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Microbiological, Biochemical and Molecular Characterisation of Strains Isolated from Smoked and Dried *Clarias gariepinus* Fish Sold at the N'Djamena Market (Chad)

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ABSTRACT

In Africa, and particularly in Chad, fish consumption remains relatively low despite its richness in proteins, vitamins, and essential fatty acids. In this study, a total of 16 samples of processed *Clarias gariepinus* fish (smoked and dried), collected from the Dembé and Taradona markets in N'Djamena, were analyzed. The main objective of this work is to characterize, from a microbiological, biochemical, and molecular perspective, the strains isolated from these processed fish. Microbiological analysis was carried out using standardized methods. Presumptive *Escherichia coli* strains were identified using API 20E test strips along with the API WEB software. Antibiotic susceptibility was assessed in accordance with the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM). Furthermore, the isolated *E. coli* strains were subjected to molecular analysis by PCR. The microbiological quality assessment revealed the presence of *E. coli* strains at an average concentration of $1.3 \times 10^3 \pm 3.2 \times 10^2$ CFU/g. The antibiogram showed the presence of resistant, intermediate, and susceptible strains to the tested antibiotics. The strains were confirmed by PCR, validating the initial identification. These results highlight the need for strict control measures and awareness campaigns on good hygiene practices in fish processing, in order to improve the sanitary quality

of the marketed products and protect consumer health.

1. INTRODUCTION

At the scale of the African continent, and more specifically in the countries of the West and Central African sub-region, smoking and drying are two main traditional techniques used for processing fishery products. Used individually or in combination, these methods are widely adopted by actors in the fisheries sector. Their purpose is to reduce post-catch losses, extend the shelf life of fish products, and facilitate their distribution in rural or remote areas [1, 2]. In Chad, the marketing of processed fish mainly involves the sale of smoked and dried fish, often displayed in the open air on stalls or placed on plastic sheets laid directly on the ground. In some cases, these products are stored in enclosed shops characterized by poor ventilation. A lack of hygiene during handling, along with the use of rudimentary equipment for fish processing, is frequently observed. These practices can promote contamination by microbial agents or various volatile chemical compounds [3, 4]. Food safety is a major global concern, especially in developing countries where food processing and preservation practices are often artisanal. In Africa, numerous cases of microbiological and physicochemical contamination of food products—particularly those sold in markets—have been reported, leading to frequent foodborne illnesses [5]. Fish, as a highly perishable food, is not only a significant source of animal protein but also a potential carrier of pathogenic bacteria for humans. Contamination can occur at various stages of the production chain, particularly during storage, processing, or preparation [6]. Escherichia coli is a Gram-negative bacterium belonging to the Enterobacteriaceae family. It is naturally found in the intestinal microbiota of humans and warm-blooded animals, where it plays an important role in digestion. However, its presence in fishery products is an indicator of fecal contamination, often linked to poor water quality or inadequate hygiene during handling, transport, or processing of the fish. Some strains of *E. coli* are pathogenic to humans. Several pathotypes are distinguished, including: Enterotoxigenic *E. coli* (ETEC): responsible for watery diarrhea, especially in travelers. Enterohemorrhagic *E. coli* (EHEC), with the most well-known strain being O157:H7: it can cause hemorrhagic colitis and, in severe cases, hemolytic uremic syndrome (HUS). Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC): also associated with gastroenteritis [7]. Notably, *Salmonella* and Shigella species are frequently found in fish and aquatic organisms, posing major public health challenges [8]. In rural areas, traditional methods such as drying and smoking are commonly used. However, these techniques are often applied without strict adherence to good hygiene practices, which compromise not only the sanitary quality of the products but also their textural and sensory properties [9-14]. To ensure the safety of processed fishery products, it is crucial to control and improve current processing and preservation methods [15]. This study is part of that effort, with the general objective of evaluating the microbiological quality of traditionally processed fish. Specifically, it aims to:

- ✓ Perform microbiological analysis of processed fish;
- ✓ Biochemically identify isolated bacterial strains using API and APT WEB methods;
- ✓ Assess the antibiotic susceptibility of the strains using the disk diffusion method and the Vitek 2 Compact automated system;
- ✓ Carry out molecular characterization of isolated *Escherichia coli* strains.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in the Dembé and Taradona markets, located in the city of N'Djamena. Geographically, N'Djamena lies between 11˚ and 12˚8' North latitude and 14˚2' and 15˚2' East longitude. It is bordered to the north by the Hadjer-Lamis region, to the east and south by the Chari-Baguirmi region, and to the west by the Logone River. According to the general housing census [16], the city has an estimated population of 951,418 inhabitants. N'Djamena is divided into 10 districts, with an average maximum

temperature of 35.8˚C and an annual average rainfall of 509.8 mm [17], The study involved microbiological analyses of food products found in these markets, with particular focus on the bacterial strains isolated from fish samples. Fish samples were collected from the Dembé and Taradona markets and analyzed at the Food Quality Control Center (CECOQDA). The microbiological characterization of the isolated strains helped identify the pathogens and understand their profiles. The analyses included API tests, antibiotic susceptibility testing (antibiograms), and PCR assays, the latter being performed at Joseph KI-ZERBO University for more accurate identification of the pathogens (Figure 1).

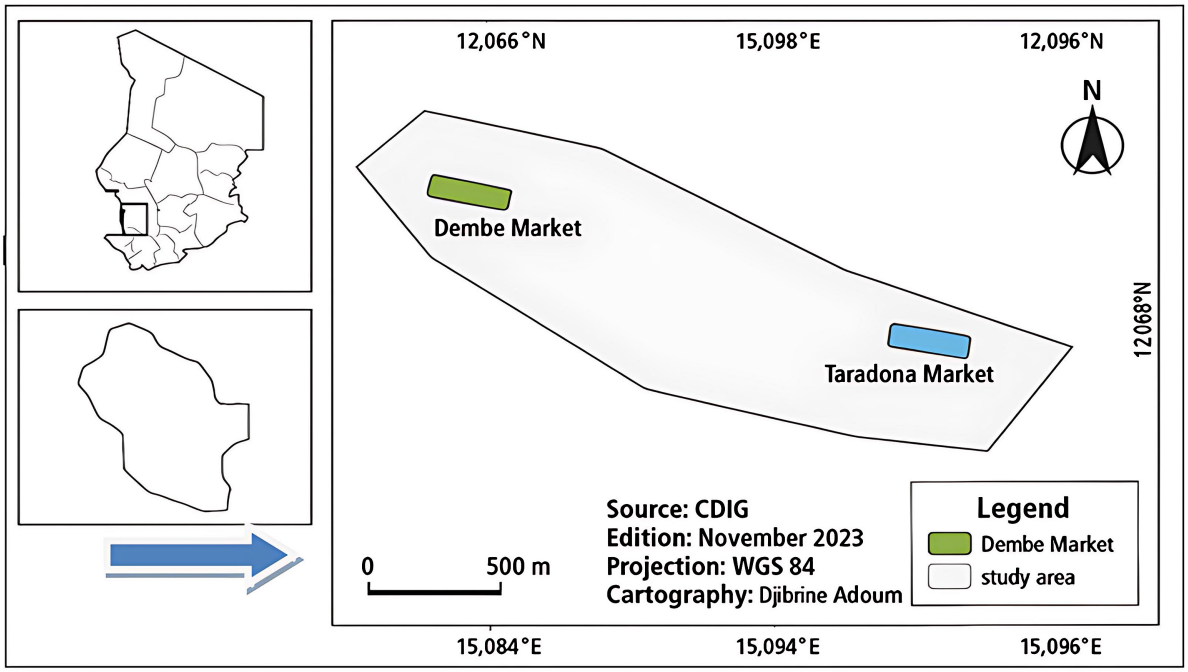


Figure 1. Location of study areas.

