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Prevalence of *Klebsiella pneumoniae* isolated from public universities' restaurants of Abidjan, Côte d'Ivoire

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ABSTRACT

This study was conducted to determine the prevalence of *Klebsiella pneumoniae* in cooked and sold food samples obtained from private and public on campus restaurants of Félix Houphouët-Boigny University and Nangui Abrogoua University of Abidjan, Côte d'Ivoire, to identify the antibiotic resistance genes and to determine the resistance profile of *K. pneumonia*e. Bacteriological analyses consisted of the enrichment of buffered peptone broth followed by culture on Violet Red Bile Glucose Agar (VRBG) medium. The characteristic colonies were subjected to biochemical identification by analytical profile index (API) 20E kit. Twenty-four *K. pneumoniae* were isolated from cooked and served samples. The frequencies of *K. pneumoniae* isolates were 8.3% (2/24) in rice samples, 20.8% in fish soup samples, 4.2% in attiéké samples, 37.5% in raw fresh vegetable samples, and 29.2% in fried fish samples. Thirteen *K. pneumoniae* strains harbored the beta-lactamase gene (blaSHV). Antimicrobial resistance profile of *K. pneumoniae* against nine antibiotics showed high resistance rates for amoxicillin (92.3%), amoxicillin + clavulanic acid (76.9%), ticarcillin + clavulanic acid (76.9%), ceftriaxone (92.2%), cefotaxime (92.2%), and ceftazidime (92.2%). Our findings raise concerns that food cooked and sold by university's private and public restaurants poses a serious threat to consumers' health since *K. pneumoniae* that harbored blaSHV genes was found in this food. Authorities of universities should undertake sanitation control and prevention strategies in order for restaurants to improve the food quality and hygiene of the restaurant environment and staff.

1. INTRODUCTION

Public eating places like restaurants and canteens, fast foods, and popular foods on street have become today the major elements of urban development. They allow more than 80% of the cities' populations to satisfy their nutritional needs [1]. There is no law or legislation on the universities' public restaurants. Despite their social-economic importance, the hygienic affairs in the common eating places systems are still a major worry for the consumers. In fact, taking into account the huge quantity of daily cooked foodstuffs, low instruction, and training of manpower, the hygiene rules are most of the time neglected in our African countries particularly, where the lower level of training is recorded [2].

If the universities' public eating places are kept up and continue to grow, this means an increase in needs. However, the multiplication of serious food-infected diseases has been reported in recent years in several African countries, related to the proliferation of restaurants according to the WHO in 2009. Actually, more than 250 diverse diseases coming from food poisoning are described and the bacteria are reported to be the cause of 2/3 of these epidemics [3]. Among the predominant bacteria, enterobacteria are involved in these diseases. Enterobacteria are the normal hosts found in human and animal digestive tubes [4].

Klebsiella pneumoniae is an important enterobacteria. It is an opportunistic pathogen causing several diseases and is acquiring resistance to several antibiotics [5,6]. This organism is responsible for about one-third of all Gram-negative infections such as urinary tract infections, cystitis, pneumonia, surgical wound infections, endocarditis, and septicemia [7–9]. It also causes necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis [10,11]. *Klebsiella pneumoniae* can be found in the respiratory and intestinal tracts of

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humans and animals. It is frequently isolated from foods, including fresh legumes, powder foods for babies, meats, fish, and street foods [12–14]**.** Infections with *K. pneumoniae* can be transmitted through contaminated food or water and can be associated with community-acquired infections or between persons and animals involved in hospital-acquired infections.

The emergence of antimicrobial resistance is causing serious health problems nowadays worldwide. In Côte d'Ivoire, bacteria resistance to antibiotics and the emergence of bacteria producing the ELSB are becoming a serious public health problem [15–17]. Some previous works had reportewd the presence and expansion of human enterobacteria strains producing ELSB [18,19]. *Klebsiella pneumoniae* has developed resistance to several antibiotics through the production of enzymes such as extended-spectrum β-lactamase (ESBLs) and carbapenemase [20,21]. The production of ESBLs is encoded by several genes such as blaSHV, blaTEM, and blaCTM [22,23].

