Methicillin resistant *Staphylococcus aureus* causing osteomyelitis in a tertiary hospital, Mwanza, Tanzania

Vitus Silago¹, Martha F. Mushi¹*, Boniface A. Remi¹, Alute Mwayi¹, Stephen Swetala², Conjester I. Mtemisika³ and Stephen E. Mshana¹

**Abstract**

**Background:** Culture results of fluid/pus from sinuses or open wound are not reliable in establishing the causative agent of osteomyelitis due to the high chances of contamination of superficial contaminants. Bone fragments obtained during surgery have been recommended as ideal sample to establish pathogens causing osteomyelitis. This study investigated pathogens causing osteomyelitis among patients undergoing orthopedic surgical treatment at Bugando Medical Centre.

**Methods:** A cross-sectional hospital-based study was conducted from December 2017 to July 2018 among 74 patients with osteomyelitis who underwent surgical treatments at Bugando Medical Centre, Mwanza, Tanzania. Bone fragments were collected using sterile 10 ml of in-house prepared brain heart infusion broth (Oxoid, UK) during surgery. Specimens were processed according to standard operating procedures within an hour of collection. Data were analyzed using STATA 13.0.

**Results:** The median age of study participants was 12 with inter quartile range of 8–20 years. The majority 45 (60.8%) of participants were male. All 74 non-repetitive bone fragment specimens had positive culture, of which 17 had dual growth of bacteria resulting to 91 bacterial isolates. Out of 91 isolates, 63 (85.1%) were *Staphylococcus aureus* (S. aureus) of which 18 (28.6%) were confirmed to be methicillin resistant *Staphylococcus aureus* strains. Fever was significantly associated with Staphylococcal osteomyelitis (100% vs. 79.6%, \( p = 0.029 \)).

**Conclusion:** About one third of cases of Staphylococcal osteomyelitis in the current study were caused by methicillin resistant *Staphylococcus aureus*. There is a need of tailoring antibiotic management of osteomyelitis based on culture and sensitivity results for the better treatment outcome of the patients.

**Keywords:** Staphylococcal osteomyelitis, Methicillin resistant *S. aureus*, Bugando Medical Centre, Mwanza, Tanzania
**Introduction**

Osteomyelitis is an inflammation of the bone that can be localized to only one part of the bone such as bone marrow, periosteum, or cortex [1]. In few occasions, osteomyelitis can disseminate to the surrounding tissues [1]. Osteomyelitis can be classified by the location within the bone, extent of dissemination and source of infection [1]. Bacteria are the commonest cause of osteomyelitis with *S. aureus* implicated in more than 75% of cases. *S. aureus* spreads to the bones through blood (hematogenous osteomyelitis) or directly as a result of open fractures [2, 3].

Staphylococcal osteomyelitis is a major global health concern because of the increasing antimicrobial resistance. Treatment of osteomyelitis is always complex because it requires a well-coordinated team of radiologist, orthopedic surgeon, and infectious diseases specialist [4]. Culture of fluid/pus from sinuses or discharging ulcers is not reliable, thus leading to the dependence of imaging and high suspicion of a surgeon in managing osteomyelitis patients. To determine the causative pathogen implicating in osteomyelitis, a sample of choice is bone fragments because culture of fluid/pus from sinuses or open is always associated with superficial bacteria contaminants [4, 5]. This study investigated the pathogens causing osteomyelitis among patients undergoing surgical treatment at Bugando Medical Centre, Mwanza-Tanzania for the purpose of generating data that can be used to devise antibiotic treatment guidelines.

**Methodology**

**Study design, participants, duration, setting and specimen collection**

It was a cross sectional hospital based study, conducted from December 2017 to July 2018 among 74 patients with osteomyelitis admitted for surgery at Bugando Medical Centre (BMC), Mwanza, Tanzania. BMC is a tertiary referral hospital with 1000 bed capacity, serving seven regions namely: Mwanza, Musoma, Simiyu, Shinyanga, Kagera, Kigoma, and Geita. The study included all patients with clinical diagnosis of osteomyelitis planned for orthopedic surgery. As part of routine clinical care at enrollment, body temperature was measured using a digital thermometer MDD 93/42/EEC, “0197” (Holding Corp. GmbH Hamburg) and all patients with body temperature above 37.5 °C were termed as febrile [6]. During surgical procedures, bone fragments were collected and placed into sterile universal bottle containing 10 ml of in-house brain heart infusion (BHI) broth (Oxoid, UK) [7] to increase the yield of pathogenic bacteria. Specimens were sent to Microbiology laboratory of the Catholic University of Health and Allied Sciences within 1 h of collection.