2.2. Sampling

Samples were collected in the city of N'Djamena, specifically from the dembe and taradona markets, located in the 6th and 7th districts, respectively. A total of 16 samples of smoked and dried *Clarias gariepinus* fish were purchased. After purchase, the samples were transported in a tightly sealed container, coded upon arrival at the laboratory, and stored under refrigeration pending analysis.

2.3. Microbiological Method

2.3.1. Preparation of Stock Solution and Decimal Dilutions

A 25 g portion of fish was weighed and placed into a sterile stomacher bag. Then, 225 mL of previously sterilized buffered peptone water (BPW) was added. The mixture was then blended and homogenized using a stomacher device (Inter Science brand, Bag Mixer model, serial number 120322348) for 30 seconds. The resulting solution was allowed to rest for 30 minutes to allow the revival of bacteria that may have been shocked during blending. This procedure was performed under a vertical laminar flow hood (FLOW ACTIVAHF, serial number Sn. 807/12), following the [18].

2.3.2. Microbiological Criteria

The microbiological criteria (CFU/g) are those of the Food Safety Division, Ministry of Health, Grand Duchy of Luxembourg, 2016 (Table 1).

Table 1. Applied microbiological criteria.

Search parameters	Methods	Microbiological criteria UFC/g (DSA, 2016)
Microorganisms at 30˚C	ISO 4833-1 (2013)	$\leq 10^5$
Coliforms at 37˚C	NF ISO 4832 (2006)	$\leq 10^2$
Escherichia coli	NF ISO 16649-2 (2001)	$\leq 10^2$
Coagulase Staphylococcus +	NF EN ISO 6888-2 (1999)	$\leq 10^2$
Yeasts - Moulds	NF V 08-059 (2009)	$\leq 10^2$
Bacillus cereus	ISO 7932 (2005)	$\leq 10^2$
Salmonella	NF EN ISO 6579 (2017)	Absence in 25 g
Anaerobic Sulphite Reducers (*ASR*)	ISO 26461-2-(2011)	$\leq 10^2$

2.3.3. Search and Enumeration of Total Aerobic Mesophilic Flora

The enumeration of total mesophilic aerobic flora is based on the pour plate method using Plate Count Agar (PCA). A series of decimal dilutions (usually from 10^{-1} to 10^{-4}) is prepared, and 1 ml of each dilution is aseptically transferred into sterile Petri dishes. Then, 12 to 15 ml of molten PCA (cooled to 45˚C) is added to each dish. The mixture is gently swirled to ensure even distribution of the microorganisms and allowed to solidify. A second layer of PCA (5 to 7 ml) is poured over the solidified medium to prevent overgrowth by motile bacteria such as Proteus, which could hinder accurate colony counting. The prepared plates are then incubated at 30˚C for 72 hours in an incubator (Binder model BD053), with the lids facing downward to avoid condensation. After incubation, all colonies that have grown between the two layers of agar are counted either with the naked eye or using a colony counter with a magnifier. Results are expressed in colony-forming units (CFU) per gram or per milliliter of sample, based on counts from two successive dilutions, following the standard [19].

2.3.4. Search and Enumeration of Coliforms

The medium used is VRBL agar (crystal violet, neutral red, bile and lactose agar), melted and cooled in a water bath. 1 ml of dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} of the sample are respectively inoculated into two petri dishes. Next, 10 to 15 ml of VRBL are poured into these dishes and homogenized by gentle circular movements. Once this first layer has solidified, a second, thinner layer is poured on. Inoculate 1 ml of the product to be analyzed or its decimal dilutions into sterile petri dishes. Pour approximately 15 ml of the medium, previously melted and cooled to 44˚C to 47˚C. Homogenize well and leave to cool on a cool, perfectly horizontal surface. Pour a second layer (about 4 ml) of this medium maintained at 44˚C to 47˚C and leave to solidify again. When solidification is complete, turn the dishes upside down and incubate in this position: For 24 h ± 2 h for coliform detection and dismemberment in the 37˚C ± 1˚C oven, Binder brand, model BD053, series 12-23810. For 24 h ± 2 h for the detection and dismemberment of thermo-Tolerant coliforms in the oven 44˚C ± 1˚C Binder brand, model KB053, series 12-01560 [20].

2.3.5. Search and Enumeration of *Salmonella*

The standard [21]. is the horizontal reference method for the detection of Salmonella pp. in a foodstuff, but also in environmental samples collected in agri-food businesses. The EPT medium will be used to detect Salmonella. According to the current standard *Salmonella* is tested in 25 g of products. The test will be carried out in 4 stages.

Pre-enrichment

Inoculate buffered peptone water (BPW) with the test sample, then incubate at 37˚C ± 1˚C for 18 h ± 2 h. In the case of large quantities, the buffered peptone water should be heated to 37˚C ± 11˚C before inoculation with the test sample.

Enrichment with selective media

Transfer 0.1 ml of the culture obtained into a tube containing 10 ml of RVS broth and 1 ml of the culture obtained into a tube containing 10 ml of MKTTn broth. Incubate the seeded RVS at 41.5˚C ± 1˚C for 24 h ± 3 h and the MKTTn broth at 37˚C ± 1˚C for 24 h ± 3 h.

Isolation

Using the culture obtained in RVS broth, inoculate the surface of XLD medium with a loop. Repeat with the second selective isolation medium. Do the same for the culture obtained in MKTTn broth. Invert the plates of XLD medium and incubate at 37˚C. For the second isolation medium, follow the manufacturer's recommendations. After 24 h ± 3 h incubation, examine the plates for the presence of Salmonella colonies, as well as atypical colonies that are likely to be Salmonella. Typical Salmonella colonies grown on XLD agar have a black centre and are surrounded by a clear transparent red halo.

Purification

Five characteristic colonies are picked from each Hektoen plate and transferred to Nutrient Agar (NA) for purification. The GN plates are incubated at 37˚C for 24 hours. Purified colonies appear whitish on reading.

2.3.6. Search and Enumeration of *Staphylococci*

The culture medium of choice for this research is Baird-Parker (BP) agar, supplemented with a mixture of egg yolk and potassium tellurite. The BP agar was melted and cooled, then poured into sterile petri dishes containing homogenized potassium tellurite and egg yolk. Once the mixture has solidified, 0.1 ml of the stock suspension or decimal dilutions are spread on the surface using a sterile glass or plastic spreader. Incubation takes place at 37˚C for 48 hours. A first reading is taken after 24 hours and a second after 48 hours incubation. For counting purposes, plates containing between 15 and 150 characteristic colonies for 2 successive dilutions are selected. Staphylococcus colonies are black, shiny, bulging, surrounded by a white precipitate and a halo of lightening. Confirmation is obtained by the catalase and coagulase tests [22].

