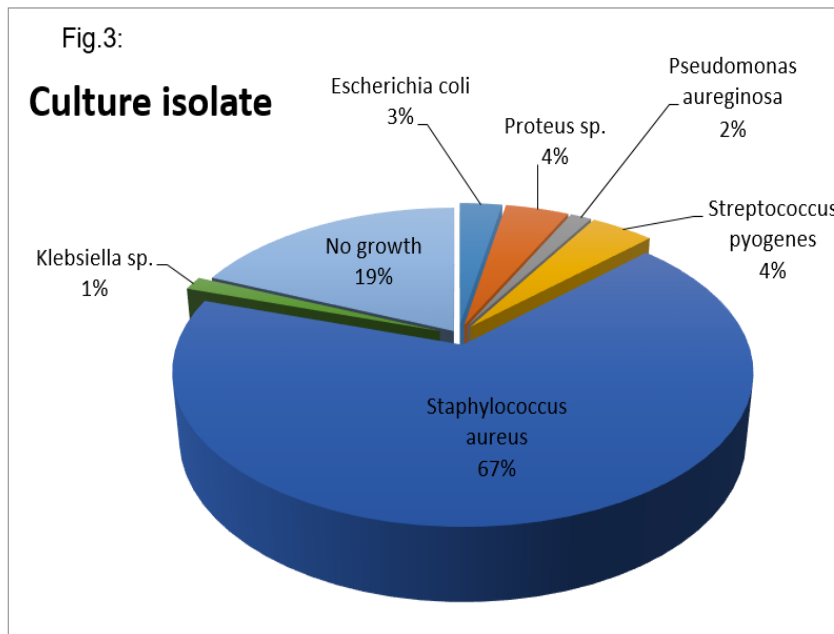


Table-3 and **Fig.3** show bacterial isolates from specimen cultures.

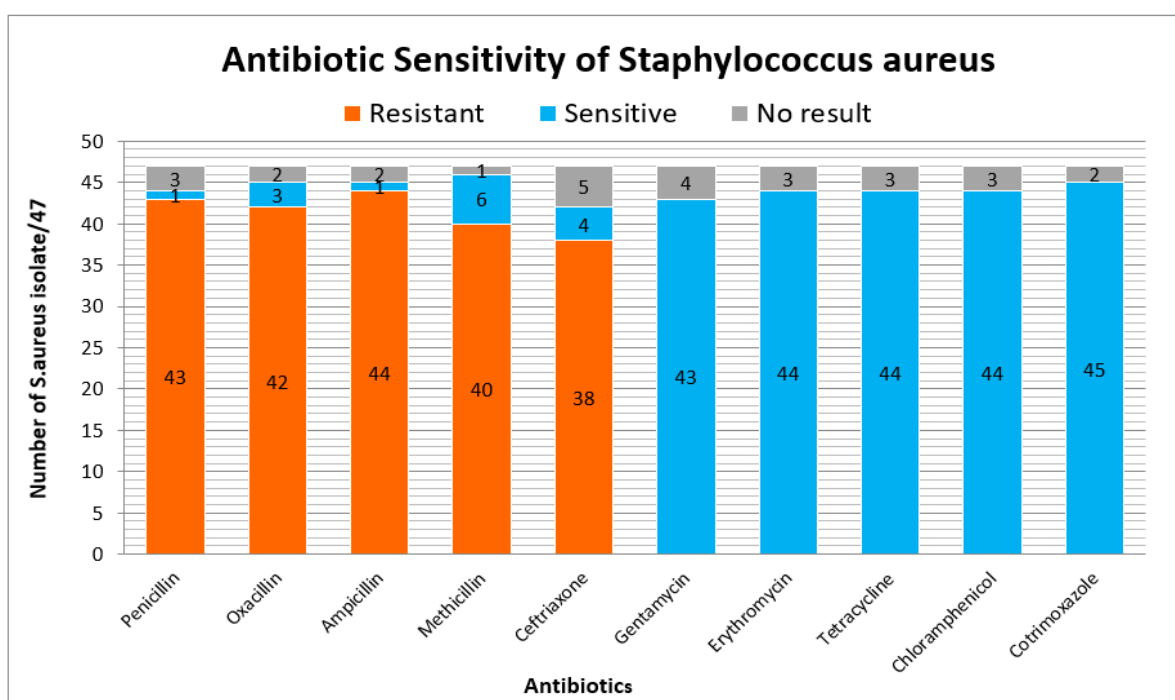


Fig.4: Compound graph shows antibiotic sensitivity of *S. aureus* isolated from collected specimen.

