Original Research Article

Antibiotic Susceptibility Profilesof Bacteria Isolated from Patients Hospitalized at the Sylvanus Olympio University Teaching Hospital in Lomé, Togo

Comment [MS1]: Throughout the article, either single antibiotic resistance rates or antibiotic sensitivity rates should be mentioned. The title and what is given in the article do not constitute integrity.

Abstract

Comment [MS2]: After the article is edited according to the suggestions, the abstract section should also be reorganised.

Background: Bacteria isolated from hospitalized patients are often responsible for healthcare-associated infections (HAI), particularly in developing countries. Thus, our study aimed to determine the antibiotic susceptibility profiles of bacteria isolated from patients hospitalized at the Sylvanus Olympio University Teaching Hospital (CHUSO) in Lomé, Togo.

Materials and methods: This was a descriptive cross-sectional study carried out on laboratory data collected from January 1, 2018, to December 31, 2019. The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing and the results were interpreted according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2018).

Results: A total of 639 samples were collected, including mainly pus (n = 339; 53.1%) and urine (n = 260; 40.7%). The samples were mainly from pediatrics (n=107; 16.7%), intensive care units (n=73; 11.4%) and surgical emergencies (n=72; 11.3%). A total of 698 bacteria were isolated, including mainly *Escherichia coli* (n=247, 35.4%), *Staphylococcus aureus* (n=123, 17.6%), and *Klebsiella pneumoniae* (n=114, 16.3%). *Enterobacteriaceae* strains were resistant to almost all antibiotics tested, except amikacin and ertapenem, which had respective resistance prevalence rates of 2.8% and 8.4%. All *P. aeruginosa* strains were susceptible to

piperacillin–tazobactam whereas 37.9% were resistant to imipenem. Among *A. baumannii* strains, 4.2% and 22.9% were respectively resistant to amikacin and imipenem, whereas 56.3% were resistant to levofloxacin. Almost all *S. aureus* strains (99.2%) were resistant to penicillin, whereas only 2.4% were resistant to rifampicin. Of the 698 bacteria isolated, the prevalence of multidrug-resistant bacteria (MDR) was 41.3% (n = 288), whereas the prevalence of ESBL-producing *Enterobacteriaceae* was 51.3% (201/392).

Conclusion: The study of clonal profiles and mobile genetic elements of bacteria isolated from hospitalized patients at CHUSO would provide useful information. The study of bacterial ecology and resistance in every prefectural, regional, and university teaching hospital would be of great importance to reduce mortality associated with hospital-acquired infections throughout the Togolese territory.

Keywords: antibiotic resistance; hospital-acquired infection; Lomé-Togo; hospitalized patient.

Comment [MS3]: This part is a suggestion. Therefore, remove this part and rearrange the conclusion section.

Comment [MS4]: The word "**Susceptibility**" would be more appropriate.

Introduction

Bacterial infections are associated with high morbidity. In 2022, Ikuta et al. [1], reported that bacterial infections were the second leading cause of death worldwide and were responsible for one in eight deaths. Bacterial infections are becoming increasingly difficult to treat because of the increasing rate of antibiotic resistance. Additionally, global surveys have shown that, in 2019, antimicrobial resistance killed more people than HIV/AIDS or malaria[2,3].

Antibiotic-resistant bacteria have contributed to the burden of hospital-acquired infections (HAI), especially in developing countries [4].HAIs have a significant impact on clinical outcomes, length of hospital stay, and extra costs of medical care [5,6].The prevalence of healthcare-associated infections is 25% in low-income countries [7,8]. The most common

HAIs are central line-associated bloodstream infections (CLABSI), catheter-associated urinary tract infections (CAUTI), surgical site infections (SSI) and ventilator-associated pneumonia (VAP) [9]. If appropriate measures are not taken, bacteria isolated from hospitalized patients cause healthcare-associated infections (HAI), particularly in developing countries[10]. Moreover, hospital environments (devices, rooms, surfaces, and air conditioning pipes) are often colonized by bacterial biofilms,which are powerful platforms for intra- and interspecific gene exchanges[11–14]. These intra- and interspecific exchanges of resistance genes in hospitals act as catalysts for antibiotic resistance [15].