**Laboratory procedures**

In the laboratory, universal bottles with samples were gently shaken 10 times, and then incubated at 37 °C for 6 h [7]. After 6 h of incubation, specimens were mixed gently and a loop full (10 μl) was inoculated on sheep blood agar and MacConkey Agar followed by aerobic incubation at 37°C for 24 h. Conventional biochemical identification tests: Gram stain, catalase and coagulase, DNase were used to identify Gram-positive bacteria while Triple sugar iron agar (TSI), Sulfur-Indole-Motility (SIM), Simmons citrate, urease, and oxidase were used to identify Gram-negative bacteria [8]. Antibiotics susceptibility testing was performed using Kirby-Bauer disc diffusion technique. Tetracycline 30 μg, gentamicin 10 μg, ciprofloxacin 5 μg, clindamycin 2 μg, and vancomycin 30 μg were used for Gram-positive bacteria while ampicillin 10 μg, trimethoprim-sulfamethoxazole 1.25/23.75 μg, tetracycline 30 μg, gentamicin 10 μg, ciprofloxacin 5 μg, amoxycillin-clavulanic acid 20/10 μg, ceftriaxone 30 μg, ceftazidine 30 μg, piperacillin-tazobactam 100/10 μg, and meropenem 10 μg were used for Gram-negative bacteria [9]. Cefoxitin 30-μg discs were used for detection of MRSA as per Clinical Laboratory Standard Institute (CLSI) recommendations [10].

Briefly, pure fresh colonies were suspended in sterile 0.85% normal saline to make a suspension equivalent to 0.5 McFarland. Using sterile cotton swab, suspension was inoculated on the entire surface of Muller Hinton agar (MHA) plate; thereafter, antibiotic discs were seeded within 15 min of inoculation. MHA plates were incubated aerobically at 37 °C for 18–24 h. Clinical and Laboratory Standards Institute (CLSI-2016) was used to interpret zones of inhibitions [10].

**Quality control**

*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains.

**Ethical considerations**

Protocol of this study was ethically approved by the joint CUHAS/BMC research ethics and review committee and given certificate number 470/2017. All participants signed informed consent and those aged below 18 years their parents/guardians consented on their behalf. Laboratory results were timely reported to the attending physicians to guide rational antibiotics therapy.

**Results**

**Socio-demographic and clinical characteristics of participants**

A total of 74 osteomyelitis patients with median age of 12 and inter quartile range of 8–20 years were enrolled. Males made the majority 45 (60.8%) of the study participants. The majority 66 (89.2%) of participants had infections of the long bones. A total of 20 (27%) and 32 (43.2%) had fever and history of antibiotic use before the index admission, respectively. The median duration of antibiotic use was 2 weeks with inter quartile range of
2–4 weeks. The most commonly used antibiotic was ampicillin-cloxacillin which was reported in 65.6% (21/35) of patients (Table 1).

**Laboratory results**
All 74 non-repetitive samples yielded growth of pathogenic bacteria on culture. Seventeen samples had dual growth making a total of 91 isolates. The majority of isolates 74 (81.3%) were Gram-positive bacteria. Of 17 patients with dual bacterial growth, 11(64.7%) had *Streptococcus pyogenes* and *S. aureus*, 3(17.6%) had *Proteus vulgaris* and *Klebsiella pneumoniae*, and 3(17.6%) had *Pseudomonas aeruginosa* with either *Klebsiella pneumoniae*, *S. aureus*, or *Citrobacter freundii*. In general, the most frequently isolated bacteria was *S. aureus* 69.2% (63/91) followed by *Streptococcus pyogenes* 12.1% (11/91) and *Klebsiella pneumoniae* 6.6% (6/91) (Fig 1). Methicillin resistant *Staphylococcus aureus* (MRSA) strains were confirmed in 18 (28.6%) of 63 *S. aureus* isolates (Table 2).

