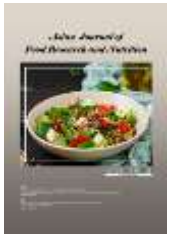

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Identification of Virulence Genes of *Samonella* and *Staphylococcus aureus* Strains from Dried Meat Isolate in N'Djamena

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Over 30% of the population in developed countries suffers from food born infectious diseases each year with diarrheal as a common symptom. Globally most of these foodborne diseases caused by *Salmonella* and *Staphylococcus aureus* have been link to meat consumption.
Virulence genes of *Salmonella* and *Staphylococcus aureus* isolated from dried meat in NDjamena/Chad was analyzed using conventional PCR in the Laboratory of Public Health Biotechnology Center, University of Yaounde.
From the analysis, 80% of the *Salmonella* isolates carried the *invA* gene, which is responsible for gastroenteritis and invasion of epithelial cells. 20% of the *Staphylococcus aureus* strains carried *coa*

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gene that causes severed toxic shock in humans. This results demonstrated the presence of virulence genes in pathogen causing bacteria strain in dried meat from N'Djamena. The presence of *Salmonella* and *Staphylococcus aureus* genes in dried is worrying, as these pathogens can cause health problems for consumers.
The study suggests the development of a user-friendly quality control manual specifically designed for producers of dried meat, with the aims to safe guarding consumer health by providing clear guidelines and procedures.

Keywords: Dried meat; virulence genes; Salmonella; Staphylococcus aureus; N'Djamena.

1. INTRODUCTION

Consuming uncontrol meat can expose consumers to food poisoning and foodborne illness which might results to public health problems in the presence of micro-organism [1]. *Salmonella* are pathogenic microorganisms that can contaminate meat when it is not properly handled, it's the one of the most important foodborne pathogens [2]. These pathogens are involved in food poisoning and meat contamination [3]. The identified invA genetic locus allows *Salmonella* to enter cultured epithelial cells. The *invA* virulence gene of *Salmonella* causes invasion of epithelial cells, and plays the role of virulence *Salmonella*. The gene is found in several strains of *Salmonella* including *Salmonella typhimurium, S. typhi, S. enteritidis, S. arizonae* [4].

The presence of *Staphylococcus aureus* in meats can be a serious problem, because this bacteria causes food poisoning ranging from simple abscesses to more severe toxic shock syndrome [5]. It is also the major cause of opportunistic pathogen and nosocomial infection worldwide, that can cause many illnesses [6,7]. *Staphylococcus aureus* causes collective foodborne illness with gastro-intestinal symptoms, this is most often due to the ingestion of toxins (enterotoxins) preformed in food by *S. aureus.* This is often manifested in the form of nausea, vomiting, abdominal pain and profuse diarrhea. These bacteria cause severe toxic shock through the action of the coa gene [8]. *S. aureus* causes infections ranging from simple abscess, severe toxic shock syndrome to plasma coagulation through the action of the *coa* gene [5].

2. METHODOLOGY

2.1 Collection of Samples

Dried meat samples (20 samples) were collected from different sales point in N'Djamena.

2.2 Isolation of *S. aureus* and *Salmonella*

For samples collected, 10 g was introduced aseptically in 90 ml of sterile diluent (0.1% peptone and 0.8% sodium chloride) and 1 ml of the suspension was diluted into 9 ml of sterile diluent and used for isolation of pathogens. Dilutions were done in accordance with the NF EN ISO 6887, 2011 standards. Coagulase positive *Staphylococci* were enumerated in Chapman Mannitol Salt Agar at 37°C for 48 h accordance to NF V08-057-1: 2004. *Salmonella* was isolated according to the standard method of NF ISO 6579: 2002 [9,10]. A portion of 25 g of sample was suspended in 225 ml of buffered peptone water then 0.1 ml of the suspension was added to 10 ml of Rappaport Vassiliadis broth and the selective isolation was carried out on Xylose-Lysine-Deoxycholate agar and Hektoen agar.

2.3 Identification with Biochemical Tests

The *Staphylococcus aureus* were confirmed by catalase, oxidase, and coagulase tests (NF V08-057-1, 2004). API®20E galleries was used for biochemical identification of suspicious isolates of *Salmonella.*

2.4 Amplification of Targeted Genes

Chelex-100 rasinemethod was used to directly extract DNA from pathogens reisolated on nutrient agar. The extracted DNA was used to amplified the targeted genes.

Conventional PCR was carried out by the Thermocycler to identify the genes.

For the amplification of the *invA* gene the pre-denaturation was done at 94°C, for 3 minutes; followed by 28 cycles of denaturation at 94°C for 30 seconds; annealing at 57°C, for 1 minute; elongation at 72°C for 1 minute 30 seconds and termination at 72°C for 10 minutes [11]. As concerns the amplification of the *cao* gene, pre-denaturation was done at 94°C, for 3 minutes followed by 30 cycles of denaturation at 94°C for

1 minute, annealing at 55°C for 1 minute; elongation in 1 minute at 72°C and termination at 72°C, for 5 minutes.

The electrophoresis machine used to 100 volts and gel electrophoresis was used to visualized and identify the amplified genes.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella* and *S. aureus*

Table 1. Results of biochemical tests of *Salmonella*

Pathogen	Catalase	Oxidase	Uréase	Lactose	Glucose	Gaz	H_2S
Salmonella	+	-	-	-	+	+	+

Table 2. Results of biochemical tests of *S. aureus*

Pathogen	Catalase	Oxidase	Coagulase	Gaz	H_2S
S. aureus	+	-	+	-	-

3.2 PCR Amplification of Target Genes

From gel electrophoresis and visualisation following PCR amplification of the *invA* gene, 8 samples carried the invA gene which is responsible for gastroenteritis and invasion of epithelial cells (Fig. 1).