Most hospital-acquired bacteria (HAB) are multidrug-resistant(MDR) and extensively drug-resistant bacteria (XDR)[16–18]. The most reported HABs are carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPa), carbapenem-resistant *Acinetobacter baumannii* (CRAb), methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamases -producing *Enterobacteriaceae* (ESBL-E), and vancomycin-resistant *Enterococcus* (VRE) [6,19].

Monitoring the susceptibility profiles of bacteria isolated from hospitalized patients is a crucial step leading to the adaptation of empirical antibiotic treatments and better prevention of outbreaksof multidrug-resistant bacteria in hospitals[20,21].

Almost all countries worldwide have established antimicrobial resistance committees to drastically reduce antibiotic resistance-associated mortality [1,22,23] and Togo is part of the same dynamic. Thus, our study aimed to determine the antibiotic susceptibility profiles of bacteria isolated from hospitalized patients atthe Sylvanus Olympio University Teaching Hospital (CHUSO) in Lomé, Togo.

Materials and methods

Study design

This was a descriptive cross-sectional study carried out on laboratory data collected from January 1, 2018, to December 31, 2019, at the Sylvanus Olympio University Teaching Hospital (CHUSO) in Lomé, Togo.

CHUSO (https://chuso.tg/) is the largest hospital in Togo, with 43 departments, 838 hospitalization beds, 205 medical staff, 474 paramedical staff, and approximately 27,000 hospitalizations annualy.

The inclusion criteria were hospitalization and antibiogram completion. Data from patients hospitalized at the CHUSO who underwent laboratory bacteriological analysis were included in this study. The collected data included patient demographics (age, sex, and diagnosis), department from which the sample came, type of sample, bacteria isolated, and antibiotic susceptibility testing results. Data from non-hospitalized patients were excluded.

Bacterial isolates

The bacteria were isolated and identified using bromocresol purple lactose agar (BPLA), UriSelect chromogenic media (Bio-Rad Laboratories, Marnes-la-Coquette, France), API20E galleries (bioMérieux, Craponne, France) for *Enterobacteriaceae*, Api10S (bioMérieux, Craponne, France) for *S. aureus* and Api20NE (bioMérieux, Craponne, France) for *P. aeruginosa* and *A. baumannii*.

Antibiotic susceptibility testing and ESBL detection

The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing and the results were interpreted according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2018). For *Enterobacteriaceae*, following antibiotic were tested: amoxicillin - clavulanic Acid (AMC, 20µg - 10µg), piperacillin (PIP, 30µg), ertapenem (ERT,10µg), ceftazidime (CTZ, 30µg), ceftriaxone (CTR, 30µg),

cefotaxime (CTA, 30µg), amikacin (AMI, 30µg), gentamicin (GEN, 10µg), nalidixic acid (NAL, 30µg), norfloxacin (NOR, 10µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), and sulfamethoxazole - trimethoprim(SXT, 23.75µg - 1.25µg).For *P. aeruginosa* and *A. baumannii*strains, following antibiotic were tested: piperacillin – tazobactam (PTZ, 100µg - 10µg), ceftazidime (CTZ, 30µg), imipenem (IMP, 10µg), amikacin (AMI, 30µg), gentamicin (GEN, 10µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), and sulfamethoxazole – trimethoprim (SXT, 23.75µg - 1.25µg).The following antibiotics were tested for *S. aureus*: penicillin (P, 10µg),cefoxitin (FOX, 30µg), kanamycin (KAN, 30µg), tobramycin (TOB, 10µg),gentamicin (GEN, 10µg), erythromycin (E, 30µg), tetracycline (TET, 30µg), ciprofloxacin (CIP, 5µg), norfloxacin (NOR, 10µg), rifampicin (RIP, 5µg), and linezolid (LNZ, 30µg).

ESBL production was detected by double-disk synergy test using AMC disc surrounded at a radius of 30 mm by CTR, CTZ and CTA.

Data analysis

Data processing was performed using STATA® statistical processing software version 15. The variables were described by their absolute (n) and relative (%) frequencies. Microsoft Excel® 2019 software was used to make the graphs.