**Susceptibility patterns**
Generally, Gram-positive bacteria were 100%, 100%, 98.7%, 100%, and 100% sensitive to gentamicin, ciprofloxacin, clindamycin, tetracycline, and vancomycin, respectively. Out of 63 *S. aureus*, 18 (28.6%) were MRSA with 10 of MRSA strains being isolated from patients with history of antibiotic use.

The proportion of resistance among Gram-negative bacteria was as follows: ampicillin (100%), amoxicillin-clavulanic acid (100%), ceftriaxone (66.7%), ceftazidime (64.7%), trimethoprim-sulfamethoxazole (41.7%), tetracycline (41%), piperacillin-tazobactam (35.5%), gentamicin (23.5%), ciprofloxacin (0%), and meropenem (0%) (Table 2).

**Factors associated with Staphylococcal osteomyelitis**
By chi-square test, only having fever was statistically associated with Staphylococcal osteomyelitis (100% vs. 79.6%, \( p = 0.029 \)) (Table 3). The sub-analysis by age (children vs. adults) found no significance difference terms of pathogens distributions and factors associated with Staphylococcus osteomyelitis (data not shown).

**Discussion**
Dependence on imaging in the management of osteomyelitis in resource limited settings [11] is associated

| Characteristics                              | Frequency (N) | Percentages (%) |
|----------------------------------------------|---------------|-----------------|
| **Median age (IQR) in years**                | 12            | 8–20            |
| Sex                                          |               |                 |
| Males                                        | 45            | 60.8            |
| Females                                      | 29            | 39.2            |
| Education level                              |               |                 |
| None formal education                        | 21            | 28.4            |
| Primary school                               | 32            | 43.2            |
| Secondary school                             | 15            | 40.3            |
| College and above                            | 6             | 8.1             |
| Referrals outside Mwanza                     |               |                 |
| No                                           | 35            | 47.3            |
| Yes                                          | 39            | 52.7            |
| Major complaint                              |               |                 |
| Sepsis                                       | 32            | 43.2            |
| Trauma                                       | 42            | 56.8            |
| Infected bone                                |               |                 |
| Long bone                                    | 66            | 89.2            |
| Short bone                                   | 8             | 10.8            |
| Infected site condition                      |               |                 |
| Discharging                                  | 65            | 87.8            |
| Swelling                                     | 9             | 12.2            |
| Fever during enrollment                      |               |                 |
| Yes                                          | 20            | 27              |
| No                                           | 54            | 73              |
| Pre-exposed to antibiotics                   |               |                 |
| Yes                                          | 32            | 43.2            |
| No                                           | 42            | 56.8            |
| Median duration (IQR) of drug use in weeks   | 2             | 2–4             |
| Type of antibiotic exposed                   |               |                 |
| Ampicillin-cloxacillin (Ampiclox)            | 21            | 65.6            |
| Ceftriaxone                                  | 5             | 15.6            |
| Ampiclox with ceftriaxone                    | 4             | 12.5            |
| Herbal medicine                              | 2             | 6.3             |
with poor prognosis of patients and morbidity such as amputation of infected bone. In the places where there is increased isolation of multidrug resistant bacteria, rational antibiotic therapy is mandatory for proper patients’ management [12]. However, in many resource limited settings, there is no routine culture for the diagnosis of osteomyelitis. Good prognosis of patients with osteomyelitis depends much on proper identification of etiological agent and timely treatment [13].

There is limited data regarding the pathogens causing osteomyelitis from developing countries. The majority of participants in this study were adolescent males, with infection of long bones as a result of trauma. The majority of these infections had purulent drainage from infection sites as previously reported elsewhere [14–16]. The escalating burden of long bones fracture in Tanzania among adolescent males is highly associated with the legalization of motorcycles as a means public transport [17, 18].