2.3.7. Search and Enumeration of *Yeasts* and Molds

The fungal flora was counted on glucose agar with Oxytetracycline (OGA). A 10 ml sample of OGA agar was poured into sterilized petri dishes. After solidification, the plates were inoculated with 0.1 ml of dilutions 10^{-1} and 10^{2} from the stock solution (SM) on the surface and then incubated at 25˚C for 3 to 5 days. For counting purposes, plates containing fewer than 150 colonies after 5 days of incubation are retained [23].

2.3.8. Search and Enumeration of Sulfite-Reducing *Anaero*bes (SRA)

Sporulated forms are sought. Two selective media can be used: Trypticase Sulfite Neomycin Agar (TSN). TSN was used for the study. TSN medium is inoculated in tubes to detect RSA. The TSN medium, melted and cooled in a water bath, was poured at a rate of 12 to 15 ml per tube. 1 ml of stock suspension at 10^{-1} and 1 ml of dilution at 10^{-2} were transferred to the sterile tubes. After homogenization and solidification, a second layer is poured. The tubes with the solidified agar are incubated anaerobically at 46˚C for 24 hours. Characteristic colonies appear black in the incubation tubes [24].

2.3.9. Enumeration of Escherichia Coli

Take a sterile Petri dish and use a pipette or micropipette to transfer 1 ml of the test sample (if liquid), or 1 ml of the stock suspension (10^{-1}) in the case of other products. If necessary, repeat these operations with the following decimal dilutions, using a new sterile pipette for each dilution. Pour approximately 15 ml of TBX medium, previously cooled to between 44˚C and 47˚C in the water bath, into the Petri dish. Carefully

mix the inoculum with the medium and allow the mixture to solidify, placing the Petri dishes on a cool horizontal surface. Repeat these same operations with sterile buffered Peptone Water (blank test). The time elapsing between depositing the inoculum in the Petri dish and adding the medium should not exceed 15 min. Turn the plates over and incubate them in an oven set at 44˚C for 18 to 24 hours. If the presence of stressed micro-organisms is suspected, incubate first for 4 h at 37˚C, then for 18 h to 24 h at 44˚C. The incubation temperature must not exceed 45˚C. [25].

2.3.10. Enumeration of *Bacillus cereus*

Take a quantity of sample, transfer to diluent and homogenize; prepare decimal dilutions; surface plating with a spreader of 0.1 ml of sample or dilution of a solid selective medium with Mannintol egg Yolk Polymyxin Agar (MYP) and incubation for 18 to 24 hours at 30˚C; count the number of typical colonies on plates containing MYP agar (large pink colonies surrounded by a zone of precipitation). If this type of colony is observed, then carry out the confirmation phase using a haemolysis test: take a few typical colonies, perform a streak inoculation on the blood agar and incubate for 24 hours at 30˚C. If it is confirmed that the colonies sampled are indeed B. cereus, then count the number of cfu from the number of colonies counted, taking into account the dilutions performed [26].

2.3.11. Calculation Formula and Expression of Results

In accordance with the [27]. standard, to ensure the accuracy of the count, only Petri dishes containing a maximum of 200 colonies, corresponding to two successive dilutions, or tubes containing a maximum of 30 well-separated colonies, should be considered. Additionally, it is required that a minimum of 10 characteristic colonies be observed to ensure the representativeness and reliability of the results obtained.

$$N = \frac{\sum C}{V \times \left[n_1 + \left(0.1 n_2 \right) \right] d}$$

where:

$\sum C$ = sum of characteristic colonies on the two selected Petri dishes;
V = volume of inoculum applied to each dish;
n_1 = number of dishes retained at the first dilution;
n_2 = number of dishes retained at the second dilution;
d = dilution rate corresponding to the first selected dilution.
Round the calculated results to two significant figures.

2.3.12. API 20E Gallery

The API (Apparatus and Procedure for Identification) gallery is used as a confirmatory test for several types of tests: Preparation of the bacterial suspension: An isolated bacterial strain is inoculated into 5 ml of sterile distilled water to obtain a suspension. This suspension is used to fill the wells of the API gallery. Each well is filled with a specific substrate that will react with the microorganism being studied. Some substrates require filling to create a meniscus, while others must be covered with paraffin oil to create an *anaerobic* condition or to maintain volatile ions in solution. The wells of the gallery are filled with water to ensure a humid chamber. The gallery is incubated at 37˚C for 24 to 48 hours. During this period, fermentation reactions or other enzymatic reactions may occur, resulting in color changes in the media. The results are interpreted based on the reactions observed after incubation. The color changes or the revelation of reactions by the addition of reagents allow for the identification of the microorganism. The identification is confirmed using the API analytical catalog and the API web identification software [28].

2.3.13. Storage of Bacterial Strains

The bacterial strains were scraped and preserved in Brain Heart Infusion (BHI) broth (BioMérieux), concentrated to 15% glycerol. They were then placed in cryotubes and stored in a freezer at −20˚C. These isolates will be used for further analyses, including antibiogram tests.

2.3.14. Resistance Sensitivity Study Using Diffusion Method and Vitek 2 Compact

The antibiogram was performed on a solid Mueller-Hinton (MH) medium (Liofilchem, ref. 61033) using the disk diffusion method. Disks impregnated with antibiotics at known concentrations were placed on the medium. The antibiotics tested included: Ampicillin, Amoxicillin, Clavulanic acid, Ticarcillin, Piperacillin, Tazobactam, Cephalothin, Cefoxitin, Cefotaxime, Ceftazidime, Ertapenem, Imipenem, Amikacin, Gentamicin, Tobramycin, Nalidixic acid, Ciprofloxacin, Ofloxacin, Nitrofurantoin, and Trimethoprim/sulfamethoxazole. The tests were conducted in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology. Following this phase, a confirmation of the results was performed using the automated VITEK 2 Compact system. Bacterial growth or inhibition was quantified by optical methods, and result interpretation was performed using specialized software. The minimum inhibitory concentration (MIC) was determined by the microdilution method [29].

2.4. Molecular Characterization of Isolated Strains

2.4.1. Revival and Purification of Isolated Strains

The strains stored in cryotubes, obtained from samples of smoked and dried fish, were revived in a nutrient medium.

2.4.2. DNA Extraction

Genomic DNA from *Escherichia coli* isolates was extracted using the boiling method, known for its simplicity, speed, and low cost. Isolated colonies grown on EMB agar at 37˚C for 24 hours were suspended in 100 µL of sterile deionized water. The bacterial suspension was vortexed briefly and then incubated in a boiling water bath at 100˚C for 10 minutes to lyse the cells and release intracellular contents. Immediately after boiling, tubes were chilled on ice for 10 minutes to stabilize the DNA and facilitate precipitation of cell debris. Samples were centrifuged at 10,000 rpm for 10 minutes at room temperature. The clear supernatant containing the genomic DNA was carefully transferred to a new sterile microtube and stored at −20˚C until further use, particularly for PCR amplification [30, 31].

2.4.3. Preparation of the Reaction Mixture and Primers Used

The organisms isolated, suspected to be *E. coli* due to their cultural and biochemical characteristics, were confirmed as *E. coli* by PCR using specific primers for the 16S rRNA gene. DNA extracted from a known *E. coli* strain was used as a positive control, while water served as a negative control. The PCR was performed according to the procedure described by Hassan *et al.* (2014). The isolates that tested positive for the *E. coli* 16S rRNA gene were then subjected to a search for the stx1 and stx2 genes by PCR using specific primers for the *E. coli* stx1 and stx2 genes (**Table 2**) [32].