Results

Comment [MS5]: There is no demographic data regarding age, gender and diagnosis in the findings section. The findings section needs to be rearranged by adding these data and making comparisons (for example, which bacteria are isolated more frequently in which diagnosis, in which age group the antibiotic sensitivity/resistance rate is high, etc.).

Distribution of samples

A total of 639 samples were collected, including pus (n = 339; 53.1%), urine (n = 260; 40.7%) and blood (n = 40; 6.3%) (Table 1). The samples were mainly from pediatrics (n=107; 16.7%), intensive care units (n=73; 11.4%), surgical emergencies (n=72; 11.3%), and

pediatric surgery (n=61; 9.5%). The distribution of the samples according to service is detailed in Table 1.

Table 1. Distribution of samples according to department.

Department	Sample						Total
	Pus		Urine		Blood		
	n	%	n	%	n	%	
Pediatrics	15	14	84	78.5	8	7.5	107
Intensive Care Units	6	8.2	56	76.7	11	15.1	73
Surgical Emergencies	64	88.9	7	9.7	1	1.4	72
PediatricSurgery	58	95.1	2	3.3	1	1.6	61
Traumatology	48	90.6	4	7.6	1	1.9	53
Gynecology-Obstetrics	32	62.8	18	35.3	1	2	51
Visceralsurgery	35	89.7	3	7.7	1	2.6	39
Medical-surgicalclinic	22	53.7	15	36.6	4	9.8	41
Oto-Rhino-Laryngology	33	100	0	0	0	0	33
Nephrology	0	0	24	88.9	3	11.1	27
Total	**339**	**53**	**260**	**40.7**	**40**	**6.3**	**639**

A total of 698 bacteria were isolated from the 639 samples. Several single samples carried two pathogenic bacteria. Among the 698 isolated bacteria, 516 (73.9%) were Gram-negative bacilli, mainly *E. coli* (n=247; 47.9%) and *K. pneumoniae* (n=114; 22.1%) (Table 2).

Table 2. Distribution of bacteria

Comment [MS6]: Distribution of bacteria isolated from samples included in the study

	Bacteria	n	%
Gram-negativebacilli(n=516)	*E. coli*	247	47.9
	K. pneumoniae	114	22.1
	A. baumannii	48	9.3
	Enterobacter spp.	31	6.4
	P. aeruginosa	29	5.6
Gram-positive cocci(n=182)	*S. aureus*	123	67.6
	Non-groupable streptococci	23	12.6
	Enterococcus spp.	18	9.9

Comment [MS7]: ??

Antibiotic susceptibility testing

Enterobacteriaceae species included in this antibiotic susceptibility testing,(n=392), were*E. coli* (n=247), *K. pneumoniae*(n=114),and *Enterobacter* spp. (n=31). *Enterobacteriaceae* strains were resistant to almost all antibiotics tested, including piperacillin (93.1%), sulfamethoxazole – trimethoprim (84.9%), amoxicillin – clavulanic acid (71.2%), third-generation cephalosporins (69.9 – 74.7%), fluoroquinolones (61.7 – 70.9%), and gentamicin (50.5%). However, amikacin (2.8%) and ertapenem (8.4%) showed good activity against the *Enterobacteriaceae* strains (Figure 1).

Most antibiotics tested showed good activity against *P. aeruginosa* strains. Thus, the following resistance prevalence has been reported: sulfamethoxazole – trimethoprim (58.6%), imipenem (37.9%), fluoroquinolones (20.7 – 24.1%), ceftazidime (20.7%) and aminoglycosides (17.2%). Furthermore, all *P. aeruginosa* strains were susceptible to piperacillin–tazobactam(Figure 2).

The prevalence of resistance in *A. baumannii* strains included: sulfamethoxazole – trimethoprim (66.7%), fluoroquinolones (56.3%), gentamicin (50%), ceftazidime (47.9%) and piperacillin – tazobactam (31.3%). Amikacin (4.2%) and imipenem (22.9%) showed good activity against *A. baumannii*(Figure 3).

Almost all *S. aureus* strains (99.2%) were resistant to penicillin, followed by tetracycline (59.3%), cefoxitin (27.6%), fluoroquinolones (16.3 – 23.6%), erythromycin (17.1%), aminoglycosides (12.2 – 14.6%), linezolid (2.4%) and rifampicin (1.6 %)(Figure 4).