The aim of this study was to determine the prevalence, identify the antimicrobial resistance genes, and determine the resistance profile of *K. pneumonia*e isolated from cooked and sold food samples.

2. MATERIALS AND METHODS

2.1. Sample Collection

This study was conducted from June to September 2017 and then from November 2017 to February 2018. It was carried out on 160 of cooked and sold food samples of rice and cassava powder called "attiéké," fish soups, fried fish, and raw fresh vegetables collected from the public and private restaurants of the two main public universities, Nangui Abrogoua University (UNA) and Félix HouphouëtBoigny University (UFHB). The choice of the private collective restaurants was based on their attendance by the students and the choice of meals consumed by them. The samples were taken in sterile bowls and sent to the laboratory for analysis.

2.2. Isolation and Identification of *K. pneumoniae*

Ten (10) g of each sample was suspended in aseptic conditions in 90 ml of buffered peptone water previously autoclaved for 15 minutes at 121°C. The sample suspensions were homogenized for 30 seconds. From the suspension, a serial dilution was conducted up to 10^{-5}. From each fold dilution, 0.1 ml was streaked directly onto VRBG agar plates and incubated for 24 hours at 37°C. Red violet colonies of 0.5 mm in size were transferred to nutrient agar plates. All the suspected *K. pneumoniae* isolates were checked for their Gram-staining reaction, indole production, and motility. The selected colonies were further identified by biochemical tests using API 20^{E} kits (BioMérieux, France).

2.3. Molecular Characterization of Beta-Lactamase Genes

The presence of acquired extended-spectrum beta-lactamase genes (blaTEM, blaSHV, and blaCTX-M_{15}) was investigated by multiplex polymerase chain reaction (PCR) assays. Representative colonies of *K. pneumoniae* were cultured on nutritive broth at 37°C for 24 hours. Then, the cell pellet was centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and 400 µl of lysed buffer was added to the cell pellet and then mixed for 1 minute. Three hundred microliters of chloroform was added and then mixed again for 1 minute. Twenty µl of sodium acetate and 400 µl of ethanol were added to the supernatant and incubated at −20°C for 1 hour. The mixture was centrifuged at 12,000 rpm for 10 minutes. The supernatant is discarded, and 200 µl of isopropanol was added to the cell pellet and then centrifuged at 12,000 rpm for 10 minutes. The next step was the elimination of alcohol by drying the pellet at room temperature or for 30 minutes. Finally, 50 µl of TE buffer was added to the pellet and incubated at −20°C until use. The total DNA in the supernatant was thereafter precipitated with 70% ethanol and used as the template for PCR.

All the oligonucleotide primers have been synthesized by Sangon Biotech (Table 1). The mixture (25 µl) used for PCR included 12.5 µl of Dream Taq TM Green PCR Master Mix (Fermentas, Waltham, MA), 2 µl of primers, and 3 µl of the template DNA. PCR amplification was carried out in a thermocycler, Bio-Rad PTC-200 (Bio-Rad, Hercules, CA), according to the following cycles: initial denaturation at 95°C for 5 minutes, followed by 40 cycles at 95°C for 30 seconds; for SHV gene, 40 cycles of hybridization for 30 seconds at 53°C; for TEM gene, 40 cycles of hybridization at 50°C for 30 seconds; for CTX-M-15 gene, 40 cycles of hybridization at 49°C for 30 seconds; and the initial elongation at 72°C for 1 minute and one final elongation cycle at 72°C for 5 minutes in a thermocycler. *Klebsiella pneumoniae* T300, obtained from Pasteur Institute of Abidjan, was used as a positive control. The amplified products were analyzed by electrophoresis on 1.5% agarose gel composed by Gold View (0.005% v/v) (SBS Genentech, Beijing, Chine) in Tris-buffered saline (TBS) buffer 1× (Tris-HCl 40 mM, 1,18 ml acetate acid, ethylenediaminetetraacetic acid (EDTA) 2 mM

Table 1: Primers used for PCR amplification.