Table 2. **Primers used for the detection of *E. coli*.**

Primers	Primer name	Sequence **5' - 3'**	Expected siz **(bp)**
Stx1	Stx 1F	CAC AAT CAG GCG TCG CCA GCG CAC TTG CT	600
	Stx 1R	TGT TGC AGG GAT CAG TCG TAC GGG GAT GC	
stx2	Stx 2F	CCA CAT CGG TGT CTG TTA TTA ACC ACA CC	372
	Stx 2R	GCA GAA CTG CTC TGG ATG CAT CTC TGG TC	

2.4.4. Amplification

PCR amplification of stx1 and stx2 genes was performed in 25 µL reaction volumes containing template DNA, specific primers, PCR buffer, dNTPs, Taq polymerase, and nuclease-free water. The thermal cycling protocol consisted of an initial denaturation at 94˚C for 5 minutes, followed by 30 cycles of denaturation at 95˚C for 20 seconds, annealing at 61˚C for 40 seconds, and extension at 72˚C for 90 seconds. A final

extension was conducted at 72˚C for 7 minutes. Amplified products were analyzed by 1.5% agarose gel electrophoresis using a 100 bp DNA ladder for size estimation Table 3.

Table 3. ***E. coli*** **amplification program.**

Primers	Denaturation Initial	30 Cycles			Elongation Final
Stx1	94˚C For 5 minutes	Denaturation	Hybridization	Elongation	72˚C For 7 Minutes
Stx2		95˚C For 20 Seconds	61˚C For 40 Seconds	72˚C For 90 Seconds	

2.4.5. Electrophoresis of *E. coli* Strains

A volume of 10 µL of each amplicon was subjected to electrophoresis on a 1.5% agarose gel, in a 0.5× TAE buffer. The electrophoresis was performed in a migration chamber for 60 minutes, under a voltage of 100 V and an intensity of 100 mA. A volume of 10 µL of molecular weight marker was used to determine the size of the amplified bands. The gel was then visualized using a UVP Transilluminator system, coupled with the UVP PhotoDoc-it Imaging System.

2.4.6. Statistical Analysis

Statistical analysis and processing of the mean and standard deviation data were carried out using mathematical formulae for microbial enumeration, API and web API software for confirmation, antibiotic analysis software (VITEK® 2 Systems Software) and analysis of the impact of the smoking and drying process on the DNA of the fish strain associated with artificial intelligence (ChatGPT).

3. RESULTS AND DISCUSSION

3.1. Results of Microbiological Enumerations

The following Table 4 and Table 5 present the results of analyses of microbiological enumerations of dried and smoked fish, *Clarias gariepinus*.

Table 4. **Results of microbiological enumeration of dried** ***Clarias gariepinus*** **fish.**

Microorganisms	Mean ± Standard deviation (cfu/g)	Microbiological criterion (cfu/g)	Compliance
TAMF **(*Total aerobic mesophilic flora*)**	$3.6 \times 10^6 \pm 6.9 \times 10^4$	$\leq 10^5$	Non-compliant
Coliforms at **37˚C**	$2 \times 10^5 \pm 1.2 \times 10^4$	$\leq 10^2$	Non-compliant
Escherichia coli	$1.7 \times 10^3 \pm 3 \times 10^2$	$\leq 10^2$	Non-compliant
Bacillus cereus	$1.4 \times 10^4 \pm 8 \times 10^2$	$\leq 10^2$	Non-compliant
Coagulase-positive Staphylococcus	$1.5 \times 10^4 \pm 4 \times 10^2$	$\leq 10^2$	Non-compliant
Yeasts and molds	$1.4 \times 10^4 \pm 8 \times 10^2$	$\leq 10^2$	Non-compliant
Salmonella spp	-	**Absent in 25 g**	Not specified

Legend: ***TAMF***: *Total aerobic mesophilic flora*; ***E. coli***: *Escherichia coli*; ***Staph***: *Staphylococcus spp.*

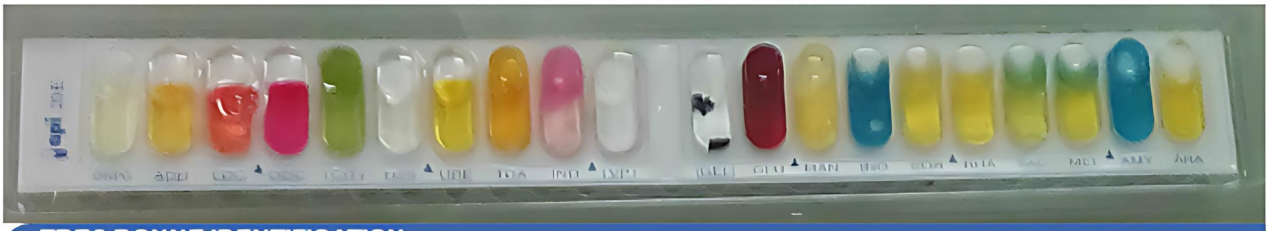
Table 5. Results of microbiological enumeration of smoked *Clarias gariepinus* fish.

Microorganisms	Mean ± Standard Deviation (cfu/g)	Microbiological Criterion (cfu/g)	Compliance
TAMF (Total Aerobic Mesophilic Flora)	$3.5 \times 10^6 \pm 2.3 \times 10^5$	$\leq 10^5$	Non-compliant
Coliforms at 37˚C	$1.8 \times 10^5 \pm 1.3 \times 10^4$	$\leq 10^2$	Non-compliant
Escherichia coli	$1.4 \times 10^3 \pm 1.5 \times 10^2$	$\leq 10^2$	Non-compliant
Bacillus cereus	$1.25 \times 10^4 \pm 6.74 \times 10^2$	$\leq 10^2$	Non-compliant
Coagulase-positive Staphylococcus	$1.6 \times 10^4 \pm 6.7 \times 10^2$	$\leq 10^2$	Non-compliant
Yeasts and molds	$1.4 \times 10^4 \pm 1.3 \times 10^3$	$\leq 10^2$	Non-compliant
Salmonella spp	-	**Absent in 25 g**	Not specified

Legend: ***TAMF***: *Total aerobic mesophilic flora*; ***E. coli***: *Escherichia coli*; ***Staph***: *Staphylococcus spp.*

3.2. Confirmation Results of Strains by API 20E and API Web

The results obtained after the incubation of biochemical tests using the API 20E gallery, with the identification of each strain and their numerical profile proposed by the API software and confirmed by API Web, are presented in the following (Figure 2).



TRES BONNE IDENTIFICATION

Galerie	API 20 E V5.0
Profil	5 1 4 0 5 7 2
Note(s)	

Taxons significatif(s)	% ID	T	Test(s) à l'encontre			
Escherichia coli 1	99.7	0.68	GLU 99%			

Taxon suivant	% ID	T	Test(s) à l'encontre			
Kluyvera spp	0.2		LDC 25%	GLU 100%	SOR 25%	AMY 99%

Figure 2. Biochemical profile of *E. coli* strain.

3.3. Results of Susceptibility Tests Using the Classical Method

The results of the antibiogram, carried out using the standard Mueller-Hinton agar disc diffusion method, showed the emergence of resistance in the isolated strains to several antibiotics, in particular tobramycin, flucytosine, amikacin and teicoplanin (Figure 3). On the other hand, a marked sensitivity to ceftriaxone was observed, suggesting the potential efficacy of this antibiotic against the strains tested.