Table 2 Antibiotic susceptibility patterns of isolated bacteria

| Antibiotic agents | Potency | Gram positive bacteria | Gram negative bacteria |
|------------------|---------|------------------------|-----------------------|
|                  |         | Sensitive | Resistant | Sensitive | Resistant |
| Ampicillin       | 10 μg   | NA        | NA        | 0%        | 100%      |
| Trimethoprim-sulphamethaxazole | 1.25/23.75 μg | NT        | NT        | 58.3%     | 41.7%     |
| Tetracycline     | 30 μg   | 100%      | 0%        | 58.3%     | 41.7%     |
| Gentamicin       | 10 μg   | 100%      | 0%        | 76.5%     | 23.5%     |
| Ciprofloxacin    | 30 μg   | 100%      | 0%        | 100%      | 0%        |
| Clindamycin      | 10 μg   | 98.6%     | 1.4%      | NA        | NA        |
| Cefoxitin (S. aureus only) | 30 μg | 71.4% | 28.6% | NA | NA |
| Vancomycin       | 30 μg   | 100%      | 0%        | NA        | NA        |
| Ceftriaxone      | 30 μg   | NA        | NA        | 33.3%     | 66.7%     |
| Cefazidime       | 30 μg   | NA        | NA        | 35.3%     | 64.7%     |
| Piperacillin-tazobactam | 100/10 μg | NA        | NA        | 64.7%     | 35.3%     |
| Amoxicillin-clavulanic acid | 20/10 μg | NA        | NA        | 0%        | 100%      |
| Meropenem        | 10 μg   | NA        | NA        | 100%      | 0%        |

NT not tested, NA not applicable
previously reported [18–20], this study has confirmed that purulent discharge from the affected part of the bone is the commonest symptoms of osteomyelitis. The purulent discharge from the affected part of the bone increases the suspicious index of pyogenic infections and call for the need of microbiological investigations [21].

The 69.2% prevalence of the Staphylococcus osteomyelitis in the current study was certainly high. However, these results align with previous reports which reported S. aureus as a major pathogen causing osteomyelitis [22, 23]. It was observed that about one third of S. aureus isolates were MRSA which is almost double to what was observed 10 years ago in the same setting among S. aureus isolates from wounds [24, 25]. These findings cement the previous observations of increasing trend of MRSA in study settings made by Moremi et al. in 2016 [26].

Over the counter use of antibiotics and irrational empirical treatment of bacterial infections in the study settings [27] might result to the selection of resistant Staphylococcus aureus strains such as MRSA. This is further supported by the fact that in the current study the majority of patients with history of antibiotic use were using ampicillin-cloxicillin hence more likelihood of selecting MRSA strains.

The current study has observed that S. aureus isolates were highly susceptible to chloramphenicol, clindamycin, and vancomycin. This could be explained by the fact that these antimicrobial agents are not used in self-medication as penicillin, ampicillin, and amoxycillin. In addition, clindamycin and vancomycin are preserved as the second line treatment regimen [11]. The standard treatment guidelines in Tanzania recommend the use of ampicillin, tetracycline, and/or erythromycin in management of gram positive bacterial infection [11]. Based on these results, clindamycin should be considered as the first line empirical therapy while waiting for culture and sensitivity results, and when gram negative pathogens are suspected Piperacillin tazobactam can be added. These recommendations are supported by a previous systemic review [28].

Only fever was significantly associated with Staphylococcal osteomyelitis ($p = 0.029$). Fever is the commonest response of infection or inflammation whereby the interaction between exogenous pyogenic bacteria and the organum vasculosum of the lamina terminalis (OVLT) induces production of cytokines and increases synthesis of prostaglandin E2 (PGE2) resulting to body temperature rise [29].

**Limitation**
Due to limited diagnostic facilities, the study did not investigate all range of pathogens that may cause osteomyelitis such fungi, *Mycobacteria* spp., and anaerobic pathogens. In addition, data regarding involvement of the joint were not collected.

**Conclusion and recommendation**
This study found high prevalence of Staphylococcal osteomyelitis among patients underwent surgical treatment at Bugando Medical Centre with a third of these patients infected with MRSA. Fever was statistically significant associated with positive Staphylococcal osteomyelitis. There is a need to tailor antibiotic management of osteomyelitis based on culture and sensitivity patterns for the better outcome of the patients.

