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Prevalence and Antibiotic Sensitivity Pattern of *Staphylococcus aureus* Isolated from Wound and Otitis Media among Patients Attending Aminu Kano Teaching Hospital, Kano, Nigeria

Suraj Ibrahim[1*], Abubakar Sunusi Adam[2], Adamu Almustapha Aliero[2,3] and Shamsuddeen Umar[1]

[1]Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, Nigeria.
[2]Department of Microbiology and Immunology, Faculty of Biomedical Sciences, Kampala International University Western Campus, Ishaka, Uganda.
[3]Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author SI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ASA and AAA managed the analyses of the study and literature searches. Author SU supervised and monitored the work. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The study aimed at determining the prevalence and antibiotic susceptibility patterns of *S. aureus* isolated from wound and otitis media among patients attending Aminu Kano Teaching Hospitals, Kano Nigeria.
Study Design: This was an experimental laboratory cross-sectional study conducted from May to June, 2013. Both out and inpatients presenting with wound and otitis media or highly suspicious of having otitis media infection was recruited in the study. Only patient attending treatment at Aminu Kano University-Teaching Hospital was included in the study. Purposive sampling technique was

**Corresponding author: E-mail: abubakarsunusi@studwc.kiu.ac.ug;*

applied to recruit patients who have satisfied the selection until the desired sample size was reached. Swab samples were collected after obtaining informed consent from the selected patients.
Methodology: A total of 150 swab samples (wounds 80 swabs and ears 70 swabs) were collected from males and females, adults and children patients at specimen collection center and brought to the Microbiology laboratory, Aminu Kano Teaching Hospital for analysis. All the swab samples were inoculated on Mannitol salt agar and incubated overnight at 37°C. After 24hrs, the isolates were identified using catalase, coagulase and DNAse test. The identified isolates were subjected to susceptibility testing using disc diffusion method.
Results: Out of 150 samples collected, 71 (47.3%) were positive for *S. aureus* from all the categories of patients. Among the antibiotics tested, Erythromycin, Cefalexin, Tetracycline and Ceftriaxone were more effective against *S. aureus* while Trimethophrim, Gentamycin, Clindamycin, Oxacillin and Cefoxitin had the highest resistance.
Conclusion: In conclusion the study revealed that *S. aureus* is more prevalent in wound patient compared to otitis media patients.

Keywords: Prevalence; antibiotics susceptibility pattern; S. aureus; wound; otitis media.

1. INTRODUCTION

Staphylococcus aureus is a Gram positive and also coagulase positive bacteria belonging to the genus Staphylococci [1]. It is an opportunistic pathogen infecting immune-compromised patients with high mortality and morbidity rates in tropical countries [2]. *Staphylococcus aureus* is the most prominent nosocomial pathogen responsible for several nosocomial infections including: gastro enteritis, respiratory and urinary tracts infections, surgical sites infections, mastitis, mild skin infections (impetigo, folliculitis, furuncle and carbuncle), invasive diseases (wound infections, osteomyelitis, and bacteraemia) [3]. *Staphylococcus aureus* is the frequent microorganism isolated from ear (otitis media) causing 50% or more of nosocomial *S. aureus* infections in many countries [4]. Ear infection (Otitis media) is the infection caused by an inflammation of the middle ear due to the colonisation of a pathogenic bacteria leading to an impaired or deficiency in hearing [5,6]. Otitis media varies in complications based on the level of severity and infectivity by the causative agents, as such it is classified into; a chronic suppurative otitis media (CSOM) and acute suppurative otitis media (ASOM) [7].

Wound is an appropriate site for proliferation of bacteria and is more vulnerable to nosocomial pathogens in comparison to otitis media; this depends on the size of the area affected by the wound which may prolong the patients stay in the hospital [8]. Wound infection is a major cause of morbidity and mortality in healthcare settings [9]. It was estimated that about 75% of mortality rate caused by wound injuries is related to infections relatively than osmotic shock and hypovolemia [10]. Microorganisms in most cases are transmitted to the wound surfaces of the newly admitted patients either by the hands of the healthcare personnel or by a certain formites and sometimes by hydrotherapy [11].