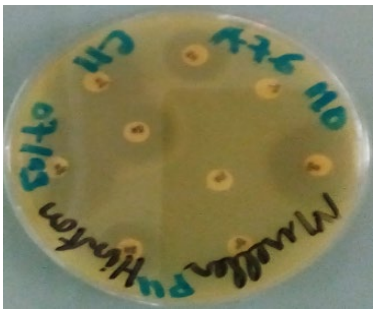


Figure 3. Résultat de sensibilité aux antibiotiques de souche *E. coli*.

Table 6 and Table 7 show the detailed results of the sensitivity tests carried out with the Vitek 2 Compact system, enabling the most effective antibiotics to be identified and resistant or multi-resistant strains of *E. coli* to be detected.

Table 6. Antibiotic sensitivity results for *E. coli* strains isolated from dried *clarias gariepinus* fish.

N°	Antibiotique	CMI	Interprétation
1	Ampicilline	>14	R
2	Amoxicilline/acide clavulanique		
3	Ticarcilline	>64	R
4	Piperacilline/Tazobactam	>63	R
5	Céfaloctine		
6	Céfoxtine	>32	R
7	Céfotaxime	>31	
8	Céftazidime	>32	R
9	Ertapéném	≤0.5	S
10	Imipéneme	≤0.25	S
11	Amikacine	6	S
12	Gentamicime	>8	R
13	Tobramycine	>8	R
14	Acide nalidixique	>16	R
15	Ciproxfloxacine	>2	R
16	Ofloxacine	>4	R
17	Nitrofuantoine	62	S
18	Triméthoprime/sulfaméthoxazole	>100	R

Legend: Sensitive (S), Intermediate (I), Resistant (R).

Table 7. Antibiotic sensitivity results for *E. coli* strains isolated from smoked *Clarias gariepinus* fish.

N°	Antibiotique	CMI	Interprétation
1	Ampicilline	>16	R
2	Amoxicilline/ acide clavulanique		
3	Ticarcilline	>64	R
4	Piperacilline/Tazobactam	>64	R
5	Céfaloctine		
6	Céfoxtine	>32	R
7	Céfotaxime	>32	
8	Céftazidime	>32	R
9	Ertapénéme	≤0.5	S
10	Imipéneme	≤0.25	S
11	Amikacine	8	S
12	Gentamicime	>8	R
13	Tobramycine	>8	R
14	Acide nalidixique	>16	R
15	Ciproxfloxacine	>2	R
16	Ofloxacine	>4	R
17	Nitrofuantoine	64	S
18	Triméthoprime/sulfaméthoxazole	>100	R

Legend: Sensitive (S), Intermediate (I), Resistant (R).

3.4. Molecular Biology Results for Transformed Fish

The genetic profiles of the *E. coli* strains characterized by PCR are shown in **Figure 4**. Electrophoretic analysis of DNA extracted from dried and smoked *Clarias gariepinus* revealed significant differences in DNA quality and integrity depending on the transformation process.

This diagram (**Figure 5**) illustrates the impact of the smoking process on the DNA of the fish strain *Clarias gariepinus* through a series of degradation mechanisms

The image (**Figure 6**) illustrates a scientific study on the effect of drying on DNA integrity in dried *Clarias gariepinus* fish.

4. DISCUSSION

The results presented in **Table 4** indicate a very high microbial load in the samples of dried *Clarias gariepinus* fish analyzed, far exceeding the microbiological standards generally accepted for ready-to-eat foodstuffs. With the exception of Salmonella spp. which was absent from the samples (complying with the criterion of absence in 25 g), all the other germs tested showed concentrations above the regulatory

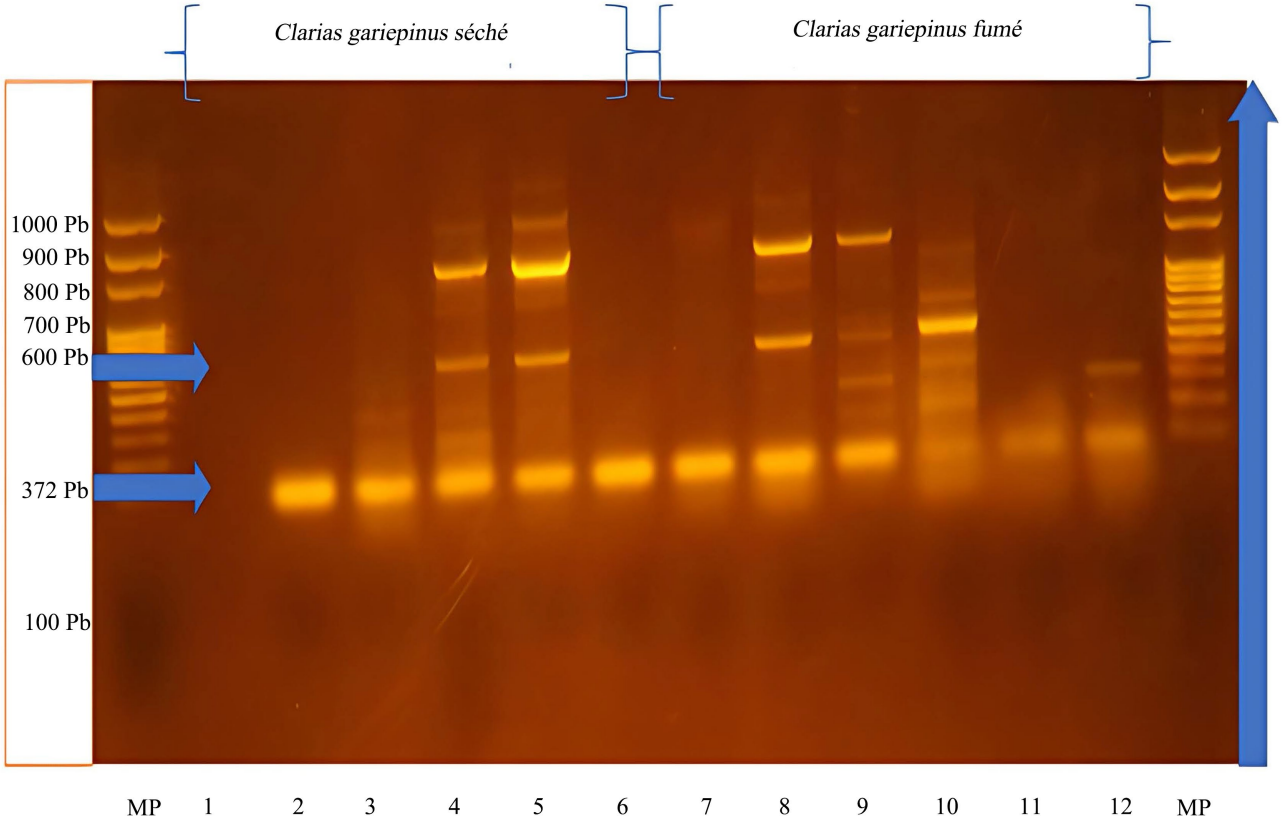


Figure 4. Genetic profiles of *E. coli* strains characterized by PCR.