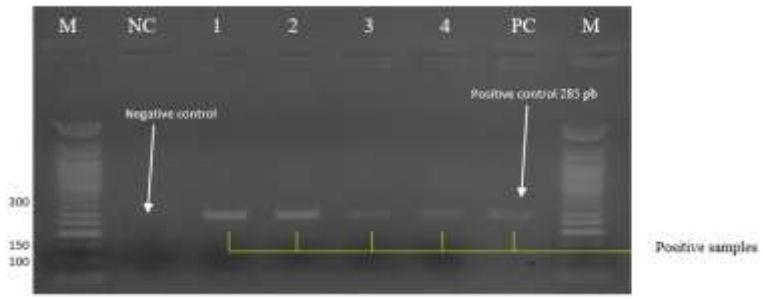

Fig. 1. Profile of PCR products of the invA gene in Salmonella strains

Legend: M: 50 bp marker; NC: Negative Control; PC: Positive Control (Salmonella typhi 15SA); samples number 1, 2, 3 and 4 carry the invA gene.

Following PCR amplification and the gel electrophoresis and visualisation of the *coa* gene 2 samples carried the coa gene (Fig. 2).

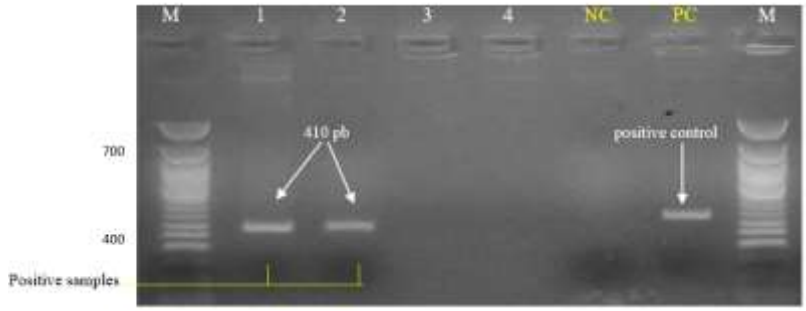

Fig. 2. Profile of PCR products of the *coa* gene of *S. aureus*

Legend: M: 50 bp marker; NC: Negative Control; PC: Positive Control (S. aureus NCTC 10652); samples 1 and 2 are positive for the coa gene (two bands are between 400 and 700 bp); samples 3 and 4 are negative. S. aureus NCTC 10652 was also used for the positive control of the coa gene [12].

Table 3. Prevalence of amplified virulence genes

Pathogen isolated	Gene	Number of samples	Positive samples	Negative samples
Salmonella spp	*invA*	10	8(80%)	2(20%)
S. aureus	*coa*	10	2(20%)	8(80%)

Legend: Positive sample: presence of the gene after molecular analysis; Negative sample: absence of the gene after molecular analysis

Of the 10 *Salmonella* strains isolates analyzed, 8 samples carry the *invA* gene. For *Staphylococcus aureus* samples, 2 strains carry the coa gene. 80% of Salmonella isolated carry the *invA* gene and 20% of *Staphylococcus aureus* samples carry the coa gene Table 3.

4. DISCUSSION

In this study, 80% of *Salmonella* carried the *invA* virulence gene. This finding is similar to the result obtained by Stella *et al.,* [13] who showed that 96% of *Salmonella spp* carry the *invA* gene. However, the results obtained are in disagreement with Nwiyi *et al.,* [14] in Nigeria, who indicated that 100% of *Salmonella* strains isolated carry the *invA* gene. This can be explained by the fact that their isolated *Salmonella* strains all came from wastewater discharged from farms.

The *invA* gene was present in all tested *Salmonella enterica* strains isolated from food chain links in Poland, according to Wójcicki *et al*.,[2].

The pathogens isolated in this study were similar to those reported by Fasanmi et al., (2010) and Anbessa [15,16]. where *Salmonella* and *S. aureus* were found in meat in Nigeria and Ethiopia. *Salmonella* are pathogenic bacteria for the consumer, the *invA* virulence gene of *Salmonella* causes an invasion of epithelial cells [17]. The unclean environment (67%) and the lack of training in Good Hygiene Practices (66.25%) could contribute to the contamination of dried meats by microorganisms as in the case of meat analyzed by Ayalew *et al*. in Ethiopia [1]. The presence of microorganisms in meat could also come from clothing, the environment or contaminated materials, as in the studies of meat processed in Uganda by Bagumire *et al.* [18]. Regarding *Staphylococcus aureus*, our results are slightly below the studies of Hassan *et al*., and Kav *et al*., who found 48% and 31% of samples positive for the toxic shock syndrome of *coa* gene [19,12]. This result could reflect the fact that they had used the 23SrRNA detection gene from *S. aureus* before looking for the *coa* gene. Most *S. aureus* give a single band between 300 and 800 bp [20]. This was the case with our results which gave a single band for the positive samples.

Coa gene of *S. aureus* is also isolated from ready-to-eat seafood revealed several virulence gene in food pahogene [21].

The high level of *Staphylococcus* in beef and camel meat samples indicates the presence of cross-contamination which is generally related to the materials and clothing used [22]. The results of the presence of *S. aureus* in meat samples at 32.5% in slaughter houses in South Africa is similar with our results [23].

5. CONCLUSION

Molecular analyzes showed us the presence of virulence genes in the identified pathogens. These virulence genes, responsible for gastroenteritis (80%) and toxic shock syndrome poisoning (20%), can cause real public health problems among consumers.

To resolve this problem, the development of a protocol for dry meat management would be a solution. Also, the established manual of procedure for microbia control in dried meat from N'Djamena with suggestions for easy-to-apply method, could be used for meat quality control management, in order to preserve the health of the consumer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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