Antimicrobial resistance among pathogenic microorganism has become a major public health concern both in the healthcare and community settings [12]. The Center for Disease Control and Prevention (CDC) reported that *S. aureus* is the most recurrently identified antimicrobial drug-resistant pathogen in the US causing about 10% of all Staphylococcus infections and more than 50% of nosocomial Staphylococcal infections [13]. By 2012, the prevalence has increased to more than 34% of clinical isolates of *S. aureus* while in Europe 26% was reported, and 45% in the western Pacific [14]. In developing countries of Africa, literature have shown that the prevalence of resistant *S. aureus* has been on the rising thereby increasing concerns due to the related increase in morbidity and mortality rates [15]. In Sub-Sahara Africa, a multinational surveillance project in eight African hospitals found that 14.8% of clinically significant *S. aureus* strains were resistant to most common antibiotics used in healthcare settings [16].

In Nigeria the situation is not different as this leads to increase of mortality and economic burden exponentially [17]. Literatures have shown that, there is no comprehensive burden of antimicrobial resistance within the country [17, 18]. In some study carried out in some part of the country showed that, *S. aureus* was resistant to ampicillin ranged between 0 to 95.6% with the least resistance in Oyo State and the greatest resistance in Cross River State [19,20]. In

another studies conducted by [21] on *Staphylococcus* species isolated from HIV-infected Adults Attending a Lagos Teaching Hospital and Among Neonates attending University College Hospital, Ibadan respectively showed variable degrees of resistance to the aminoglycosides with less resistance to amikacin than to gentamicin. Omoregie et al. [22]; Adejuyigbe et al. [23] also reported resistance of *Staphylococcus* species isolated among young children in Benin City and Obafemi Awolowo University Teaching Hospital, Ile-Ife respectively to the quinolones with variable resistance to ciprofloxacin ranging between 0% in Osun State and 73.4% in Edo State. In studies conducted by [24,25] from two States in the North-Eastern and North-Western zones of the country showed that *S. aureus* isolated among Suspected Neonatal Septicaemia at a Teaching Hospital in Kano and Maiduguri showed less resistance of 0% and highest resistance of 60.5% with higher resistance to chloramphenicol in all studies. In Oyo State, [20] reported, methicillin resistant *S. aureus* (MRSA) isolates were highly resistant to gentamicin but 100% sensitive to amikacin, ciprofloxacin and chloramphenicol. It appears that, burden of antimicrobial resistance in *S. aureus* within the country is not well documented due to the lack of appropriate antimicrobial stewardship and AMR surveillance within the country and this has stultified the perception of the full impact of antimicrobial resistance in the country [17,18]. Therefore, this study intended to investigate the prevalence and antibiotic susceptibility pattern of *S. aureus* isolated from wound and otitis media from patients attending Aminu Kano Teaching Hospital, Kano Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Aminu Kano Teaching Hospital within Kano State metropolis North-Western part of Nigeria located on latitude 11.7574° N and longitude 8.6601° E with populations of 3,626,068 [26].

2.2 Study Design

This was a cross-sectional study conducted from May to June, 2013. Both out and inpatients presenting with the wound and otitis media or highly suspicious of having otitis media infection was recruited in the study. Only patient attending treatment at Aminu Kano University-Teaching Hospital was included in the study. Purposive sampling technique was applied to recruit patients who have satisfied the selection until the desired sample size was reached. Swab samples were collected after obtaining informed consent from the selected patients.

2.3 Sample Size Determination

Sample size was calculated using a formula (by Kish and Lesley 1979); $n = z^2p\,(1-p)/d^2$. Where: z = Z score for 95% confidence interval = 1.96, p = prevalence, d = acceptable error (5%). Twenty five (25%) prevalence of *S. aureus* among Suspected Neonatal Septicemia at a Teaching Hospital in Kano was used [24].

$$\frac{n = (1.96)^2 x 0.25(1 - 0.25)}{(0.05)^2} = \frac{0.7203}{0.0025} \approx 288$$

Sample size of ≈ 288 was obtained after substituting the above parameters. But due to the limited number of wound and otitis media patient during the stipulated approved period of study by both research committee of Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, and Health service management board of Aminu Kano Teaching Hospital, Kano State Nigeria, only 150 swabs samples were collected and used for this study.

