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Prevalence of bacterial contamination on wild meat processing and cooking surfaces in rural Cameroon

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ABSTRACT

The transmission of food-borne pathogens from wildlife to humans presents a significant public health challenge. The recent COVID-19 pandemic has highlighted the critical need to enhance our understanding of wild animals' role in transmitting infectious diseases. The handling and consumption of wild meat carry inherent risks of contracting foodborne illnesses. We analysed the prevalence of bacterial pathogens encountered in wild meat processing in four villages in southern Cameroon, highlighting the critical role of hygienic practices in preventing disease. We collected 100 samples from various utensils and surfaces involved in wild meat preparation and assessed them for bacterial contamination. We isolated 577 bacterial strains, of which 154 (27 %) were pathogenic, with a high prevalence (75 %) of pathogenic bacteria on commonly used utensils, with cooking pots identified as significant reservoirs of bacteria. Antimicrobial resistance among the order Enterobacteriales included high levels of resistance to ampicillin, amoxicillin-clavulanic acid, ciprofloxacin, cotrimoxazole, and gentamicin. The study also explores the impact of cleaning practices, the materials of cooking utensils, and the potential economic consequences of foodborne illnesses. The results underscore the urgent need for improved sanitation measures and provide insights into the health risks posed by wild meat consumption. They also serve as a foundation for comparative studies and the development of region-specific interventions. Following safe handling and cooking guidelines is critical to safeguarding public health and mitigating the risks associated with food-borne diseases, particularly in regions where wild meat is a significant part of the diet. Our results reinforce the need to implement the Standard Operating Procedures (SOP) recently approved by the Ministry of Livestock, Fisheries and Animal Industries of Cameroon, providing comprehensive guidelines for safe handling, preparing and consuming wild meat.

1. Introduction

The heightened global awareness of disease spillover from wildlife to humans, exemplified by the COVID-19 pandemic, has brought increased scrutiny to the health risks associated with wild meat consumption [1]. While the threat of emerging infectious diseases (EIDs) linked to wildlife—such as zoonotic viruses like SARS-CoV-2 and Ebola—remains a valid global concern, it is foodborne pathogens, including *Salmonella*, *Escherichia coli*, and *Trichinella*, that represent a more immediate, frequent, and well-documented risk to public health, particularly in communities regularly handling and consuming both domestic and wild meat [2–4]. In contrast to the episodic nature of zoonotic spillover

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events, foodborne illnesses occur with far greater regularity. They are a leading cause of diarrhoeal disease globally, disproportionately affecting low-resource settings where food safety practices and regulatory oversight are often lacking [5]. Despite this, data on the burden of foodborne diseases linked explicitly to wild meat remain limited, hindering the development of targeted public health interventions [4].

Wild meat is crucial in food security and livelihoods for millions in tropical regions [6]. For many rural and Indigenous communities, it is a primary source of protein and essential micronutrients, particularly where alternative animal protein sources are scarce or unaffordable. Wild meat harvesting and trade also provide vital economic opportunities, supporting household incomes and local economies, especially in remote areas with limited employment alternatives. Balancing food safety, nutrition, and economic reliance on wild meat is essential for developing policies that mitigate health risks while sustaining livelihoods.

A World Health Organization (WHO) study estimates that as many as 31 foodborne hazards led to 600 million illnesses, 420,000 deaths, and 33 million Disability-Adjusted Life Years (DALYs) lost annually [4]. The primary causes of illness were diarrhoeal disease agents, including norovirus, *Campylobacter* spp., and non-typhoidal *Salmonella enterica*, which also contributed significantly to mortality. Other major contributors included *Salmonella Typhi*, *Taenia solium*, hepatitis A virus, and aflatoxin.

Children under five bear 40 % of the foodborne disease burden despite comprising only 9 % of the global population [5]. The long-term effects of these illnesses, including malabsorption, malnutrition, growth delays, and chronic anaemia, can severely impact physical and cognitive development [5,7]. These findings highlight the urgent need to address the health and socioeconomic consequences of contaminated food, particularly in settings where wild meat remains an essential component of diets and livelihoods.

The primary pathways for foodborne pathogen contamination in wild meat stem from two key sources: first, direct contact with animal body fluids during carcass handling, and second, consumption of raw, undercooked, or spoiled meat. These risks are compounded by village-level food-handling practices, where butchering, processing, and cooking involve multiple social actors [8]. Additional factors—including contaminated food-processing surfaces, clothing, or utensils, feeding domestic animals with animal viscera, improper food disposal, and the direct presence of infants during meat preparation—further facilitate pathogen transmission.

Although food safety regulations have improved hygienic practices for domestic meats [9], many tropical countries lack adequate safeguards for wild meat [10]. Addressing these gaps in hygiene practices could reduce the burden of foodborne diseases and lower the probability of widespread zoonotic disease outbreaks. As with any other food source, ensuring the safety of wild meat requires a systematic approach to hazard identification and control throughout the supply chain—from harvesting and handling to processing and consumption. Standardised Hazard Analysis and Critical Control Point (HACCP) management systems can enhance food safety if adequately implemented [10]. However, the scarcity of reliable data on foodborne diseases linked to wild meat, particularly in Africa, limits the development of effective strategies to protect consumer health.