Genes	Type	Primer sequences	Reheat temperature	Amplicon height (bp)
SHV	Fw	5′-ACTATCGCCAGCAGGATC-3′	53°C	356
	Rev	5′-ATCGTCCACCATCCACTG-3′		
TEM	Fw	5′-GATCTCAACAGCGGTAAG-3′	50°C	786
	Rev	5′-CAGTGAGGCACCTATCTC-3′		
CTX-M_{15}	Fw	5′-GTGATACCACTTCACCTC-3′	49°C	255
	Rev	5′-AGTAAGTGACCAGAATCAG3′		

Source: Neuberger *et al*. [24].

pH 8,0), and the bands were visualized using a system of capture, Image Quant 350 (GE Healthcare, Waukesha, WI).

2.4. Antibiotic Susceptibility Tests

The susceptibility test of *K. pneumoniae* to antibiotics was determined on Mueller Hinton agar by diffusion method on disk Kirby–Bauer according to Clinical and Laboratory Standards Institute instructions. Commercially available antimicrobial discs that were used included amoxicillin (30 µg), amoxicillin + clavulanic acid (30 µg), ticarcillin + clavulanic acid (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ertapenem (30 µg), gentamicin (15 µg), tetracycline (10 µg), and ciprofloxacin (30 µg). The measurement of the inhibition zone diameter around the antibiotic discs was carried out and compared with the Tables of international measurements.

Table 2: Identification of enterobacteria.

Identified species	*Number of isolates*	*Occurrence frequency (%)*
Klebsiella pneumoniae	24	20.3
Klebsiella oxytoca	21	17.8
Serratia liquefaciens	18	15.3
Enterobacter cloacae	14	11.8
Enterobacter aerogenes	11	9.3
Pantoea spp	11	9.3
Serratia odorifera	4	3.9
Escherichia coli	2	1.7
Shigella spp	2	1.7
Kluyvera spp	2	1.7
Cronobacter spp	1	0.8
Enterobacter amnigenus	1	0.8
Citrobacter koseri	1	0.8
Buttiauxella agrestis	1	0.8
Enterobacter asburiae	1	0.8
Burkholderia cepacia	1	0.8
Raoultella ornithinolytica	1	0.8
Rahnella aquatilis	1	0.8
Serratia rubidaea	1	0.8
Total	118	100

3. RESULTS

3.1. Identification of *K. pneumoniae*

A total of 24 strains of *K. pneumoniae* out of 118 enterobacteria were isolated from cooked rice, "attiéké," fried fish, fish soup, and raw fresh Vegetables (Table 2).

3.2. Contamination Rate of Food Samples with *K. pneumoniae* Isolates

The contamination rates of *K. pneumoniae* in cooked and sold food at the universities' private and public restaurants are presented in Table 3. In total, 160 cooked food samples were tested in this study, and *K. pneumoniae* was isolated from 24 samples. Overall, 15% of the food samples were positive for *K. pneumoniae*. *Klebsiella pneumoniae* was isolated from 8.3% of rice samples, 20.8% of fish soup samples, 4.2% of "attiéké" samples, 37.5% of raw fresh vegetable samples, and 29.2% of fried fish samples.

At UNA, 13 (54.2%) *K. pneumoniae* strains were isolated from public and private restaurants. *Klebsiella pneumoniae* was mostly isolated from all the food samples: rice samples [2 (8.3%)], fish soup samples [4 (16.7%)], "attiéké" [1 (4.2%)], raw fresh vegetables [3 (12.5%)], and fried fish [1 (4.2%)] from private restaurants. *Klebsiella pneumoniae* was isolated only from fried fish samples [2 (8.3%)] from the public restaurant. At UFHB, 11 (45.8%) *K. pneumoniae* strains were isolated from public and private restaurants. *Klebsiella pneumoniae* was mostly isolated from three food samples fish soup samples [1 (4.2%)], raw fresh vegetables [2 (8.3%)],

Table 3: Contamination rates of *K. pneumoniae* isolates from all the food samples.

	Sources					
	Rice	**Sauce**	**"Attiéké"**	**Vegetables**	**Fish**	**Total**
Number of strains *N* (%)	2 (8.3)	5 (20.8)	1 (4.2)	9 (37.5)	7 (29.2)	24 (100)

Table 4: Contamination rates of *K. pneumoniae* isolates per sold food from universities restaurants.