2.4 Inclusion and Exclusion Criteria

Any wound and otitis media patients who attend Aminu Kano Teaching Hospital and agreed to consent for the study were included. Those who are bellow age of consent (bellow 18 years) or unconscious their parents or Guidant was asked to consent on their behalf. Patients who fail to consent, terminally ill, or who fails to give swab sample were not included.

2.5 Ethical Consideration

Ethical clearance and approvals to carried out this work were obtained from research committee of Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, and Health service management board Kano, Ministry of Health Kano state and Aminu Kano Teaching Hospital, Kano state Nigeria. The procedures were followed in accordance with the ethical standards of the committee on human Experimentation, and World Medical Association [27]. Oral and written consents were obtained from the participants before sample collection; participation was voluntary and free.

2.6 Sample Collection

A total of 150 samples were collected from specimen's collection center and brought to the Microbiology laboratory, Aminu Kano Teaching Hospital for analysis. Eighty (80) samples were collected from wound while 70 from otitis media. Out of 80 samples collected from wound, 40 were collected from males and 40 from females respectively. While out of 70 samples collected from otitis media, 35 were collected from males and 35 from females respectively. The swab samples were collected after debridement of the wound boundaries and washing with physiological saline, using a sterile swab sticks dipped in 0.85% saline solution, the inmost part of the wound was swabbed and transported to Microbiology laboratory in a Stuart media [28].

2.7 Isolation and Identification of *Staphylococcus aureus*

The swab samples were inoculated on a sterile plates containing Mannitol salt agar in a Bio-safety cabinet to avoid contamination. The plates were incubated for 24hrs at 37°C. After the incubation, the colonies that fermented the Mannitol sugar turning the media into yellow coloration were sub-cultured in new sterile plates to get a pure culture. The pure colonies were subjected to Gram-staining, Catalase, Coagulase and DNAse test for confirmation [29].

2.8 Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of the pure colony of *S. aureus* isolates were determined using disc diffusion method, the antibiotic discs used in this study were: Ciprofloxacin (10 µg), Gentamycin (10 µg), Erythromycin (15 µg), Oxacillin (5 µg), Clindamycin (10 µg), Cefalexin (10 µg), Tetracycline (30 µg), Cefoxitin (10 µg), Ceftriaxone (2 µg) and Trimethophrim (25 µg). Inoculums suspension of a test organism (*S. aureus*) was prepared by inoculating pure colonies into sterile test tubes containing 2 ml of normal saline. The suspension was then adjusted by standardising the turbidity to that of 0.5 McFarland standards. The standardised suspension was inoculated on to the sterile plates containing freshly prepared Mueller Hinton agar using a sterile cotton swab. The surfaces of the plates were then impregnated with the antibiotic discs using a sterile Forcep. The plates were incubated for 24hrs at 37°C. After incubation, the inhibition zones were measured in mm using ruler in accordance to the guidelines of clinical laboratory standard institute [30].

2.9 Data Analysis

Data obtained was presented in Tables, Pie chart and bar graph. *S. aureus* rate was calculated as the proportion of individuals positive for *S. aureus* out of the sample populations. Chi-squire test was used to determine the significant differences on the prevalence of *S. aureus* between the Wound and Otitis media patients. However, distribution of *S. aureus* was compared between Male and Female, and different age groups. P value (p = 0.05) was considered significance at 95% confidence of interval. All analysis was carried out using SPSS (version 20) and WinPepi (Version 11.65).

3. RESULTS AND DISCUSSION

Staphylococcus aureus is the most frequent cause of nosocomial infections and is most likely to infect immunosuppressed patients such as infants, surgical patients, elderly and malnourished individuals, diabetic patients and other chronic diseases [31]. It appears that, burden of antimicrobial resistance in *S. aureus* within the African countries is not well documented due to the lack of appropriate antimicrobial stewardship and AMR surveillance within the regions and this has stultified the perception of the full impact of antimicrobial resistance in the regions [17,18]. This study therefore, determined the prevalence and antibiotic susceptibility pattern of *S. aureus* isolated from wound and otitis media from patients attending Aminu Kano Teaching Hospital, Kano Nigeria.