This study investigates bacterial contamination associated with the cooking and processing wild meat in four villages in southern Cameroon. The importance of wild meat hunting and consumption in these and other communities in the region is well documented [11,12]. The relevance of this research extends beyond the immediate study area, offering insights applicable to other sub-regional contexts in Sub-Saharan Africa where similar challenges in wildlife consumption and food safety exist. By examining bacterial risks linked to wild meat preparation, this study provides a foundation for developing region-specific interventions that enhance food safety without undermining wild meat's nutritional and economic importance. The methodology and findings presented here can serve as a reference for comparative studies across diverse ecological, cultural, and socio-economic settings, informing policy and practice at multiple levels.

2. Methods

2.1. Ethics statement

The sample collection was approved by the Cameroonian Ministry of the Scientific Research and Innovation and granted by the research permit n° 000210/MINRESI/B00/C00/C10/C13, and by the Hospital Clinic of Barcelona Ethics Committee (CEIC), reference HCB/2023/0934. Free Prior and Informed Consent (FPIC) was applied by consultation with the four communities involved in the study. It was later formally established through agreement letters where communities expressed their will to participate.

2.2. Study sites

This study was conducted in four settlements (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeast Cameroon (Fig. 1), situated within moist evergreen forests, part of the western Congo Basin. The region's terrain is sloping with gently rolling hills and is characterised by a four-season equatorial climate. Rainfall averages 1500–2000 mm per year, and the mean temperature remains steady year-round, averaging 25 °C, fluctuating slightly with the seasons [13].

Mintom is a town inhabited by around 6000 people and is the administrative centre for villages around its immediate vicinity. All other settlements were Baka Pygmy villages. From population censuses conducted by us in ten villages within the study area, including those in this study, an average of four (range 1–17) persons lived in the occupied dwellings [10]. Villagers primarily rely on harvesting and trading non-timber forest products, particularly wild meat.

2.3. Bacterial sampling

To detect the presence of bacteria, 100 samples were collected from utensils and surfaces associated with preparing and handling wild meat (Table 1). Samples were taken using a dry swab rolled over the whole surface or immersed in the corresponding water or broth. The community provided utensils at each site. The type of utensils and the number of samples are given in Table 1. We employed Thermo Scientific™ Oxoid™ Amies Agar Gel Transport Swabs. Samples were preserved and sent from Cameroon to Spain at room temperature for processing.

2.4. Laboratory analyses

The collected samples were evenly spread onto Blood agar and McConkey agar plates and incubated for 24 h at 37 °C. Following incubation, all distinct colonies were isolated and characterised using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF). In instances of no correlation with the MALDI-TOF database, additional analysis was conducted through 16S polymerase chain reaction (PCR) amplification and sequencing. The identified bacterial colonies were then stored in skimmed milk and frozen at −80 °C for future use.

2.5. Antimicrobial resistance profiles

Bacterial preservation involved inoculating pure colonies onto Luria Bertani agar plates, followed by overnight incubation at 37 °C for 24 h without CO_2. The Kirby-Bauer disc diffusion method, a widely used technique in microbiology for assessing antimicrobial activity, was employed to evaluate bacterial susceptibility [14].

Antimicrobial susceptibility profiles of identified strains were assessed using the Kirby-Bauer disc diffusion method or broth

