

## Original Research Article

### **Identification of virulence genes of *Salmonella* and *Staphylococcus aureus* strains from dried meat isolate in N'Djamena**

#### **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 food born diseases caused by *Salmonella* and *Staphylococcus aureus* have been link to meat consumption.

In order to identify the virulence genes of *Salmonella* and *Staphylococcus aureus* responsible for food born diseases in dried meat consumers in N'Djamena/ Chad, isolated, extracted and analyzed DNA using conventional PCR from May to December 2020 in the laboratory of Public Health Biotechnologies of the Biotechnology Center of the University of Yaounde 1.

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* gene that causes severe 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. As a follow up from this finding an easy to used dried meat quality control manual of procedure was developed in view of preserving the health of consumers

**Keywords:** dried meat, virulence genes, *Salmonella*, *Staphylococcus aureus*, N'Djamena.

#### **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 *S. thyphimurium*, *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 Foodborn 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].

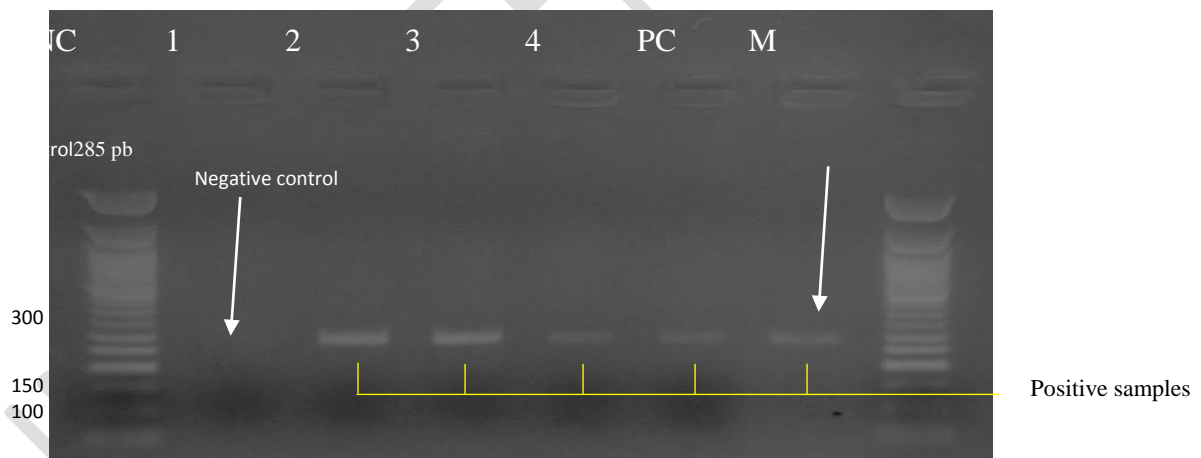
## **Methodology**

Dried meat samples were collected from different sales point in N'Djamena. Coagulase positive *Staphylococci* and *Salmonella* were isolated from the dried meat samples in accordance to NF V08-057-1: 2004 and the standard method of NF ISO 6579: 2002[9, 10]. Chelex-100 rasin method was used to directly extract DNA from the bacterian culture reisolated on nutrient agar. The extracted DNA was used to amplified the targeted 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 *coa* 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. Gel electrophoresis was used to visualize and identify the amplified genes.

## Results and discussions

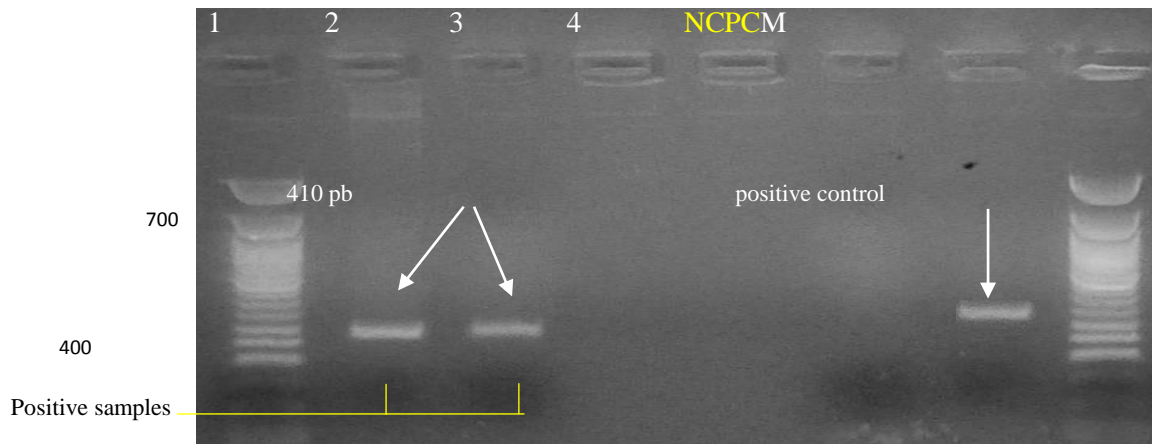
From gel electrophoresis and visualization following PCR amplification of the *invA* gene, 8 samples carried the *invA* gene which is responsible for gastroenteritis and invasion of epithelial cells (Figure 1).



**Figure 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 visualization of the *coa* gene 2 samples carried the *coa* gene (Figure 2).



**Figure 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].

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 1.

**Table 1:** Prevalence of amplified virulence genes.

Pathogenisolated	gene	Number of samples	Positive samples	Negativesamples
<i>Salmonella spp</i>	<i>invA</i>	10	8(80%)	2(20%)
<i>S. aureus</i>	<i>coa</i>	10	2(20%)	8(80%)

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

## Discussion

In this study, 80% of *Salmonella* carried the *invA* virulence gene. This finding is similar to the result obtained by Stella *et al.*, (2015) [13] who showed that 96% of *Salmonella spp* carry the *invA* gene. However, the results obtained are in disagreement with Nwiyiet *al.*, (2015) [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 Wójcickiet *al.*, (2022)[2].

The pathogens isolated in this study were similar to those reported by Fasanmi *et al.*, (2010) and Anbessa, (2013) [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.* , (2015) 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 Bagumireet *al.* (2017) [18]. Regarding *Staphylococcus aureus*, our results are slightly below the studies of Hassan *et al.*, (2010) and Kavet *al.*, (2011) 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 23S rRNA 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 pathogens[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 slaughterhouses in South Africa is similar with our results [23].

## 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 methods, could be used for meat quality control management, in order to preserve the health of the consumer.

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