	Sources										
	Rice		**Fish soup**		**"Attiéké"**		**Vegetables**		**Fried fish**		**Total**
	P	**C**	**P**	**C**	**P**	**C**	**P**	**C**	**P**	**C**	
UNA isolates *N* (%)	2 (8.3)	0	4 (16.7)	0	1 (4.2)	0	3 (12.5)	0	1 (4.2)	2 (8.3)	13 (54.2)
UFHB isolates *N* (%)	0	0	1 (4.2)	0	0	0	2 (8.3)	4 (16.7)	1 (4.2)	3 (12.5)	11 (45.8)

P: Private restaurant; C: Regional Center for University Works (CROU) restaurant.

Table 5: Contamination rates of *K. pneumoniae* isolates per sold food from private and public restaurants.

	UNA			UFHB		
	Rice and fish soup	"Attiéké"-vegetables-fried fish	Total	Rice and fish soup	"Attiéké"-vegetables-fried fish	Total
Public restaurant *N* (%)	0	2 (8.3)	2 (8.3)	0	7 (29.2)	7 (29.2)
Private restaurants *N* (%)	6 (25)	5 (20.8)	11 (45.8)	1 (4.2)	3 (12.5)	4 (16.7)

UNA: University of Nangui Abrogoua; UFHB: University of Félix Houphouët-Boigny.

and fried fish [1 (4.2%)] from private restaurants. *Klebsiella pneumoniae* was isolated from two food samples, raw fresh vegetables [4 (16.7%)] and fried fish samples [3 (12.5%)] from public restaurants (Table 4).

The contamination rates of *K. pneumonia* isolates per meal served in private and public restaurants were reported in Table 5. In the public restaurants, *K. pneumonia*e was isolated only from 2 (8.3%) and 7 (29.2%) of "attiéké"–vegetables–fried fish samples from UNA and UFHB, respectively. *K. pneumonia* was isolated from 6 (25%) of rice–fish soup samples and 5 (20.8%) of "attiéké"–vegetables–fried fish samples served in the private restaurants of UNA. *Klebsiella pneumoniae* was isolated from 1 (4.2%) of rice–fish–soup samples and 3 (12.5%) of "attiéké"–legume–fried fish samples served in the private restaurants of UFHB.

3.3. Antimicrobial Resistance Determinants of the *K. pneumoniae* Isolates

According to the results of antimicrobial susceptibility testing, 24 strains were tested for ESBL genes and 3 β-lactamase genes were amplified. As shown in Figure 1, 13 out of 24 strains isolated carried only the blaSHV gene. BlaTEM and blaCTX-M-15 genes were not detected in these isolates.

3.4. Antibiotic Resistance Profile of *K. pneumoniae*

Antimicrobial susceptibility testing was conducted for the 13 *K. pneumoniae* isolates producing the blaSHV gene, and detailed information on the resistance rates to all of the tested antimicrobials is listed in Table 6. The susceptibility profile of *K. pneumoniae*

Table 6: Antimicrobial resistance rate of (n = 13) *K. pneumoniae* ESBL isolated.

	Frequency *N* (%)		
Antibiotics	**Sensitive**	**Intermediate**	**Resistant**
Amoxicillin	0	0	12 (92.3)
Amoxicillin–clavulanic acid	0	3 (23.1)	10 (76.9)
Ticarcillin–clavulanic acid	0	3 (23.1)	10 (76.9)
Ceftriaxone	0	1 (7.7)	12 (92.2)
Cefotaxime	0	1 (7.7)	12 (92.2)
Ertapenem	10 (76.9)	3 (23.1)	0
Gentamicin	3 (23.1)	2 (15.4)	8 (61.5)
Tetracycline	11 (84.6)	0	2 (15.4)
Ciprofloxacin	1 (7.7)	0	12 (92.2)