| Table 3 Factors associated with Staphylococci osteomyelitis |
|-------------------------------------------------------------|
| Characteristics                                             | Staphylococcal osteomyelitis | Chi² | p value |
|                                                             | Positive n (%) | Negative n (%) |     |         |
| Age category                                                |               |                |     |         |
| Children                                                    | 44 (89.8)      | 5 (10.2)       | 0.963 | 0.124   |
| Adult                                                       | 19 (76.0)      | 6 (24.0)       |     |         |
| Sex                                                         |               |                |     |         |
| Females                                                     | 26 (89.7)      | 3 (10.3)       | 0.7699 | 0.380   |
| Males                                                       | 37 (82.2)      | 8 (17.8)       |     |         |
| Referral outside Mwanza                                      |               |                |     |         |
| No                                                          | 30 (85.7)      | 5 (14.3)       | 0.0176 | 0.894   |
| Yes                                                         | 33 (84.6)      | 6 (15.4)       |     |         |
| Education level of participant                              |               |                |     |         |
| Non-formal                                                  | 19 (90.5)      | 2 (9.5)        | 2.5445 | 0.467   |
| Primary level                                               | 28 (87.5)      | 4 (12.5)       |     |         |
| Secondary level                                             | 12 (80.0)      | 3 (20.0)       |     |         |
| College and above                                           | 4 (66.7)       | 2 (33.3)       |     |         |
| Complaint                                                    |               |                |     |         |
| Sepsis                                                      | 28 (87.5)      | 4 (12.5)       | 0.2492 | 0.618   |
| Trauma                                                      | 35 (83.3)      | 7 (16.7)       |     |         |
| Condition                                                    |               |                |     |         |
| Discharging                                                  | 57 (87.7)      | 8 (12.3)       | 2.2615 | 0.097   |
| Swelling                                                     | 6 (66.7)       | 3 (33.3)       |     |         |
| Fever during enrollment                                     |               |                |     |         |
| Yes                                                         | 20 (100.0)     | 0 (0.0)        | 4.7854 | 0.029   |
| No                                                          | 43 (79.6)      | 11 (20.4)      |     |         |
Abbreviations
BHI: Brain heart infusion; BMC: Bugando Medical Centre; CLSI: Clinical Laboratory Standard Institute; MHA: Muller Hinton agar; MRSA: Methicillin resistant Staphylococcus aureus; OVLT: Organum vasculosum of the lamina terminalis; SIA: Sulfur-Indole-Motility; TSI: Triple sugar iron agar

Acknowledgements
Authors acknowledge the department of Microbiology and Immunology, Well Bugando School of Medicine, Catholic University of Health and Allied Sciences, Bugando.

Authors’ contributions
VS, SS, MFM, and SEM designed this study. BAL and SS collected data. VS, BAL, AM, and CIM performed laboratory procedures. VS, CIM, MFM, and SEM analyzed data. MFM, VS, and CIM wrote the first draft of manuscript. SEM critically revised the manuscript. All authors approved the final draft to be published.

Funding
No fund was received for this work.

Availability of data and materials
The data is available upon request and the request should be made to the Director of Research and Innovation, Catholic University of Health and Allied Sciences, Bugando.

Ethics approval and consent to participate
Protocol of this study was ethically approved by the joint CUHAS/BMC research ethics and review committee and given certificate number 470-2017. Only consented participants were enrolled in this study, participants aged below 18 years their mothers’/guardians’ consented on their behalf. Laboratory results were timely reported to attending physicians to guide rational antibiotics therapy.

Consent for publication
None applicable

Competing interests
The authors declare that they have no competing interests.

Author details
1Microbiology and Immunology department, Well Bugando School of Medicine, Catholic University of Health and Allied Sciences, P. O. Box 1464, Mwanza, Tanzania. 2Department of Surgery, Bugando Medical Centre, P. O. Box 1370, Mwanza, Tanzania. 3Central Pathology Laboratory, Bugando Medical Centre, P. O. Box 1370, Mwanza, Tanzania.