Out of 150 swabs samples collected and inoculated on Mannitol salt agar, 71(47.4%) were positive for *S. aureus* by fermenting the Mannitol sugar turning the media into yellow. All the 71(47.33%) suspected isolates were positive for catalase, coagulase and DNAse tests.

Out of 71(47.33%) positive *S. aureus* isolates, 49(32.7%, n=150; 95% confidence interval [CI] 25.24 to 40.79) were obtained from wound patients and (Table 1). This was in line with findings of Okon et al. [15], on their study dynamic of *S. aureus* from north-eastern Nigerian from samples collected in 2007and 2012 who reported the prevalence of 32% (n =75) in 2007 and 41% (n= 75) in 2012 of *S. aureus* from ear swab samples collected from

Medical microbiology laboratories serving the University of Maiduguri Teaching Hospital (Borno State, 500 beds, hospital A), the Federal Medical Centre Gombe (Gombe State, 420 beds, hospital B) and the Federal Medical Centre Nguru (Yobe State, 150 beds, hospital C). However, Almeida et al. [32] reported the prevalence of 20% (n=125) from wound swab collected from patient hospitalised in a remote and underdeveloped inland region of northeastern Brazil with extreme poverty. Out of 71(47.33%) of *S. aureus* obtained from this study, 22(14.7%, n=150, 95% confidence interval [CI] 9.42 to 21.36) were from otitis media patient (Table 1). The prevalence of *S. aureus* reported in this study was in line with findings of [15] on their study dynamic of *S. aureus* from north-eastern Nigerian from samples collected in 2007and 2012 who reported the prevalence of 10·7% (n =75) in 2007 and 2·7% (n= 75) in 2012 of *S. aureus* from ear swab samples collected from Medical microbiology laboratories serving the University of Maiduguri Teaching Hospital (Borno State, 500 beds, hospital A), the Federal Medical Centre Gombe (Gombe State, 420 beds, hospital B) and the Federal Medical Centre Nguru (Yobe State, 150 beds, hospital C). From outside the country, [33] reported prevalence of 19% (n=86) and 45% (n = 68) *Staphylococcus aureus* isolated from middle ear fluids of chronic suppurative otitis media and chronic rhinosinusitis patients undergoing ear surgery respectively. The prevalence of *S. aureus* from otitis patient reported in this study was lower compared to the findings of Basnet et al., 2017 who reported the prevalence of *Staphylococcus aureus* 36.11% (n=263) from pus of Otitis Media patient attending ENT department of Bharatpur Hospital, Brazil. Ahmad [34] also reported the prevalence of 45.1% (n= 164) of *S. aureus* isolated from chronic suppurative otitis media patients attending Department of Ear, Nose and Throat (ENT). However, Khan et al. 2013 reported higher prevalence of isolation rate 83.2%(n=250) of *S. aureus* from otitis media patients attending Departments of ENT, Mufti Mehmood Memorial Teaching Hospital and DHQ Teaching Hospital, Dera Ismail Khan.

Fig. 1 showed the Prevalence of *S. aureus* isolated from Male and Female wound and otitis media patients attending Aminu Kano Teaching Hospital. Out of the 71(47.33%) of *S. aureus* positive culture obtained, 43(28.67%, n=150) were male while 23(18.67%, 150) were female with 95% confidence interval, 0.34 to 0.84. This was in line with findings of [32] who reported higher prevalence of *Staphylococcus aureus* in male 15/63 (23.80%) than in female 10/62 (16.10%) in patient with Chronic Suppurative Otitis Media. This was contrary to the findings [35] who reported a slight differences of prevalence growth of *S. aureus* in female patients 109/121 (90.1%) than in male patients 107/119 (89.9%) from ear swabs samples.