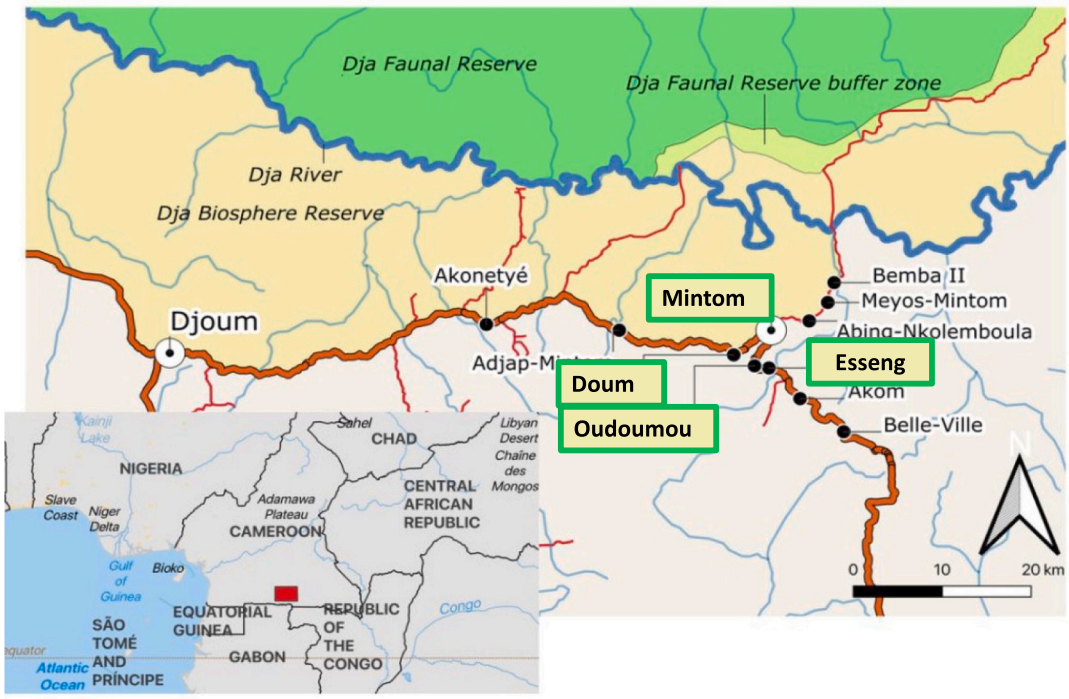

Fig. 1. Map of study area showing the location of villages (in yellow rectangles) in which samples were taken for this research.

Table 1
Distribution of samples collected, percentage of samples positive and number of pathogenic strains on utensils, surfaces and water in four settlements (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeast Cameroon.

Sample type	Minton	Oudomo	Doum	Esseng	TOTAL	% bacteria positive	% with pathogenic bacteria	n° pathogenic bacteria strains/sample type
Cooking and meat processing surfaces								
Broth	1	1	1	1	4	100	75	2.75
Cooking pot surface	4	7	5	5	21	100	85	2.24
Meat	1	0	0	2	3	100	100	2.00
Smoking basket	0	1	0	0	1	100	100	1.00
Meat contact surfaces	2	4	3	4	13	100	70	0.85
Water-related								
Wastewater	3	0	0	0	3	100	100	3.00
Cooking pot water	1	2	0	0	3	100	67	1.67
Water pump	4	0	0	2	6	100	67	1.33
Cutting tools and surfaces								
Machetes	1	6	7	6	20	100	75	1.20
Cutting surfaces	2	3	2	6	13	100	54	1.08
Meat transportation								
Hunter bag	1	2	3	5	11	100	81	1.55
Transport bag	1	0	1	0	2	100	50	0.50
No. pathogenic bacteria	35	40	38	41	154			
No. non-pathogenic bacteria	124	120	99	80	423			

microdilution method following Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2023, 2015) [14]. Bacterial inoculum was prepared on Mueller-Hinton (MH) II agar (Beckton Dickinson, USA) for the disc diffusion method, and antibiotic discs were placed. Briefly, bacterial colonies were resuspended in 0.9 % NaCl to achieve a 0.5 McFarland standard, equivalent to 1.5×10^8 CFU/mL and spread in Mueller-Hinton agar.

The antibiotics used were ampicillin (AM-10 μg), amoxicillin/clavulanic acid (AMC-30 μg), aztreonam (ATM-30 μg), amikacin (AN-30 μg), chloramphenicol (CHL-30 μg), ceftazidime (CAZ-30 μg), ciprofloxacin (CIP-5 μg), cefotaxime (CTX-30 μg), cefepime (FEP-30 μg), fosfomycin (FOS-50 μg), gentamicin (GM-10 μg), imipenem (IMI-10 μg), levofloxacin (LEV-5 μg), meropenem (MEM-10 μg), trimethoprim-sulfamethoxazole (SXT-1.25/23.75 μg), and tetracycline (TE-30 μg). The susceptibility to antibiotics was determined based on the measured diameter of the inhibition zone, following the guidelines outlined by the CLSI.

2.6. Statistics

The utensil/location combinations with three or four of the four screened settlements were used to fit a Generalized Linear Model (GLM) using R [15]. Data were not log-transformed but analysed using the Poisson distribution, which is adequate for count data [16]. Before applying the GLM, we checked for data overdispersion by comparing the mean, variance, and overdispersion ratio [17]. The overdispersion ratio was 0.95, which is near one and indicates no overdispersion, further supported by similar mean (0.31) and variance (0.40) values of the data. Collinearity was checked by variance inflation factors (VIF), which measures how much the variance of the estimated regression coefficients increases due to collinearity, using the R package "car" [18]. As in our case, the Generalized VIF for categorical predictors with more than two levels was 1.17, which is close to 1 and suggests that collinearity is not a problem for these predictors in the model. The proportion of zeros was calculated to check for zero inflation in the data. The percentage of zero values was 76 %, indicating zero-inflation. We attempted to fit a zero-inflated Poisson regression and subsequent Vuong test using the R package "pscl" [19]. However, computation resulted in a "computationally singular" warning, indicating a problem with the model fitting process, which remained unresolved. We then used a standard GLM for the count-based response variable describing the number of bacterial strains with the assumption of a Poisson distribution for the data and according to the formula:

$$\text{number of bacterial strains} \sim \text{Species} + \text{Utensil} + \text{Location}, \text{family} = \text{Poisson}\,(\text{link} = \log)$$

GLM coefficients represent the log of the expected bacterial counts for each level of the predictor variables, holding all other variables constant. A negative coefficient for a particular bacterial species or group, location and utensil implies that its presence is associated with a

Fig. 2. Utensils and surfaces sampled for bacterial contamination in four Baka pygmy villages (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeastern Cameroon: a) and b) machetes and cutting surfaces; c) and d) cutting surfaces; e) machetes, cooking pots and palm leaves as a contact surface with wild meat; f) traditional backpack to transport game meat; g), h) and i) cooking pots; j) game meat in small local markets; k) wastewater; and l) pump trough that supplies water to the local population.

lower count of colonies. In contrast, a positive coefficient suggests an association with a higher count.