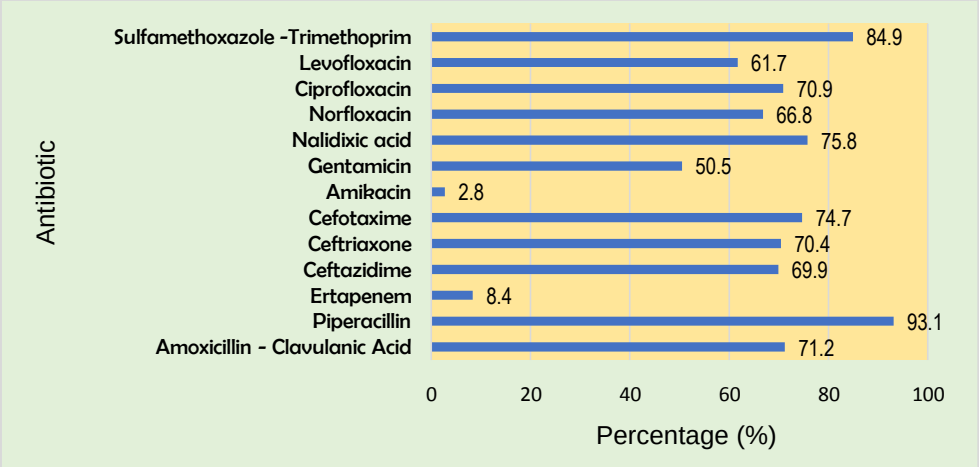

Figure 1. *Enterobacteriaceae* resistance profiles.

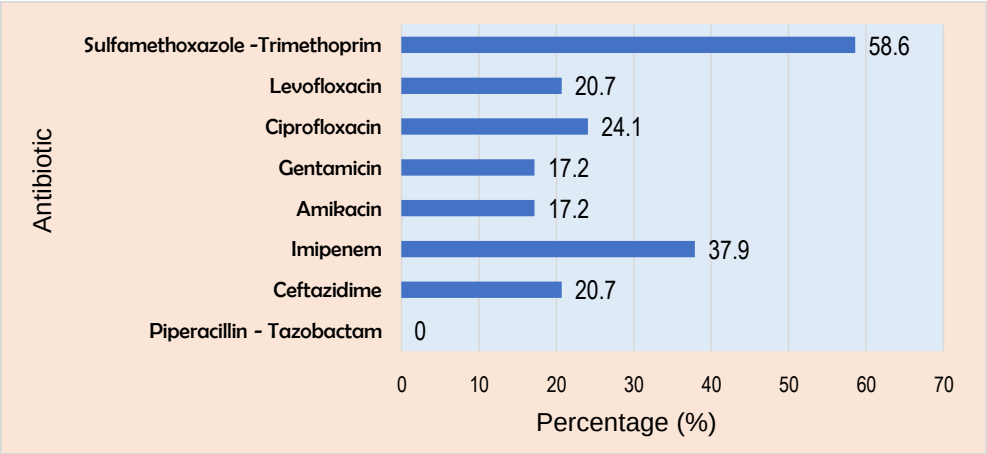

Figure 2. *P. aeruginosa*resistance profiles

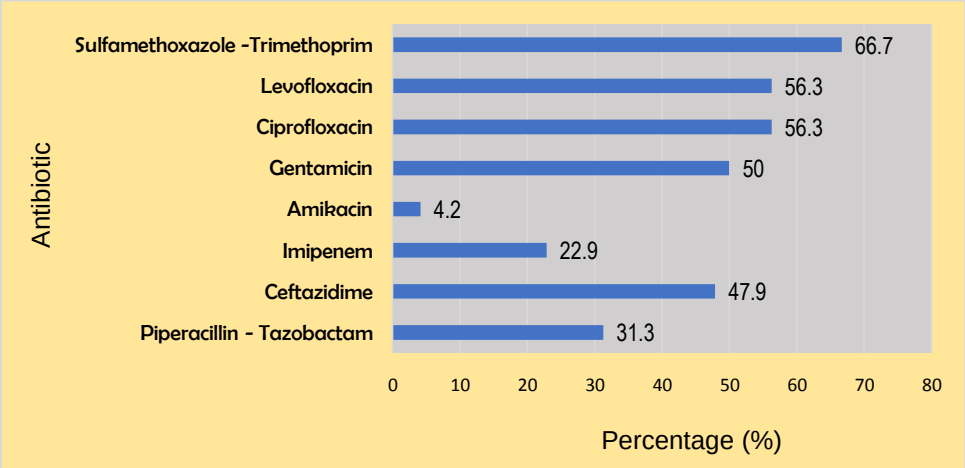

Figure3. *A. baumannii* resistance profiles.