Received: 29 October 2019 Accepted: 27 February 2020
Published online: 05 March 2020

References
1. Kavanagh N, Ryan EJ, Wlada A, Sexton G, Fennell J, O’Rourke S, Cahill KC, Kearney CJ, O’Brien FJ, Kerrigan SW. Staphylococcal osteomyelitis: disease progression, treatment challenges, and future directions. Clin Microbiol Rev. 2018;31(2):e00084–17.
2. Del Pozo EG, Collazos J, Carton J, Camporro D, Asensi V. Factors predictive of relapse in adult bacterial osteomyelitis of long bones. BMC Infect Dis. 2018;18(1):635.
3. Carek PJ, Dickerson LM, Sack JJ. Diagnosis and management of osteomyelitis. Am Fam Physician. 2001;63(12):2413–20.
4. Fritz JM, McDonald JR. Osteomyelitis: approach to diagnosis and treatment. Phys Sportsmed. 2008;36(1):50–4.
5. Jacob E, Durham L, Falt M, Williams T, Wheat L. Antibody response to teichoic acid and peptidoglycan in Staphylococcus aureus osteomyelitis. J Clin Microbiol. 1998;32(5):1224–7.
6. Ergonül Ö, Willke A, Azap A, Tekeli E. Revised definition of ‘fever of unknown origin’: limitations and opportunities. J Infect. 2005;50(1):1–5.
7. Senneville E, Melliez H, Beltrand E, Legout L, Valette M, Cazabue M, Cordonnier M, Caillaux M, Yazdanpanah Y, Mouton Y. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis. 2006;42(1):57–62.
8. Winn WC. Korein’s color atlas and textbook of diagnostic microbiology: Lippincott Williams & Wilkins; 2006.
9. Bauer A, Kirby W, Sherris JC, Tuck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493–6.
10. CLSI C. Performance standards for antimicrobial susceptibility testing: Clinical Lab Standards Institute; 2016.
11. Ministry for Health CD, Gender, Elderly and Children. Standard Treatment Guidelines & National Essential Medicines List-Tanzania Mainland. 2017.
12. Allerberger F, Arnamn S, Aspiller P, Brodt H-R, Eckmanns T, Fellhauer M, Geiss H, Janata O, Krause R, Lenmen S. Strategies to enhance rational use of antibiotics in hospitals: a guideline by the German Society for Infectious Diseases. Infection. 2016;44(3):395–439.
13. Zuluaga AF, Galvis W, Jaimes F, Vesga O. Lack of microbiological concordance between bone and non-bone specimens in chronic osteomyelitis: an observational study. BMC Infect Dis. 2002;2(1):8.
14. Gaetano A, Nunes A, Pinheiro C, Tavares J, Bastos R, Cardoso C. Treatment of long-term chronic post-traumatic osteomyelitis of the tibia: two case reports. In: Orthopaedic Proceedings; 2015: The British Editorial Society of Bone & Joint Surgery; 2015. p. 17.
15. Juutilainen V. Posttraumatic osteomyelitis. Suomen Ortopedia ja Traumatologia. 2011;34(1):38–41.
16. Vardakas K, Kontopidis I, Gkegelis I, Rafailidis P, Falagas M. Incidence, characteristics, and outcomes of patients with bone and joint infections due to community-associated methicillin-resistant Staphylococcus aureus: a systematic review. Eur J Clin Microbiol Infect Dis. 2013;32(6):711–21.
17. Chalya PL, Mabula JB, Nyagomela IH; Kanumba AB, Chandika AB, Giti G, Mawala B, Balamuka D. Motorcycle injuries as an emerging public health problem in Mwanza City, Tanzania: a call for urgent intervention. Tanzan J Health Res. 2010;12(4):214–21.
18. Mutasingwa D, Aare L. Injury registration in a developing country: A study based on patients’ records from four hospitals in Dar es Salaam; Tanzania; 2001.
19. Shrestha A, Sah SK. Chronic osteomyelitis on an old compound fracture. J Coll Med Sci Nepal. 2011;28(2):58–63.
20. Pande K. Optimal management of chronic osteomyelitis: current perspectives. Orthoped Res Rev. 2015;7:71–81.
21. Birt MC, Anderson DW, Toby EB, Wang J. Osteomyelitis: recent advances in pathophysiology and therapeutic strategies. J Orthopae. 2017;14(1):45–52.
22. Olson ME, Horswill AR. Staphylococcus aureus osteomyelitis: bad to the bone. Cell Host Microbe. 2013;13(6):629–31.
23. Babaei S. Osteomyelitis septic arthritis & soft tissue infections; 2013.
24. Mshana S, Kamugisha E, Miramb M, Chalya P, Rambau P, Mahalu W, Silago et al. Journal of Orthopaedic Surgery and Research (2020) 15:95 Page 6 of 6

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.