3. Results

3.1. Number of samples

We obtained 100 samples from a range of utensils and surfaces (Fig. 2), including cooking pots ($n = 21$), machetes ($n = 20$), cutting surfaces ($n = 13$), surfaces in direct contact with wild meat (n = 13), hunter bags ($n = 11$), samples from pipes of the water pumps commonly used by local people ($n = 6$), wild meat broth ($n = 4$), water from cooking pots with food waste ($n = 3$), meat (n = 3), wastewater (n = 3), transport bags (n = 2), and a wild meat smoking basket (n = 1) (Table 1). The number of samples collected per type varied according to the opportunity of obtaining samples in each settlement (Table 1), with the total number of samples per settlement ranging from 21 in Mintom to 31 in Esseng.

3.2. Prevalence of bacteria by surface types

All analysed samples presented bacteria. Among them, 75 % contained pathogenic bacteria, with the highest percentage of pathogenic bacteria in cooking pots (85 %), hunter bags (81 %), machetes (75 %), broth (75 %), and cutting surfaces (70 %), presented the highest prevalence (Table 1). Among utensils, the percentage of cooking pots containing pathogenic bacteria was significantly higher than the rest ($p = 0.0006$).

3.3. Identification of bacterial strains

A total of 577 bacterial strains were isolated, 508 of which were identified using MALDI-TOF and 69 through 16S sequencing. One hundred fifty-four strains (27 % of all identified bacteria) were classified as pathogenic due to their potential to cause infection. The distribution of pathogenic bacteria per sample per village varied, with the lowest in Oudomo (1.2), followed by Esseng (1.6) and Doum (1.8), while the highest was recorded in Minton (2.2).

GLM revealed no statistically significant association between the count of bacterial colonies and location (Esseng: $p = 0.12$, Mintom: $p = 0.48$, Oudomo: $p = 0.93$). Six of 15 bacterial groups were significant with five showing a negative coefficient (*Clostridium difficile*, *Enterococcus* spp., *Mycobacterium* spp., *Neisseria* spp. and *Streptococcus parasanguis* each with GLM coefficient = −2.303 and $p = 0.028$) and one a positive coefficient (*Enterobacter* spp. with GLM coefficient = 0.916 and $p = 0.014$). One of six utensils or surfaces was significant with a positive coefficient (cooking pots with GLM coefficient = 1.167 and $p = 0.0006$).

The average number of strains (± SD) isolated per sample type varied between 0.25 ± 0.50 in wild meat smoking baskets and 5.25 ± 1.26 in cooking pots (Table 1). All bacteria isolated from cooking pots, smoking baskets, and wastewater were pathogenic, with above 70 % for machetes, broth, meat contact surfaces, and hunter bags and 50–69 % in cooking pot water, water pumps, and cutting surfaces.

Enterobacter cloacae was detected in 31 % of the samples, followed by *Klebsiella* spp. (*K. pneumoniae, K. oxytoca* and *K. varicola*) (23 %), *Pseudomonas* spp. (*P. aeruginosa* and *P. putida*) (21 %), *Stenotrophomonas maltophilia* (18 %), *Bacillus cereus* (15 %), and *Achromobacter* spp. (13 %). Less common forms were *Citrobacter freundii* and *Escherichia coli* (8 % each), *Serratia marcescens* (7 %), *Acinetobacter* spp. (5 %), and *C. difficile*, *Enterococcus*, *Mycobacterium*, *Neisseria,* and *S. parasanguis* (1 % each). Fifty percent (77/154) of the pathogenic bacteria detected belonged to the order Enterobacteriales (*E. coli, Klebsiella, Enterobacter, Citrobacter*, and genera *Serratia*).

Whereas the presence of *Enterobacter* ($p = 0.014$) was significantly higher than the others: *C. difficile* ($p = 0.028$), *Enterococcus* (p = 0.028), *Mycobacterium* (p = 0.028), *Neisseria* (p = 0.028) and *S. parasanguis* (p = 0.028) were significantly lower.

3.4. Antimicrobial resistance

Antimicrobial resistance among the order Enterobacteriales included 74 % resistance to AM, 48 % to AMC, 19 % to CIP, 18 % to SXT, 11 % to GM, and only 5 % to TE. In the case of *Stenotrophomonas* spp. results indicated that 16.7 % and 56 % were resistant to CAZ and SXT, respectively. However, only 24 % of the *Pseudomonas* strains were resistant to ATM, and the resistance to other antibiotics was less than 4.8 % (one strain). The complete results of the antimicrobial resistance by bacteria and antibiotics are shown in Table 2. In addition, five *Enterobacter* strains, five *Klebsiella* strains and three *Serratia* strains showed multidrug-resistant (MDR) profiles (Table 3) and Table 4.)