- 72% (50/70) of collected specimen were pus and blood from infected bones and soft tissue or joint, while 20% (14/70) were curetting with blood from infected bone.
- 49% (34/70) of specimen were collected from infected long bones of the leg (tibia/fibula) while 30% (21/70) were from the right and left femurs, totaling to 79% (55/70) of sampled specimens being from long bones of the lower limbs.
- Coagulase-positive *Staphylococcus aureus* was isolated in 67% (47/70) of the collected specimens with no organism grown in 19% (13/70) of specimens.
- Of the 47 *Staphylococcus aureus* isolates, 91.5% (43/47) were resistant to penicillin, 85.1% (40/47) were resistant to methicillin, 89.4% (42/47) were resistant to oxacillin, 93.6% (44/47) were resistant to ampicillin and 80.9% (38/47) were resistant to ceftriaxone.
- Staphylococcus aureus* isolates showed 91.5% (43/47) sensitivity to gentamycin, 93.6% (44/47) sensitivity to erythromycin, tetracycline and chloramphenicol, and 95.7% (45/47) sensitivity to co-trimoxazole.
- Sensitivity to ciprofloxacin was not tested for.

4- DISCUSSION

The most common isolate from infected bone, joint and soft tissue specimens in this study was coagulase-positive and methicillin-resistant *Staphylococcus aureus* (67%). This observed etiological predominance is consistent with results from similar culture studies on pediatric and adult osteomyelitis throughout the world (12, 30, 31). MRSA was isolated mainly from pus and blood sampled from subperiosteal, intracortical, intramedullary or surrounding soft tissue collections mostly along long bones of the lower limbs. The high vascularity of growing long bones in children is suggested to predispose them to hematogenous bacterial exposure and infection (32-34). Predominant metaphyseal infection of the long bones is consistent with proposed mechanisms of bone infection including metaphyseal sluggish blood flow, paucity of immune cells and adherence potential of *Staphylococcus aureus* to metaphyseal cartilage, with either a subperiosteal, medullary, or articular

progression and suppuration (23). Non-bone specimen like pus and blood may be less sensitive in ascertaining etiology of osteomyelitis than bone culture as indicated by a recent study that showed a false positive rate of 36% for non-bone culture and a lower concordance (38%) between bone and non-bone culture for staphylococcus aureus (35). However, the need for infection control measures to limit extension of superficial infections to the bone by contiguity, break interpersonal transmission chains and minimize emergence potential for MRSA is of far greater clinical importance (8, 23, 36). Hypervirulent MRSA remains an important cause of severe forms of infections including pediatric osteomyelitis in developing countries (8).

There was 91.5% penicillin resistance and 81.5% methicillin resistance by the *Staphylococcus aureus* isolates. Most of the study subjects presented directly from the community with no prior antibiotic treatment in the preceding days, indicating a largely community-associated MRSA (37). Whole genome sequencing with relevant spatiotemporal analyses would be necessary to provide information on the molecular epidemiology of the successful clone (s) of MRSA strains (38, 39), which can then be correlated with clinical epidemiological data to explore provenance of MRSA as either community or hospital associated, and thereby inform infection control interventions and therapeutic efforts (8, 36).

The potential community-associated MRSA observed in this study may be attributable to imprudent drug distribution and use by unsanctioned drug providers, suboptimal self-medication, and unguided overuse, underuse or misuse of antimicrobial agents by rural clinicians without adequate diagnostic support to inform pathogen susceptibility patterns (16, 40). Limited availability and affordability of drugs with an increasing

use of counterfeit, substandard or expired drugs in developing countries also contributes to emergence potential of antimicrobial resistance (41-44). *Staphylococcus aureus* in this study also demonstrated a high level of resistance to ceftriaxone besides penicillins, which is an important third-generation cephalosporin commonly used together with penicillins for empirical treatment of pediatric osteomyelitis and other severe gram-positive infections in resource-constrained settings. This suggests possible presence of a multidrug resistant MRSA, genotypically related or unrelated to previously recognized strains in the community or hospital (38, 39, 45), and suboptimal efficacy of local penicillin-based regimes employed routinely for treatment of pediatric osteomyelitis in low-resource settings without the capacity to test and determine microbial susceptibility patterns. *Staphylococcus aureus* was sensitive to Co-trimoxazole, erythromycin, gentamycin and chloramphenicol which can alternately be used in safe and affordable regimes, together with surgery, for effective treatment of pediatric osteomyelitis in Melanesian children (15, 46).