However, the results of this study showed that children within the age groups of 1-10 had the highest prevalence of *S. aureus* 10(6.66%) while those above 50 years old age had the lowest prevalence 2(1.33%) (Table 2). This was in line with findings of [32] who reported prevalence of 16/62 (25.80%) and 9/61 (14.30%) among ≥ 65 and < 65 years age respectively. However, [35, 36, 37, 38] also reported higher prevalence of *S. aureus* among otitis media patient 1-10 years than <50years age. Basnet et al. [35] added that the prevalence of *S. aureus* increased as age decreased and decreased as age increased.

The result of antibiotics susceptibility study on *S. aureus* isolated from both wound otitis media patients showed that, Erythromycin, Cefalexin, Tetracycline and Ceftriaxone had 90.1%, 69%, 69% and 69% sensitivity against *S. aureus* respectively, while Trimethophrim, Gentamycin, Clindamycin, Oxacillin and Cefoxitin had 69%, 43.7%, 50.7% and 50.7% highest resistance respectively. Erythromycin (90.1%) was the most effective among the sensitive antibiotics, while Trimethophrim (69%) showed the highest percentage of resistance by the *S. aureus* as shown in Fig. 2. The effectiveness of erythromycin against *S. aureus* in this study is in agreement with the report of [34,39] who reported 92.3% and 70% sensitivity of *S. aureus*

Table 1. Prevalence of *Staphylococcus aureus* in wound and otitis media isolated from patients attending AKTH Kano

Sample type	Number of samples collected	Number of positive samples (%)	CI 95%	P-value
Wound	80	49 (32.7)	25.24 to 40.79	0.001
Otitis media	70	22 (14.7)	9.42 to 21.36	
Total	150	71 (47.4)		

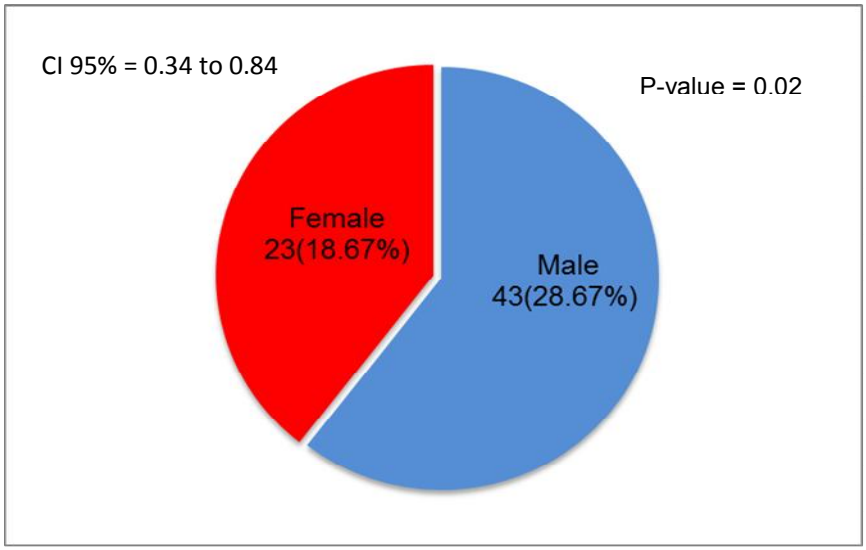

Fig. 1. Prevalence of *S. aureus* from both wound and otitis media patients among males and females

Table 2. Prevalence of *Staphylococcus aureus* isolated from wound and otitis media patients attending AKTH according to age

Patients based on age group	Sources of samples		Total n=150 (%)
	Wound n =80 (%)	Otitis media n=70 (%)	
<10	16(20%)	9(12.86)	25(16.66)
11-20	11(4.66)	5(7.14)	16(10.66)
21-30	8(1.00)	0(0.00)	8(5.33)
31-40	6(7.50)	3(4.29)	9(6.00)
41-50	5(6.25)	1(1.43)	6(4.00)
>50	3(3.75)	4(5.71)	7(4.66)
Total	**49(61.25)**	**22(31.43)**	**71(47.33)**