4. Discussion

Public health conditions in sub-Saharan Africa present a complex landscape shaped by a range of challenges and ongoing efforts to enhance health outcomes [20]. The region grapples with a high burden of infectious diseases, and this is exacerbated by limited access to essential healthcare services. Deficient healthcare infrastructure, including limited access to quality facilities, a shortage of healthcare professionals, and inadequate medical supplies, hinder the effective delivery of essential health services. In addition, disparities in healthcare access persist, with rural populations facing more significant trials than their urban counterparts.

Foodborne illnesses stand out as one of the most critical health problems in the Global South, in terms of frequency, economic costs and deaths [2]. These represent a persistent and substantial threat to public health in urban and rural societies. Rural communities are likely to face the highest health risk from foodborne diseases, given the greater persistence of contact with various sources of infection, not just from domestic animals but also from peri-domestic animals, those that live near human habitation [20], and from wildlife. Peri-domestic animals include a wide range of species, not only rats but also dogs and cats, which access food and household waste, including discarded viscera and may spread those micro-organisms.

A systematic mapping review of the literature on wild meat handling and zoonotic disease transmission (1996–2022) indicated that out of 43 zoonotic pathogens reported, 17 were bacteria, 15 viruses, and 11 parasites [21]. Most studies were undertaken in Europe, less in North America, and very few in Africa, South America, and Asia. These numbers indicate only the frequency of pathogens appearing in studies. However, the prevalence and impact of helminths and worm infections in contrast to bacterial infections is unknown, mainly in tropical countries. Helminths are the most common infectious agents of humans in the Global South and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis [22]. Changing climate will perpetuate, or perhaps exacerbate, public health issues and economic stagnation due to parasitic diseases [23]. The occurrence of parasitic worms versus bacterial contamination will depend on factors such as living conditions, the presence of vectors, and the availability and use of water, sanitation, and hygiene facilities.

Bacteria can colonise various food preparation surfaces, utensils, domestic dishcloths, sponges, and other cleaning materials, which are well studied in high-income countries [24–26] and can be transferred into food [27]. Knowledge of the propensity of bacteria in countries in the Global South is available for prepared foods (e.g. [28] in urban settings, where sanitation involves establishing a regimen to ensure the production of safe, wholesome food in a clean environment, posing minimal health threats to consumers. There is, however, little information on their spread to, and persistence on, surfaces in situations where there is no adequate cold chain, as in our study, thus making it difficult to quantify the actual burden of such pathogens in these environments or to estimate the risks they pose to consumers.

Table 2
Pathogenic bacteria species found by utensil in four settlements (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeast Cameroon.

Bacterial species	Cooking pot	Cooking pot water	Machetes	Cutting surfaces	Meat contact surfaces	Meat	Broth	Hunter's bag	Water pump	Wastewater	Smoking basket	Transport bag
Achromobacter spp.	2	2	5	4	0	0	1	1	0	0	0	0
Acinetobacter spp.	2	0	0	0	1	0	1	0	1	0	0	0
Bacillus cereus	3	1	3	1	3	0	1	0	0	2	0	0
Citrobacter spp.	4	0	2	0	0	0	1	1	0	0	0	0
Enterobacter spp.	7	1	5	4	4	3	2	7	1	0	0	1
Escherichia coli	6	1	0	0	1	0	0	2	0	0	0	1
Klebsiella spp.	8	0	1	2	4	1	1	2	2	2	1	0
Neisseria spp.	1	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas spp.	8	1	5	0	0	2	2	1	2	3	0	0
Serratia spp.	7	0	0	1	1	0	1	0	0	0	0	0
Stenothrophomonas spp.	10	0	2	3	0	0	1	4	3	1	0	0
Strptococcus parasanguis	1	0	0	0	0	0	0	0	0	0	0	0
Clostridium spp.	0	0	1	0	0	0	0	0	0	0	0	0
Enterococcus hirae	0	0	1	0	0	0	0	0	0	0	0	0
Streptococcus hominis	0	0	0	0	0	0	0	1	0	0	0	0
Mycobacterium spp.	0	0	0	0	0	0	0	1	0	0	0	0
Bacillus subtilis	0	0	0	0	0	0	0	0	0	1	0	0

Table 3
Antimicrobial resistance by antibiotics and bacteria identified in utensils and surfaces in four settlements (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeast Cameroon.