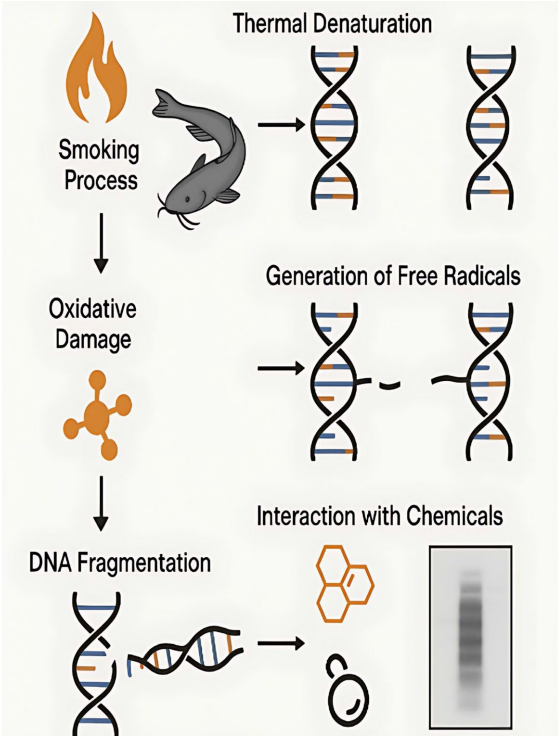


Figure 5. Mechanism of DNA degradation by smoking.

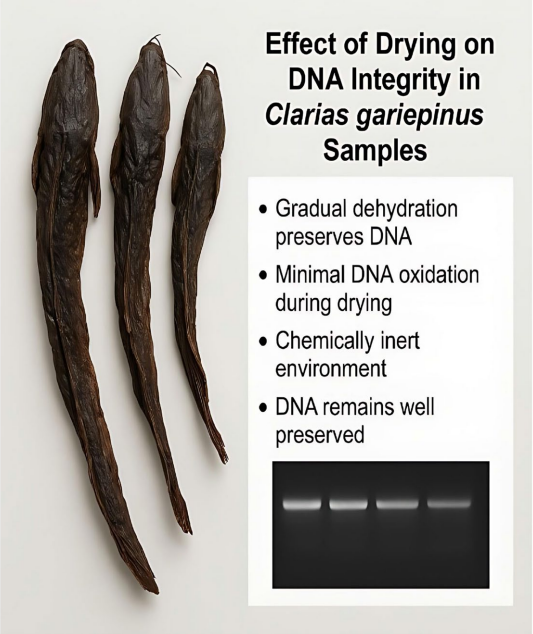


Figure 6. Drying process for biological material.

thresholds, indicating widespread non-compliance. The aerobic mesophilic total flora (TAMF) (3.6×10^6 cfu/g) is an overall indicator of microbiological quality and here reflects excessive contamination, probably due to poor hygiene conditions during product handling, storage or processing. The significant presence of total *coliforms* at 37˚C (2×10^5 cfu/g) and Escherichia coli (1.7×10^3 cfu/g) reflects probable faecal or environmental contamination, highlighting a lack of hygiene control in the production chain. The detection of Bacillus cereus at significant concentrations (1.4×10^4 cfu/g) is cause for concern, as this microorganism is known for its ability to produce toxins responsible for food poisoning, particularly in products stored at room temperature. Similarly, the high presence of coagulase-positive Staphylococcus aureus (1.5×10^4 cfu/g) suggests contamination of human origin, particularly by carrier handlers. This germ is capable of producing heat-stable enterotoxins responsible for serious food poisoning. The yeasts and moulds (1.4×10^4 cfu/g) identified at levels well in excess of the maximum permitted criteria can not only alter the organoleptic characteristics of the product, but also, in some cases, produce mycotoxins that are dangerous to human health, particularly under unsuitable storage conditions. Overall, these results highlight major shortcomings in food hygiene and safety practices, whether in the handling, processing or storage of the fish products analyzed. These non-conformities pose a significant health risk to consumers, justifying urgent corrective measures. These findings are consistent with previous studies by [33-35].

The results obtained in this study show widespread microbial contamination of processed fish smoked *Clarias gariepinus* (**Table 5**), with values well above the limits set by international standards (Codex Alimentarius, EC Regulation no. 2073/2005). The total aerobic mesophilic flora (3.5×10^6 cfu/g) is a general indicator of the hygienic quality and freshness of the product. Such a concentration reflects massive bacterial proliferation, generally due to prolonged storage time, a break in the cold chain or a lack of hygiene during processing (drying, smoking, manual handling). This level far exceeds the threshold of 10^5 cfu/g, suggesting that the product is probably unfit for consumption. The high concentration of total coliforms at 37˚C (1.8×10^5 cfu/g) and Escherichia coli (1.4×10^3 cfu/g) confirms faecal contamination, often linked to: the use of non-potable water during the washing stages; the absence of disinfection of equipment; direct handling by dirty hands; cross-contamination via uncleaned surfaces. The presence of *E. coli*, a specific indicator of recent faecal pollution, constitutes a direct danger to human health. Certain enterotoxigenic strains can cause severe gastroenteritis, and even renal complications in susceptible individuals (haemolytic uraemic

syndrome). The presence of Bacillus cereus at a concentration of 1.25×10^4 cfu/g is also a cause for concern. This spore-forming germ can survive heat treatment, particularly smoking and drying, and proliferate if storage conditions are inadequate. It is responsible for two types of food poisoning: an emetic form due to the cereulide toxin; and a diarrhoeal form due to the production of enterotoxins in the intestine. The detection of coagulase-positive staphylococci of up to 1.6×10^4 cfu/g is also a warning signal. These are generally Staphylococcus aureus, often transmitted by human carriers (nose, skin, wounds). This germ can produce heat-stable enterotoxins that are not destroyed by cooking or smoking, causing rapid food poisoning (2 to 6 hours after ingestion). The significant presence of yeasts and moulds (1.4×10^4 cfu/g) is a sign of poor drying or high residual moisture. This can also be the result of storage in damp conditions, which are conducive to fungal growth. Some moulds are not only responsible for the sensory alteration (smell, taste, texture) of the product, but can also produce mycotoxins, such as aflatoxin, which is recognized as a human carcinogen (class I by the IARC). The absence of results for Salmonella spp. is a major gap in the health assessment of the product. Salmonella is one of the major food safety pathogens. The standard requires a strict absence in 25 g of product. Screening for Salmonella is therefore essential for determining the safety of a product of animal origin. The absence of this test compromises a complete risk assessment. These results are in line with several studies carried out on processed fish in sub-Saharan Africa. For example, [36] reported similar contamination in samples of smoked fish in Benin, with a predominance of *E. coli* and Staphylococcus aureus. Similarly [37], identified a high presence of toxigenic moulds in fish stored in markets in Togo. These results indicate that locally processed fish products, although nutritious and widely consumed, can be potential vectors of food-borne diseases if hygiene standards are not respected. It is crucial to provide a framework for small-scale processing, to strengthen regulations, and to set up self-monitoring systems among producers in order to guarantee consumer safety. They highlight the urgent need to improve hygiene practices at all stages of the production chain, from slaughter and smoking to final distribution by [38, 39].