Given the general etiological similarity between the pediatric and adult populations, similar antibiotic regimes can also be used for treatment of staphylococcal osteomyelitis in Melanesian adults as well (47). Changing environmental conditions during storage and transport of specimens may have potentially affected organism survival and in turn culture results as suggested by a 19% no-growth result. This could also be due to sterile specimen sampled from patients already on an alternate effective antibiotic treatment for 24 to 48 hours.

4-1. Limitations of the study

The microbial culture and sensitivity-testing facility being about 200km from the hospital posed a logistical challenge for optimal specimen storage and transport by

road between the hospital and testing facility, which inevitably affected the number of samples suitable for testing.

4-2. Recommendation

- Safe and affordable antibiotic regimes based on isolate sensitivity patterns should be used together with surgery for effective treatment of osteomyelitis in Melanesian children, as well as adults. Erythromycin, co-trimoxazole, gentamycin, tetracycline and chloramphenicol can be used in regimes deemed appropriate for empirical treatment of pediatric osteomyelitis in settings without microbial culture and antimicrobial-sensitivity testing facilities.
- The need for microbial culture and antibiotic sensitivity-testing facilities in provincial hospitals or regional health facilities to inform antibiotic use in developing countries like Papua New Guinea cannot be over emphasized.
- Appropriate policy and funding by governments and relevant health authorities in the region is necessary to build diagnostic capacity towards informing antibiotic use and effective treatment of osteomyelitis in order to reduce the high burden of disease in the region.
- Whole genome sequencing and spatio-temporal analyses of MRSA isolates is necessary for information on provenance and epidemiological patterns of MRSA to inform infection control interventions and therapeutic responses in the community and hospital.

5- CONCLUSION

This study demonstrated coagulase-positive methicillin-resistant staphylococcus aureus to be the leading cause (67%) of hematogenous osteomyelitis in Melanesian children. Staphylococcus aureus was isolated mainly from pus and blood (72%) collected from

infected long bones of the lower limbs (79%). Children in the study presented largely from the community, indicating a predominantly community-associated MRSA. Of the 47 staphylococcus aureus isolates, 91.5% were penicillin resistant, 85.1% methicillin resistant, 89.4% oxacillin resistant, 93.6% ampicillin resistant and 80.9% ceftriaxone resistant, indicating presence of a potential multidrug resistant MRSA strain. The staphylococcal isolates demonstrated more than 91% sensitivity to chloramphenicol, tetracycline, co-trimoxazole, gentamycin and erythromycin, which could alternately be used in antibiotic regimes, together with surgery, for effective treatment of pediatric osteomyelitis to reduce the high burden of disease in the region.

6- CONTRIBUTORS STATEMENT

I. Aglua

Study design, data collection coordination, and analysis, synthesis of results, write-up, editing and submission.

J. Jaworski and B. Urakoko

Study design, Specimen collection, surgical procedural oversight, surgical diagnosis and therapeutic procedure descriptions and editing, literature review.

J. Drecore

Initiation of study, editorial, literature review, referencing, language and grammatical editing. Logistical support for samples storage and transfer to testing facility.

H. Poka

Paediatric care oversight, paediatric definitions and clinical diagnosis and disease classification, editing.

A. Michael

Microbiology support: specimen culture, antimicrobial sensitivity testing and reporting of results, editing of microbiology section.

A. Greenhill

Microbiology oversight, editing section on microbiology and overall paper editing.

7- FUNDING

This study was funded by the Simbu Children's Foundation (SCF) through its child health advocacy program in an attempt to identify the common disease-causing agent (s), understand antibiotic sensitivity of disease-causing agents and effectively treat osteomyelitis amongst children to reduce the high disease burden in the region.

8- ABBREVIATIONS

CLSI: Clinical Laboratory Standards Institute.

DALY: Disease-Adjusted Life Year.

MRSA: Methicillin-Resistant Staphylococcus Aureus.

PNGIMR: Papua New Guinea Institute of Medical Research.

S. aureus: Staphylococcus aureus.

SCF: Simbu Children's Foundation.

SJNM-KUGH: Sir Joseph Nombri Memorial-Kundiawa General Hospital.

9- CONFLICT OF INTEREST

No financial or nonfinancial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

10- ACKNOWLEDGMENT

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3. Dr D. Hasola and Dr C. Munguas for participating in the initiation of the study and data collection.

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