Bacteria)	No. isolates	AM	AMC	AN	ATM	CHL	CAZ	CIP	CTX	FEP	FOS	GM	IMI	LEV	MEM	SXT	TE
Enterobacter spp.	31	28 (90.3)	24 (77.4)	0	0	0	0	0	0	0	0	3 (9.7)	0	0	0	6 (19.4)	2 (6.5)
Klebsiella spp.	23	16 (70.0)	3 (13.0)	0	0	1 (4.3)	0	0	0	0	0	4 (17.4)	0	0	0	30 (90.0)	0
Citrobacter spp.	8	7 (87.5)	3 (37.5)	0	0	0	0	0	0	0	0	0	0	0	0	4 (50.0)	0
Serratia spp.	7	5 (71.4)	5 (71.4)	0	0	0	0	0	0	0	0	1 (14.3)	0	0	0	0	2 (28.6)
Stenotrophomonas spp.	18	nr	nr	0	nr	nr	3 (16.7)	0	0	0	0	nr	0	1 (5.6)	0	10 (56.0)	0
Pseudomonas spp.	21	1 (4.8)	1 (4.8)	0	5 (24)	1 (4.8)	0	0	0	0	0	1 (4.8)	0	0	1 (4.8)	0	0

Data is presented as Number of resistant bacteria (percentage). Ampicillin (AM), amoxicillin/clavulanic acid (AMC), amikacin (AN), aztreonam (ATM), chloramphenicol (CH), ceftazidime (CAZ), ciprofloxacin (CIP), cefotaxime (CTX), cefepime (FEP), fosfomycin (FOS), gentamicin (GM), imipenem (IMI), levofloxacin (LEV), meropenem (MEM), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE). nr, natural resistance.

Table 4
Multidrug-resistant (MDR) profiles identified among bacteria in utensils and surfaces in four settlements (Doum, Oudoumou, Mintom, Esseng) on the Djoum-Mintom road in southeast Cameroon.

Bacterial genera	MDR profile	Sample	Settlement
Enterobacter spp.	AM-AMC-SXT	Meat	Esseng
	AM-AMC-GM-SXT	Cooking pot	Esseng
	AM-AMC-SXT-TE	Water pump	Minton
	AM-AMC-SXT-TE	Meat	Minton
	AM-GM-SXT	Cooking pot	Oudoumou
Klebsiella spp.	AM-GM	Cooking pot	Esseng
	AM-AMC-GM	Cooking pot	Esseng
	AM-SXT	Water pump	Esseng
	AM-CHL-SXT	Wastewater	Minton
	AM-SXT	Meat	Minton
Serratia spp.	AM-AMC-SXT-TE	Wood surface	Minton
	AM-AMC-SXT	Cooking pot	Oudoumou
	AM-AMC-GM-SXT	Cooking pot	Oudoumou

Ampicillin (AM), amoxicillin/clavulanic acid (AMC), chloramphenicol (CH), gentamicin (GM), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE).

There is a significant knowledge gap regarding bacterial pathogens and antimicrobial-resistant bacteria in meat from wild animals or wildlife. The limited literature on studies conducted in sub-Saharan Africa shows the presence of several potentially dangerous zoonotic pathogens in meat from different wild species, including those of the genera *Bacillus, Brucella,* and *Coxiella* [29], *Klebsiella pneumonia* and *Micrococcus caseolyticus* [25], *Escherichia coli* and Shiga-toxin *E. coli* (STEC), *Campylobacter coli* [30], and *Salmonella* spp. [31,32].

Among foodborne pathogens, the most common bacteria include *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes, Bacillus* spp., *Yersinia* spp., *Campylobacter* spp., *Clostridium botulinum, Clostridium perfringens, E. coli, Staphylococcus aureus and Vibrio cholerae* [29]. However, studies of bacterial contamination in worldwide poultry and retail poultry products have focused on *Campylobacter* [33,34]. In chickens sold in Yaoundé, Cameroon, contamination with *Campylobacter*, *E. coli*, and *Salmonella* has been widely reported [35]. In Ogun state, Nigeria, high levels of *Bacillus cereus* contamination have been found in several retailed foods [36]. Samples from raw meats sold in butcher shops in Ethiopia showed high levels of aerobic mesophilic, Staphylococci, Enterobacteriaceae, total coliform, faecal coliform, aerobic spore formers, and yeasts and moulds [37]. Further characterisation of the aerobic mesophilic flora indicated a dominance by Enterobacteriaceae,

Staphylococci spp. and *Bacillus* spp. with a significant prevalence of *S. aureus*, *E. coli*, and *Salmonella* in meat and swab samples.

Our study found a high prevalence of pathogenic bacteria, not dissimilar to the strains reported in the various studies of retailed meat available in some African countries. The fact that bacteria that can cause food poisoning and other illnesses were found on a few different surfaces, not only on meat, is enough to generate caution. Without refrigeration and proper hygiene, bacteria can rapidly proliferate in warm and moist conditions. A single bacterial cell on a food item left unrefrigerated overnight can multiply into millions by morning [38]. Although survival and transfer of bacteria in controlled laboratory conditions decline after drying on soiled surfaces and cloths, Gram-positive and some Gram-negative species can persist for up to four hours and sometimes up to 24 h [39]. Moreover, when contaminated surfaces or cloths come into contact with fingers, a stainless-steel bowl, or a clean laminate surface, sufficient organism transfer occurs, posing a potential hazard if in contact with food. This result confirms the ease with which contaminated surfaces can be the source of infection of foods, preparation areas and cooking utensils. Besides cooking and meat preparation surfaces, we also obtained samples from water-related surfaces, namely water pumps, water in cooking pots and wastewater. We found that water from these sources also contained pathogenic bacteria (and more so in sewage, as expected), though less than the other samples. This finding confirms that others, e.g., in Cajamarca, Peru [40], show that household water contamination at the point of use can be a source of thermotolerant coliform and antibiotic-resistant bacteria.