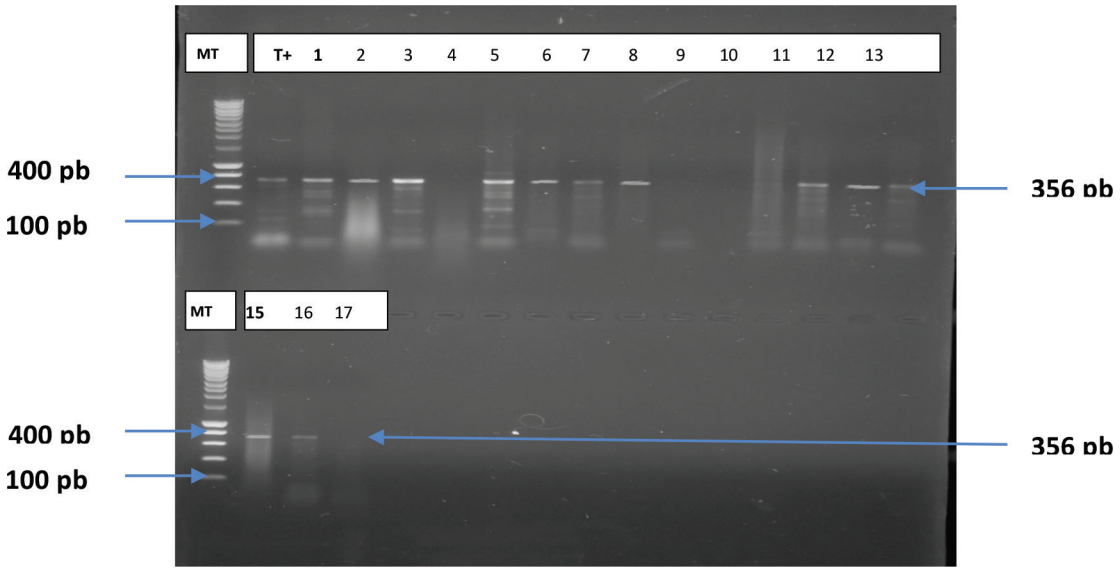

Figure 1: Electrophoretic profile indicating the presence of the gene in *K. pneumoniae* isolates MT: marker height (100–1,000 bp), lines 1–17: isolates tested, and T+: positive control.

strains revealed high resistances to beta-lactams: amoxicillin (92.3%), amoxicillin + clavulanic acid (76.9%), ticarcillin + clavulanic acid (76.9%), ceftriaxone (92.2%), cefotaxime (92.2%), and ceftazidime (92.2%). The resistance rates observed for the other antibiotics were 61.5% to aminosids and 92.2% to quinolones. None of the strains were resistant to tetracycline (84.7%) and ertapenem (67.9%).

4. DISCUSSION

This study revealed a high prevalence of enterobacteria with a dominance of *K. pneumoniae* in cooked and sold foods in private and public restaurants of the two universities. Haryani *et al*. [25] also found a high prevalence of *K. pneumoniae* in street foods. According to Mélanie [26], the high prevalence of *K. pneumoniae* was due to the lack of hygiene in the cooking of foods and to the contamination of raw products (hand preparation, use of poor water quality, and insufficient cleaning of fish and vegetable). Barro *et al*. [1] reported that, in collective food, the hygiene rules are most of the time neglected in accordance with the enormous quantity of foods daily cooked and the low level of education of restaurant staff. According to Tereza Gelbíčová *et al*. [27], *Klebsiella* spp is not generally considered as a major foodborne pathogen but a hygiene indicator with regard to food production. Our study revealed the presence of *K. pneumoniae* in cooked food from private and public restaurants. According to the Public Health Agency of Canada [28] and Sri Harminda *et al*. [29], this may result from its thermotolerance. These authors found that complete inactivation of the bacteria may not always occur at 60°C.

A high prevalence of *K. pneumoniae* was observed in the samples of "attiéké" with vegetables (tomato, pepper, and onion) and fried fish in the public and private restaurants of the two universities. These results are in agreement with the results from the survey of Falomir *et al*. [30] on ready-to-eat food samples, which showed that *K. pneumoniae* was detected in mixed vegetables rice dishes (25%, 13 out of 52). Anoman [31] also reported the presence of pathogens into "garba" (a kind of "attiéké") with raw fresh vegetables and fried tuna fish sold in Côte d'Ivoire. Belomaria *et al*. [32] also found that fresh vegetables are more involved in toxic food poisoning. Consumption of raw or slightly cooked vegetables can increase the risk of foodborne disease. According to some researchers [33,34], poor hygienic practices and improper handling and cooking are considered major factors for contamination of foods. The presence of *K. pneumoniae* in the cooked and sold foods by the university restaurants indicated potential fecal contamination, possible cross-contamination between food handlers, food preparation, surfaces, and the food itself [25]. The attiéké meal is also served with fried tuna or horse mackerel fish. These types of fish can be contaminated with harmful enteric bacteria. According to Duflos [35], histamine-producing bacteria such as enterobacteria are commonly found in tuna fish. Insufficient cooking and improper handling and storage after cooking by the kitchen staff can contaminate the fish product. Also, *K. pneumoniae* may be spread from person to person or from person to item during food preparation and serving.