The identification of the isolated bacterial strain was performed using the API 20 E system, version 5.0, designed for the biochemical characterization of Enterobacteriaceae. The biochemical profile obtained, coded 5140572 (**Figure 2**), corresponds to a highly reliable identification of *Escherichia coli* 1, with an identity percentage of 99.7%. This level of confidence allows for a definitive classification of the strain as *E. coli*. The discriminative biochemical tests contributing to this identification include glucose fermentation (GLU), which was positive at 99%, consistent with the typical metabolic profile of *E. coli*. The second proposed taxon, *Kluyvera* spp, presented a negligible identity percentage (0.2%) and several discrepancies in test results (LDC, SOR, AMY), ruling it out as a plausible alternative. These results confirm the relevance of the API 20 E system for rapid and reliable identification of Enterobacteriaceae in both research and clinical diagnostic settings. The identification of *E. coli* is particularly important in the context of urinary tract infections, gastrointestinal diseases, or systemic infections, depending on the source of isolation and associated virulence factors. It is recommended to complement this identification with an antibiotic susceptibility test (antibiogram) for a complete clinical interpretation, especially considering the frequent occurrence of antibiotic resistance within this species. The use of the API 20 E system has provided a highly reliable and accurate identification of the isolated bacterial strain as *Escherichia coli*, with a confidence level of 99.7%. The system's biochemical tests, particularly glucose fermentation, effectively supported this identification, while the alternative taxon, *Kluyvera* spp, was ruled out due to significant discrepancies in test results. This confirms the API 20 E system as a valuable tool for the rapid and precise identification of Enterobacteriaceae, especially in both research and clinical diagnostic settings. Our collaborative result [40, 41].

The analysis of antibiotic susceptibility profiles of *Escherichia coli* strains isolated from dried *Clarias gariepinus* fish (**Figure 3** and **Table 6**), reveals a marked resistance to several classes of antibiotics. The data show that most isolates are resistant to beta-lactam antibiotics, including ampicillin (>14 µg/mL), ticarcillin (>64 µg/mL), piperacillin/tazobactam (>63 µg/mL), cefoxitin (>32 µg/mL), and ceftazidime (>32 µg/mL). This broad resistance suggests the possible production of extended-spectrum beta-lactamases (ESBLs) or other enzymatic resistance mechanisms. However, the isolates remain susceptible to carbapenems, such as ertapenem (≤0.5 µg/mL) and imipenem (≤0.25 µg/mL), indicating the likely absence of carbapenemase activity. This sensitivity, while reassuring, also underscores the potential overreliance on these last-resort

antibiotics. Among aminoglycosides, susceptibility was observed for amikacin (6 µg/mL), while resistance was recorded for gentamicin and tobramycin (>8 µg/mL), potentially due to the expression of specific resistance genes (aminoglycoside-modifying enzymes). A high level of resistance to quinolones and fluoroquinolones was also observed, including nalidixic acid (>16 µg/mL), ciprofloxacin (>2 µg/mL), and ofloxacin (>4 µg/mL). This is particularly concerning, as these molecules are commonly used to treat enteric infections, and their ineffectiveness may complicate therapeutic management. Nitrofurantoin remained effective (62 µg/mL) against the tested strains, which is consistent with its preserved activity for urinary tract infections, although its use is limited in other contexts. Lastly, strong resistance to trimethoprim/sulfamethoxazole (>100 µg/mL) was recorded, further reducing the range of effective oral treatment options. These findings highlight a concerning multidrug resistance profile in *E. coli* isolated from fish intended for human consumption. This may result from fecal contamination, improper handling during processing, or uncontrolled antibiotic use in aquaculture. The situation underscores the urgent need to adopt a "One Health" approach, integrating human, animal, and environmental health, to effectively combat antimicrobial resistance. Our result corroborated with [42].

The analysis of antibiotic susceptibility of the *Escherichia coli* strain isolated from smoked *Clarias gariepinus* fish (**Table 7**), reveals a concerning resistance profile. Most of the antibiotics tested show high minimum inhibitory concentration (MIC) values, indicating strong bacterial resistance. The strain exhibits resistance to several classes of antibiotics, including: Beta-lactams: High resistance was observed to ampicillin (>16 µg/mL), ticarcillin (>64 µg/mL), piperacillin/tazobactam (>64 µg/mL), cefoxitin (>32 µg/mL), and ceftazidime (>32 µg/mL), suggesting potential production of extended-spectrum beta-lactamases (ESBLs). Aminoglycosides: Both gentamicin and tobramycin show loss of efficacy (MIC > 8 µg/mL). Quinolones and fluoroquinolones: High resistance was also noted to nalidixic acid, ciprofloxacin (>2 µg/mL), and ofloxacin (>4 µg/mL), suggesting plasmid-mediated or chromosomal resistance mechanisms involving qnr genes or mutations in DNA gyrase. Sulfonamides: A MIC > 100 µg/mL for trimethoprim/sulfamethoxazole indicates strong resistance. On the other hand, the strain remains susceptible to carbapenems (imipenem ≤ 0.25 µg/mL, ertapenem ≤ 0.5 µg/mL) and amikacin (MIC = 8 µg/mL), indicating that these drugs may still be effective therapeutic options. Interestingly, nitrofurantoin also shows activity (MIC = 64 µg/mL), despite being commonly reserved for urinary tract infections. These results indicate multidrug resistance of the isolated *E. coli* strain, which raises a major public health concern. The presence of such resistant strains in food products like smoked fish may reflect inappropriate use of antibiotics in aquaculture or post-processing contamination. Strengthening microbiological monitoring and limiting unregulated antibiotic use in the food production chain is therefore urgently needed. Our result corroborated with [43-45].

The electrophoretic analysis of DNA extracted from *Clarias gariepinus* subjected to two processing methods (drying and smoking) reveals notable differences in DNA quality and integrity (see figure). In dried fish samples (lanes 1 to 6), the DNA appears as clear and well-defined bands, mainly around 372 bp and 600 bp. The sharpness of the bands, along with the absence of smearing or diffuse signals, suggests that the drying process has relatively well preserved the DNA integrity, limiting fragmentation. In contrast, the DNA extracted from smoked fish (lanes 7 to 12) displays weaker and sometimes blurred bands, with slight smearing observed, particularly in lanes 10 and 11. These alterations indicate partial degradation of the DNA, likely caused by high temperatures, exposure to smoke, and oxidation during the smoking process. These factors are known to induce DNA breakage. These findings highlight that drying is more suitable than smoking for preserving the genetic integrity of fish. They also emphasize the importance of choosing appropriate post-harvest processing methods, especially when high-quality DNA extraction is required for applications such as traceability, authentication, or biological research. Our result corroborated with [46, 47].

The mechanism of DNA degradation by the *Clarias gariepinus* smoking process (**Figure 6**) involves several steps, each of which has a direct impact on the structure and integrity of the DNA. Here is a detailed explanation of the steps involved: During the smoking process, the high temperatures that are generated during the combustion of organic substances lead to the denaturation of biological macromolecules, including DNA. This means that the bonds between the nitrogenous bases of DNA (the purine and pyrimidine bases) are altered, leading to the breakdown of the double helix structure. The combustion of organic

materials generates free radicals, highly reactive molecules that can attack biological structures, including DNA. These free radicals can cause breaks in DNA chains, specifically by attacking the phosphodiester bonds that link nucleotides together. Oxidation, another product of combustion, can also affect DNA. Free radicals or oxidation products can induce cuts in DNA chains, creating single or double breaks. Double-strand breaks are of particular concern because they can lead to genetic rearrangements or loss of genetic information. Smoke contains chemical compounds such as phenols and aldehydes, which are capable of interacting with DNA. These substances can create adducts, *i.e.* covalent bonds between DNA and chemical molecules. These modifications can disrupt the normal processes of DNA replication and repair, leading to mutations or DNA damage. The combination of heat, free radicals, oxidation and chemicals leads to DNA fragmentation. On an electrophoresis gel, this fragmentation manifests itself as smearing, *i.e.* blurred migration of the DNA. This results from the presence of fragments of varying sizes, created by cuts in the DNA [48].