Our study underscores the intricate relationship between the handling of foods and the prevalence of bacterial pathogens. We highlight an alarming number of pathogenic bacteria found in different surfaces and utensils, enough to describe the situation in our study settlements, which are of critical public health concern. We identified pathogenic strains such as *Clostridium difficile*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Mycobacterium*, indicating potential health hazards, mainly through diarrheal infections. These pathogens represent a significant risk to human health, as they can be transmitted between wildlife and domestic and peri-domestic animals. Domestic and peri-domestic animals can serve as reservoirs for these pathogens, facilitating their spread and persistence. This continuous cycle of pathogen transmission between wildlife and other animals linked to humans increases the potential for outbreaks and poses a substantial threat to public health. Effective measures are necessary to monitor and control the circulation of these pathogens to protect both animal and human populations.

The presence of *Enterobacter* on cooking surfaces poses a serious public health concern due to the risk of foodborne illnesses. Enterobacter species, known for opportunistic pathogenicity, can lead to infections if contaminated food is consumed. Cross-contamination is a significant issue, as the bacteria can be transferred from surfaces to ready-to-eat foods, utensils, or hands, increasing the potential for the spread of infections. The resilience and persistence of *Enterobacter* on surfaces create challenges in maintaining a hygienic cooking environment. The risk of healthcare-associated infections is elevated in settings with vulnerable populations, such as hospitals. Additionally, antibiotic resistance in *Enterobacter* raises concerns about the difficulty in treating diseases.

In our study area, notably among the Baka Pygmies (as observed in [41]), individuals with a robust immune system may experience minimal impacts from certain bacteria. However, most people in this region, particularly the Baka, face nutritional challenges [42] that likely result in compromised immune systems. Weakened immunity increases vulnerability to foodborne illnesses, potentially resulting in symptoms such as diarrhoea, vomiting, abdominal pain, or more severe health issues [43]. Detecting pathogenic strains in the environment raises pertinent questions about establishing a cost-effective, safe threshold, especially given the potential for severe infections such as wounds, digestive, respiratory and urinary infections, and sepsis [44].

The elevated bacterial levels found in our study extend beyond food surfaces and utensils since we found bacteria in water-related features. Such heightened bacterial presence in food- and water-related contexts underscores the urgency of addressing inadequate hygiene measures to mitigate the risks associated with foodborne illnesses. Although we did not investigate adherence to hygienic practices, our observations and results suggest that everyday items like cooking pots and food preparation surfaces are not cleaned with water (and not with soap) since neither is generally available, serving as reservoirs for bacteria. However, this requires water with adequate microbiological quality. Our study found pathogenic bacteria in commonly used waters that would cause additional cross-contamination when washing surfaces, utensils, and meat. Therefore, acquiring appropriate behaviours does not guarantee the reduction of pathogenic bacteria if the availability of microbiologically adequate water is not guaranteed. The broader public health impact is notable, as outbreaks or sporadic foodborne illnesses can strain healthcare systems and result in adverse health outcomes. To address these risks, strict adherence to food safety practices, regular cleaning, and sanitation of cooking surfaces are crucial to prevent the contamination and transmission of Enterobacterales in food preparation environments.

The significance of bacteria, mainly those resistant to multiple antibiotics as found in our study, presents a growing challenge for disease control and public health safety. Antibiotic resistance is one of the biggest threats to public health worldwide. Currently, antibiotic-resistant bacteria kill 700,000 people each year [45]. In the Global South, several factors aggravate this problem, such as the high level of misuse of antibiotics, the unregulated sale of medicines, the high rates of self-medication, and the inappropriate use in animals and agriculture, among others [46]. Thus, the percentage of farms using antimicrobials in animal production is 100 % in Cameroon, with the most commonly used tetracyclines, aminoglycosides, and penicillin [47]. A recent report estimates that AMR could cost LMICs 5 % of their gross domestic product and lead 28 million people into poverty by 2050 [48].

The National Situation of Antimicrobial Resistance and Consumption Analysis from 2017 to 2019 [49] reported that combinations of sulphonamides and trimethoprim were the most frequently consumed Anatomical Therapeutic Chemical (ATC) class in Cameroon overall for the review period (2017–2019) at 46.5 % in 2017, 15.2 % in 2018 and 16.1 % in 2019. Thus, the top five most consumed antimicrobials were trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid, doxycycline, amoxicillin and fluconazole, accounting for 68 % of total consumption share.