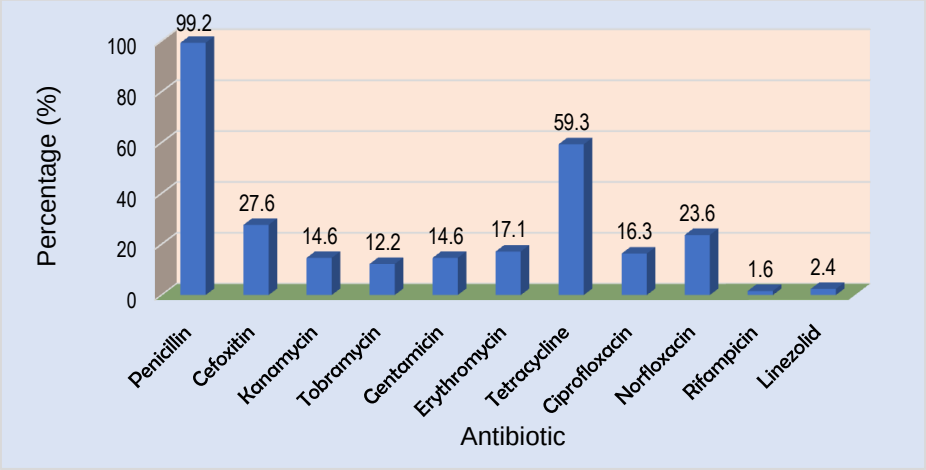

Figure 4. *S. aureus* resistance profiles.

Resistance phenotypes

Among the 698 bacteria isolated, the prevalence of MDR was (288/698; 41.3%), whereas the prevalence of ESBL-producing *Enterobacteriaceae* was (201/392; 51.3%). The prevalence of methicillin resistant *Staphylococcusaureus*(MRSA) was 27.6%.

Discussions

Comment [MS8]: The last paragraph of the discussion ("conclusion") should highlight the conclusions drawn from your personal information. General and known inferences and limitations.

This study reported bacteria from patients hospitalized in ten departments. Most samples were obtained from the pediatric department (16.7%). This predominance can be explained by the age and immaturity of the babies' immune systems, which favor infections.

Almost half of the samples were pus (53.05%), followed by urine (40.69%). The same trend was reported in Guinea Conakry, where Keita et al. found a predominance of pus (67.7%) and urine (16.1%) [24]. These two types of samples were mainly reported in a study by Kakupa et al. in DRC [25]. Fasla and Khaleq, in Morocco reported Cerebrospinal Fluid (CSF) (34.69%), followed by pus (29.59%) [26]. The fact that Fasla and Khaleq. reported only patients from emergency department, may explain this discrepancy.

A total of 471 Gram-negative bacteria and 164 Gram-positive bacteria have been reported. This trend was also reported in Cameroon and Morocco [27,28]. Among the Gram-negative bacteria isolated, *Enterobacteriaceae* occupied the first place. A similar trend was reported in Morocco by Mortaji[29] and Soraa[28]. This predominance of *Enterobacteriacae* can be explained by the fact that they are mostly commensal in the human digestive tract and widely found in the environment. Moreover,*Enterobacteriaceae*are often the predominant bacteria found in clinical settings[30–32].