The process of drying biological materials, such as dried *Clarias gariepinus* samples (**Figure 6**), involves exposing them to moderate heat, air flow and a low-humidity environment, which differs from more extreme conditions such as smoking. Here's how this process affects DNA: During drying, water is slowly eliminated from biological materials. This gradual dehydration does not damage the molecular structure of DNA. Unlike high heat or chemicals, which can cause significant damage to DNA, the gradual removal of moisture maintains the integrity of the DNA. Because the drying process does not involve high temperatures, combustion products or reactive chemicals (such as those found in smoke), DNA oxidation is minimized. The absence of intense oxidative stress means that DNA strand breaks and chemical modifications are rare. Oxidative damage is one of the main causes of DNA degradation, and its absence ensures better preservation. The drying environment is chemically inert, with no toxic fumes, radicals or reactive compounds likely to alter the structure of the DNA. In the absence of aggressive chemical agents, there are no significant modifications or alterations to the DNA molecules. The DNA remains well preserved throughout the drying process thanks to the absence of high heat or chemicals. This makes it suitable for a variety of downstream molecular biology applications. When analyzed on an agarose gel, DNA extracted from dried samples appears as clear, intact bands with little or no smearing. This indicates that the DNA has been preserved in its original form and has not been fragmented or degraded by the drying process. The high-quality DNA that remains after drying is ideal for applications such as PCR, sequencing and authentication studies. The integrity of the DNA allows for accurate analysis and reliable results in these applications [49].

5. CONCLUSIONS

The microbiological analysis of dried and smoked *Clarias gariepinus* fish samples reveals significant contamination, indicating inadequate handling and storage practices. The total viable count far exceeds the acceptable safety threshold, accompanied by the presence of several dangerous pathogens such as *E. coli*, *Bacillus cereus*, and *coagulase*-positive Staphylococcus aureus. These results suggest that improper cold chain management, insufficient hygiene, and defective temperature control are likely contributing factors to the microbial proliferation observed in the samples. The detection of *yeasts* and molds also indicates suboptimal storage conditions, particularly in high-humidity environments. Although the absence of *Salmonella* spp. is a favorable result, the overall high microbial load and the presence of fecal and pathogenic bacteria raise serious public health concerns, especially regarding foodborne illnesses. These results underscore the urgent need to strengthen hygiene protocols, improve storage practices, and implement more rigorous monitoring of cold chain conditions to ensure the safety and quality of dried and smoked fish products. Future studies should focus on improving processing methods and implementing more effective control measures to reduce microbial contamination in dried and smoked fish.

The use of the API 20 E system has provided a highly reliable and accurate identification of the isolated bacterial strain as *Escherichia coli*, with a confidence level of 99.7%. The system's biochemical tests, particularly glucose fermentation, effectively supported this identification, while the alternative t**axon**, ***Kluyvera* spp**, **was** ruled out due to significant discrepancies in test results. This confirms the API 20 E system as a

valuable tool for the rapid and precise identification of Enterobacteriaceae, especially in both research and clinical diagnostic settings.

The antibiotic susceptibility analysis of *Escherichia coli* strains isolated from processed *Clarias gariepinus* fish reveals a worrying multidrug resistance profile. The high resistance to commonly used antibiotics including beta-lactams, aminoglycosides, fluoroquinolones, and sulfonamides suggests the possible dissemination of resistance mechanisms such as ESBL production and plasmid-mediated gene transfer. The preserved susceptibility to carbapenems, amikacin, and nitrofurantoin provides limited but critical therapeutic options. These findings highlight the potential public health risks associated with the consumption of contaminated fish products and emphasize the urgent need for improved surveillance, stricter regulation of antibiotic use in aquaculture, and the implementation of a comprehensive "One Health" strategy to mitigate the spread of antimicrobial resistance.

This study evaluates *Escherichia coli* isolates from post-harvest processed *Clarias gariepinus* fish (drying and smoking). Electrophoretic analysis reveals that the samples exhibit clear and well-defined DNA bands, mainly at 372 bp and 600 bp, indicating good isolation of the strains and the genetic integrity of *E. coli*.

The drying process is gentle on DNA, ensuring its integrity and good preservation for future use. The absence of significant oxidative or chemical damage allows for the extraction of high-quality DNA suitable for molecular analyses. When visualized on an agarose gel, DNA extracted from dried fish shows clear bands with no signs of degradation, indicating that its structure is well preserved. However, although drying helps preserve DNA, this method proves insufficient for eliminating bacterial strains, particularly in the context of contamination in fish sold in open-air markets. In contrast, results from our study characterizing *Escherichia coli* strains isolated from dried and smoked Clarias indicate that the smoking method is more effective in eliminating these bacteria. Therefore, smoking is recommended as a post-harvest treatment method to improve the microbiological safety of fish intended for consumption.

The comparison between DNA degradation during smoking and preservation during drying reveals significant differences in how each process impacts the integrity of DNA. Smoking, due to the high temperatures, free radicals, oxidative stress, and chemical exposure from smoke, leads to the denaturation, fragmentation, and chemical modification of DNA, resulting in degraded and damaged DNA. This is evident in the smearing patterns observed on electrophoresis gels. In contrast, the drying process, which involves gradual dehydration under moderate conditions, preserves DNA integrity. The absence of intense heat, oxidative stress, and reactive chemicals ensures that DNA remains intact, as seen in clear, sharp bands on agarose gels. The preserved DNA from drying is well-suited for molecular biology applications such as PCR, sequencing, and genetic studies, offering accurate and reliable results. Therefore, drying is a far more effective method for maintaining DNA integrity compared to smoking, making it the preferred technique for DNA preservation in biological studies.

In order to improve the microbiological quality of locally processed fish products and enhance consumer health safety, the following recommendations are made:

Reinforcing hygiene during processing: The adoption of rigorous hygiene practices, such as the exclusive use of drinking water, the systematic cleaning of equipment, the reduction of direct manual handling, and the control of drying and smoking conditions (temperature, humidity), is essential to limit microbial contamination.

Technical training for processors: Targeted training programmes on Good Hygiene Practice (GHP) and Good Manufacturing Practice (GMP) should be organized on a regular basis. These training courses should be accompanied by simple, accessible teaching aids, written in local languages, to make it easier to grasp the concepts of hygiene.

Introduction of improved technologies: The use of improved smokehouses (e.g. Chorkor or Altona models) and hygienic processing materials (stainless steel grates, washable containers) is essential. In addition, the introduction of rapid microbiological screening kits could encourage self-monitoring by artisans.

Strengthening the regulatory and institutional framework: It is recommended that national quality standards be developed for locally processed fish products, that official market controls be stepped up, that

small-scale processors be given a legal framework (registration, recognition, support), and that food safety issues be incorporated into technical and vocational training curricula.

Raising consumer awareness: Information campaigns should be run to educate consumers about the risks associated with consuming poorly processed products. The introduction of a minimum traceability or labelling system could also increase transparency and responsibility in the production chain.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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