In a systematic review and meta-analysis carried out [50] on AMR from a One Health perspective in Cameroon, they found that the high resistance rates observed among humans are against SXT, TE, and AMC; the same happens in animals (food-producing animals are more resistant to AM, AMC, SXT, and TE). Nine studies related to AMR in environmental samples were included in the analysis, but none studied Enterobacteriacea.

The fact that higher AMR rates found in our study were against AM, AMC, CIP, and SXT could be related to the previous data on antimicrobial consumption and AMR in different sectors. Thus, their multiple interactions can easily transfer AMR bacterial strains among humans, animals, and the environment. In the present case, the factors contributing to pathogenic strains on utensils and surfaces include cross-contamination due to unhygienic food handling practices, insufficient handwashing education, the presence of animals and children in food preparation areas, and the type of surfaces used.

One critical question from the study pertains to the efficacy of current cleaning practices, specifically the role of soap in reducing bacterial load. While washing with water alone may remove some bacteria, soap is crucial for eliminating pathogens, especially those forming biofilms resistant to simple rinsing. Moreover, the type of pot used for cooking may influence bacterial growth due to differences in surface porosity and heat retention. The economic implications of improper meat

handling and resulting foodborne illnesses are noteworthy, encompassing medical expenses and lost work productivity.

Recommendations for future research should focus on determining contamination sources, understanding factors contributing to the survival and growth of pathogenic strains, and quantifying acceptable levels of bacterial presence. Studying local behaviours that minimise or mitigate risks and developing culturally sensitive strategies for mitigation are essential. Considering the ecological, cultural, and socioeconomic context is also imperative for tailoring interventions effectively. These can be relatively low-cost and have the potential to make a significant impact on public health. Regular handwashing with soap and water is a robust preventive measure that helps eliminate harmful bacteria and viruses from the hands, which is crucial in a region where infectious diseases are prevalent. Proper handwashing reduces the risk of pathogen transmission and prevents cross-contamination during food preparation, thus promoting overall personal hygiene and community health. Equally, thoroughly washing surfaces and utensils is essential since this practice removes residual contaminants, including bacteria and viruses. It prevents cross-contamination between raw and cooked foods and ensures safe food handling. Clean utensils play a vital role in minimising the multiplication of bacteria, reducing the incidence of foodborne illnesses.

Fostering behavioural change requires a concerted effort to promote awareness and education. Campaigns designed to underscore the significance of handwashing and utensil hygiene can be pivotal in encouraging the adoption of these practices into daily routines. This educational outreach informs individuals about the importance of such habits and empowers them to take charge of their health and that of their communities. Community engagement also plays a critical role in this process, catalysing spreading awareness and fostering a collective sense of responsibility. Integrating these initiatives with water and sanitation programs is paramount, with access to clean water indispensable for effective handwashing and utensil cleaning.

Nevertheless, a persistent challenge in this endeavour is the availability of soap. While local soap production offers a viable solution, procuring ingredients, particularly oils, may present complications. Oils hold dual significance as they are crucial for soap production and serve as essential components in many communities' nutrition through their cooking use. Addressing this challenge requires a nuanced approach that balances the need for hygiene with the nutritional requirements of the local population.

The overarching objective of our investigation into the presence of bacteria on food processing and cooking surfaces must be the formulation of comprehensive and realistic guidelines. These must mitigate immediate health risks associated with foodborne diseases among rural societies, particularly poorer populations. Such guidelines are paramount to safeguarding vulnerable populations, with a particular emphasis on children who are inherently more susceptible to the adverse effects of these diseases. We can establish a robust framework that directly addresses health risks by delineating clear and evidence-based recommendations for sanitation and hygiene practices related to food processing and cooking surfaces. These guidelines should encompass meticulous protocols for the cleaning and disinfection of surfaces, ensuring the removal of harmful bacteria that could otherwise compromise the safety of the food supply chain. Moreover, the emphasis should extend beyond eliminating bacterial contamination and delve into preventive measures. Educating communities, especially caregivers and food handlers, on proper hygiene practices will be integral. This educational aspect should highlight the significance of handwashing, utensil hygiene, and the overall cleanliness of food preparation environments. As we progress, ongoing research and collaboration between the scientific community, public health authorities, and local communities will be essential in effectively refining and implementing these guidelines. This holistic approach holds the promise of fostering healthier environments, enhancing food safety, and ultimately improving the overall quality of life for individuals, particularly the most vulnerable.

CRediT authorship contribution statement

Sara M. Soto: Writing – review & editing, Methodology, Funding acquisition. **Laura Castellsagués:** Writing – review & editing, Investigation. **Victoria Ballén:** Methodology, Investigation. **Yaiza Gabasa:** Writing – review & editing, Methodology. **Pedro Mayor:** Writing – review & editing, Validation, Investigation, Conceptualization. **Guillermo Ros Brull:** Writing – review & editing, Methodology, Conceptualization. **Stephan M. Funk:** Writing – review & editing, Formal analysis, Data curation. **Julia E. Fa:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

This is to declare that none of the authors in the submitted manuscript "Prevalence of bacterial contamination on wild meat processing and cooking surfaces in rural Cameroon" have any interests to declare.

Data availability

Data will be made available on request.

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