In our study, 13 strains of *K. pneumoniae* isolates harbored the blaSHV gene only. Perilli *et al*. [36] and Dayan *et al*. [37] also found that the blaSHV gene was the most dominant ESBL genotype in their studies carried out in Italy and Spain, respectively. However, ESBL blaCTX-M is the most prevalent gene found in a high proportion of samples from different parts of the world [38,39]. But in our study, blaCTM was not detected in the isolates. Finding *K. pneumoniae* isolates that carry antimicrobial resistance genes could potentially be an indirect public health hazard, regardless of their pathogenicity, because the available genetic pool of resistance is increased [40]. Interestingly, 13 out of 24 strains of *K. pneumoniae* isolates positive for the blaSHV gene were resistant to beta-lactams (amoxicillin, amoxicillin + clavulanic acid, ticarcillin + clavulanic acid, ceftriaxone, and cefotaxime). These data corroborate the finding of Gadou [41] who reported that 96.7% of *K. pneumoniae* strains were resistant to ceftriaxone and 95.6% to cefotaxime. Mathlouthi *et al*. [42] also reported that 84% of *K pneumoniae* strains were resistant to ceftriaxone and 97% to cefotaxime. This study reports that *K. pneumoniae* from cooked food samples can be resistant to multiple antibiotics. The major reasons for *K. pneumoniae* resistance to antibiotics including beta-lactam antibiotics are the production of a broad-spectrum beta-lactamase (ESBL), the changing of the permeability barrier or the target site represented by penicillin-binding protein, or alteration of outer membrane protein [43]. Additionally, *K. pneumoniae* have many efflux pumps that expel the antibiotic to the outside [44,45]. In this study, *K. pneumoniae* was also found to be highly resistant to quinolones at the rate of 92.2% and to aminosids at 61.5%. The resistance of *K pneumoniae* to aminosids and quinolones has also been reported by Gadou [41], at the respective resistance rates of 90% and 86.7%. Kouassi-M'bengué *et al*. [46] also found that the resistance rates were 85.7% for quinolones and 66.7% for aminosids. These results were in disagreement with those of Zhang *et al*. [47] who found that more than 90% of 61 *K. pneumoniae* isolates from retail foods in China were susceptible to quinolone antibiotics, including ciprofloxacin. The resistance of *K. pneumoniae* to most of the tested antibiotics mostly the beta-lactams may be the consequence of the abusive consumption of antibiotics in Côte d'Ivoire. Beta-lactams have always been the drugs of choice for the treatment of *Klebsiella* infections in healthcare institutions around the world, because aminoglycoside-modifying enzymes, macrolides esterases, and efflux systems render many other classes of drugs ineffective [48].

5. CONCLUSION

This study shows that *K. pneumoniae* recovered from cooked and sold foods by private and public restaurants of two universities of Côte d'Ivoire harbored the blaSHV gene and were resistant to beta-lactams. Food containing antimicrobial-resistant *K. pneumoniae* has the potential to become a public health risk to consumers. The study also shows the importance of good hygiene and handling practices during food preparation to reduce the risk of consumers being contaminated by antimicrobial-resistant *K. pneumoniae*. Authorities of universities should undertake sanitation control and prevention strategies in order for restaurants to improve the food quality and hygiene of the restaurant environment and staff. Restaurant staff should be trained in good hygiene practices. A study of the pathogenicity of different species of *K. pneumoniae*

isolated from foods should be undertaken to better estimate the risk associated with their consumption.

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7. CONFLICTS OF INTEREST

None declared.

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9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. PUBLISHER'S NOTE

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