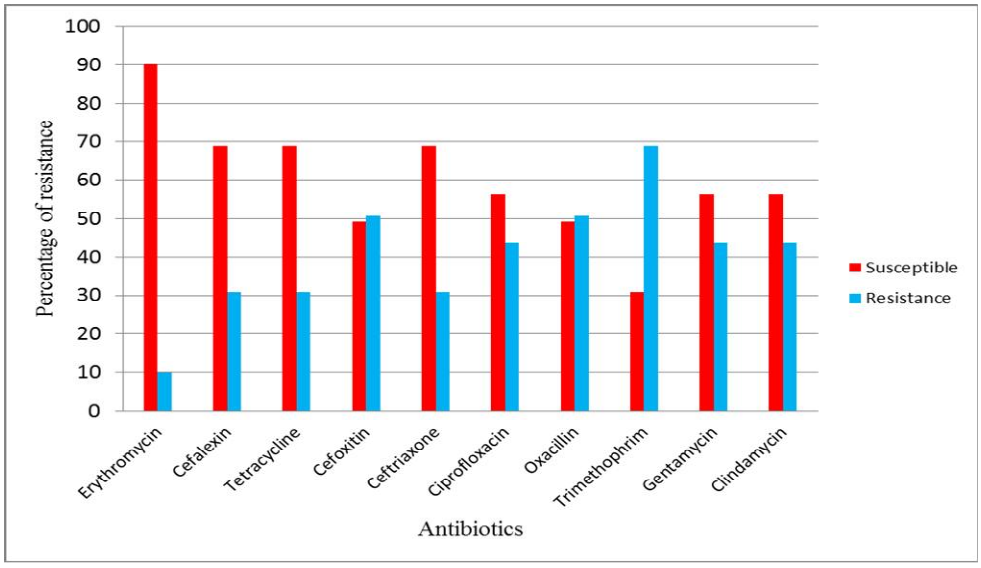

Fig. 2. A Bar-chat showing the antibiotics susceptibility pattern of *S. aureus* isolated from wound and otitis media patients attending Aminu Kano Teaching Hospital

to erythromycin respectively. This was contrary to the findings of [35] who reported 52.2% and 37.9% sensitivity of *S. aureus* isolated from Chronic Suppurative Otitis Media Patients to Erythromycin.

The higher resistance of *S. aureus* to Trimethophrim-sulfamethoxazole and gentamicin (69%, 43.7%) reported in this study was in line with findings of [38] who reported 67.4% and 41.3 resistances of *S. aureus* to Trimethophrim-sulfamethoxazole and gentamicin respectively. This was contrary to the findings of [35] who reported lower resistance 26.9% and 8.1% of *S. aureus* to Trimethophrim-sulfamethoxazole and gentamicin respectively.

S. aureus was also found to be resistant to oxacillin in this study with 50.7%, this was found to be higher than the report of Iroha et al. [40] who reported 15% of resistance by *S. aureus* to oxacillin. In a review conducted by Emaneini et al. [41] on the prevalence of methicillin-resistant *Staphylococcus aureus* isolated from burn patients reported the prevalence of 77.9% from 555 papers reviewed.

4. CONCLUSION

This study revealed the prevalence of 47.33% of *S. aureus* among wound and otitis media patient attending Aminu Kano Teaching Hospital, Nigeria. Prevalence *S. aureus* is more among children in comparison to adults. Erythromycin was the most effective among the antibiotics tested while Trimethophrim-sulfamethoxazole was having the highest percentage of resistance. Cefoxitin also showed a 50.7% of resistance which indicated the presence of methicillin resistant *S. aureus* among the resistant isolates. Before given any medication to a patient an antibiotic sensitivity test must be done, this will help in the choice of potent, safe and efficient drug. The subsequent research should focus on antibiotic susceptibility pattern of Methicillin resistant *S. aureus* with the use of polymerase chain reaction to detect the mecA gene which is responsible for the resistance.

ETHICAL APPROVAL AND CONSENT

Ethical clearance and approvals to carried out this work were obtained from research committee of Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, and Health service management board Kano, Ministry of Health Kano state and Aminu Kano Teaching Hospital, Kano state Nigeria. The procedures were followed in accordance with the ethical standards of the committee on human Experimentation, and World Medical Association [27]. Oral and written consents were obtained from the participants before sample collection; participation was voluntary and free.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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