Among the*Enterobacteriaceae*, *E. coli*, were resistant to ciprofloxacin (70.9%), gentamicin (48.2), carbapenems(8.4%) and amikacin (2.8%). Mlaga et al.[33]also reported *E. coli*strains with decreased susceptibility to fluoroquinolones and gentamicin, and highsusceptibility to amikacin and carbapenems. Sbiti et al. in 2017 [34], also reported notable resistance to ciprofloxacin (92.5%) and good susceptibility to carbapenem (3.4%) and amikacin (6.1 %). The high rate of resistance to fluoroquinolones in *Enterobacteriaceae* compromises the use of this class of antibiotics in probabilistic antibiotic therapy. This can be explained by the extensive use of fluoroquinolones as an empirical treatment for urinary infections.

Fluoroquinolones and aminoglycosides showed good activities against *S. aureus*and rifampicin whereas linezolid showed excellent activity. Tălăpan et al. [35]reported in Romania, resistance profiles very similar to ours concerning rifampicin, linezolid, fluoroquinolones, penicillin and tetracycline. Rao et al.[36]reported similar resistance patterns for gentamicin, rifampicin, and linezolid.

P. aeruginosa strains were more than 30% resistant to carbapenems, 24% to ciprofloxacin and 17% to amikacin. Abdallah et al in Tunisia reported almost similar results, 19.6% resistant to carbapenem, 21.6% to ciprofloxacin and 19.2% to amikacin[37]. Contrary to our results, a high prevalence of ceftazidime resistance (66–70%) has been reported in

Libya, Tunisia, and Egypt [38]. A concernwas the prevalence of carbapenem-resistant *P. aeruginosa* (37.9%). These carbapenem-resistant *P. aeruginosa* (CRPa) could be the starting point for hospital colonization and the intra- and inter-specific transfer of carbapenem-resistant genetic determinants. If appropriate solutions are not taken, CRB epidemics could affect CHUSO as reported worldwide [39–42].

Concerning*A. baumannii*strains, our results are similar to those obtained byMusyoki et al.[43]in Kenya for amikacin. Amikacin appears to be a good therapeutic option for*A. baumannii*infection in Africa. However, in Turkey and Iran, high prevalence rates of amikacin resistance ranging from 66.7% to 100% have been reported [44,45].Piperacillin-tazobactam is known to be a key antibiotic against *A. baumannii*[46]. Contrary to our results (resistance rate of 31.3%), several studies have reported a high prevalence of resistance to piperacillin-tazobactam, ranging from 62 to 100% in Benin, Tanzania, Ghana, Morocco, and South Africa[47,48].

More than half of the *Enterobacteriaceae* strains were ESBL producers. According to the prevalence of ESBL-producing *Enterobacteriaceae* reported by Mlaga et al. [33] (25.95%; strains collected from 2009 to 2010), and Toudji et al. [49] (22.4%, strains collected from 2009 to 2011), the prevalence of ESBL-producing *Enterobacteriaceae* is constantly increasing at CHUSO. The evolution of ESBL-producing *Enterobacteriaceae* prevalence at CHUSO will need to be carefully monitored every year.

Conclusion

We reported various resistance profiles of bacterial species isolated from hospitalized patients at the CHU Sylvanus Olympio, Lomé, Togo. Around a quarter of the *P. aeruginosa* and *A. baumannii* strains were resistant to carbapenems. In addition, ciprofloxacinshould no longer be prescribed as a probabilistic antibiotic in hospitalized patients in theCHUSO.Moreover,

amikacin may be a good alternative for carbapenem-resistant bacteria. However, its prescription must be closely monitored to delay the appearance and generalization of clones resistant to amikacin in Togo. Furthermore, Tobramycin, rifampicin, and linezoloid may be good probabilistic antibiotics for *S. aureus*-associated infections. Finally, the study of bacterial ecology and bacterial resistance in every prefectural, regional, and university teaching hospital would be of great importance to reduce mortality associated with hospital-acquired infections throughout the Togolese territory.

Availability of data and materials

Data and materials used in this work are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The Institutional Review Board (IRB) of the Sylvanus Olympio Teaching Hospital approved the study protocol, and the collected data were only used for clinical research purposes. The information was kept confidential and was not used for any other purpose. Patient information was coded to protect their identities. Since retrospective data were used, the IRB of the Sylvanus Olympio Teaching Hospital obtained all participants' consent for any further research. The authors confirmed that all the methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

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Comment [MS9]: Spelling in sources 27, 28, 30 should be corrected. Species names should be written in italics.