Original Research Article

Identification of virulence genes of *Samonella*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 borndiseases 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 consummers 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 severed toxic shock in humans.This results demontratedthe 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 preseving the health of consumers

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

Introduction

Comment [U1]: Incorrect spelling 'borne'

Comment [U2]: Recast this sentence in this manner 'Virulence genes of *Salmonella* and *Staphylococcus aureus* isolated from dried meat in Njamena/Chadwas analyzed using conventional PCR in the Laboratory of Public Health Biotechnology Center, University of Younde.

Comment [U3]: Incorrect spelling 'demonstrated'

Comment [U4]: Recast in thismanner '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.

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 thisbacteria 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 Foodbornillness 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 formof nausea, vomiting, abdominal pain and profuse diarrhea. These bacteria cause severe toxic shock through the action of thecoa 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 rasinemethod was used to directly extract DNA from the bacterianculture reisolated on nutrient agar. The extracted DNA was used to amplified the targeted genes.

Comment [U5]: Incorrect spelling 'foodborne'

Comment [U6]: Your Methodology needs clear subheadings :
-Collection of samples
-Isolation (serial dilution and method used in plating)
-Identification (biochemical tests used) (remember it is important to show how the isolates was identified)
-Amplification of Targetedgenes
This sub heading should indicates how you perform your experiments equentially.

Comment [U7]: Is coagulase test the only biochemical test performed? There are other necessary test, include them.

Comment [U8]: Give brief explantations on how this method was done

Comment [U9]: Wrong spelling

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. Gel electrophresis was used to visualized and identify the amplified genes.

Comment [U10]: Wrong spelling 'electrophoresis'

Results and discussions

Comment [U11]: Your result should be different from the discussions
The results should have sub headings in such manners :
-Isolation and Identification of Salmonella and Staphylococus: this should clearly interpret the result of your biochemical tests)in table).
-PCR Amplification of targetgene: in detail sinterpretthe results of PCR amplification of the targetgenes, (image of the Gel)

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(Figure 1).

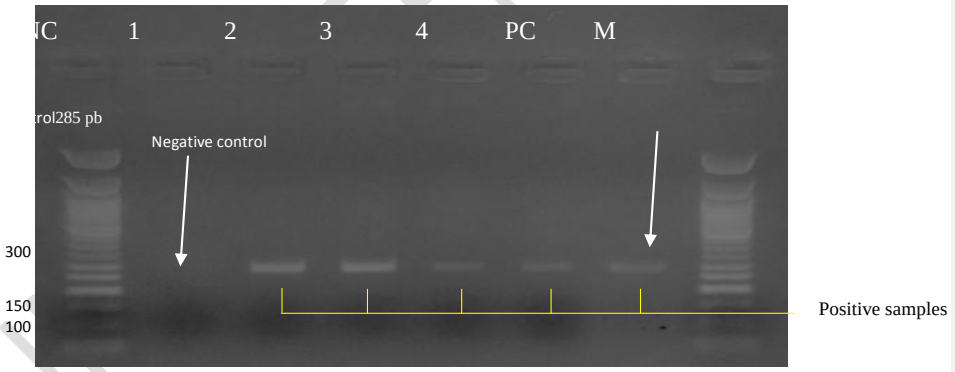

Figure 1: Profile of PCR products of the invA gene in Salmonella strains.

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 amplificationand the gel electrophoresis and visualisation of the coa gene 2 samples carried the coa gene (Figure 2).

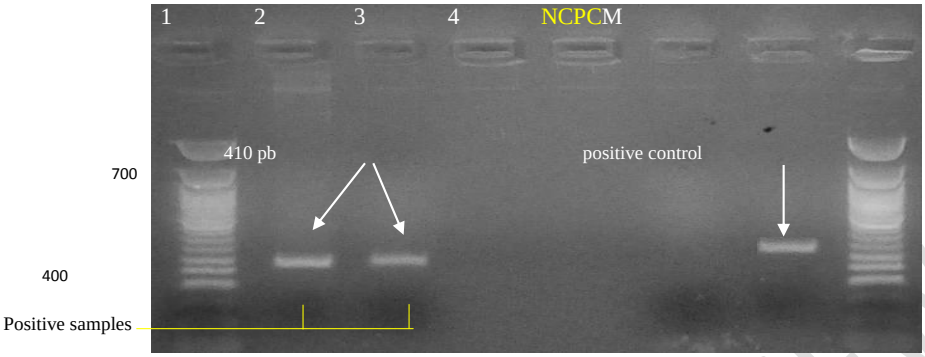

Figure 2: Profile of PCR products of the coa gene of S. aureus.

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
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.

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 Nwiyi*et 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 toWójcicki*et 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 Bagumire*et al.* (2017) [18].Regarding *Staphylococcus aureus*, our results are slightly below the studies of Hassan *et al*., (2010) and Kav*et al*., (2011) who found 48% and 31% of samples positive for the toxic shock syndrome ofcoagene[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 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 extablished 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.

Comment [U12]: Wrong spelling 'established'

Comment [U13]: Remove the 's'

References :

1 Ayalew H., Berhanu A., Berhanu S., and Biresaw S. *Microbiological assessment of meat contact surfaces at abattoir and retail houses in Jigjiga town, Somali National Regional State of Ethiopia*. Journal of the Science of Food and Agriculture. 2015;5(3): 21- 26.

2 Wójcicki, M.; Chmielarczyk, A.; Swider, O.; ´ Srednicka, P.; Strus, ´ M.; Kasperski, T.; Shymialevich, D.; Cie´slak, H.; Emanowicz, P.; Kowalczyk, M. *Bacterial Pathogens in the Food Industry: Antibiotic Resistance and Virulence Factors of Salmonella enterica Strains Isolated from Food Chain Links.* MDPIPathogens Journal. 2022; 11:(1323) 1- 25.

3 Tidjani A., Doutoum A. A., Brahim B. O, Mahamat B., Hourra D., Fatiou T., Comlan A. *Assessment of Hygiene Practices and Identification of Critical Control Points Relating to the Production of Skewered Meat Sold in N'Djamena-Chad*. Journal of Food Research. 2013;2 (5): 190 - 203.

4 Mohamed K. *Detection of Virulence Gene (invA) in Salmonella Isolated from Meat and Poultry Products.* International Journal of Genetics. 2013;3(2): 07-12.

5 Obeng A. K., Johnson F. S., Appenteng S.O.*Microbial Quality of Fresh Meat from Retail Outlets in Tolon and Kumbungu Districts of the Northern Region of Ghana.*International Journal of Science and Technology. 2013;2(6): 423 - 428.

6 Odetokun I A, et al. Mutiat A A, Rodhiat OA, Adeola OA, Aliyu NA, Ibrahim G M, Ahmed IA, and Alexandra F. Food Safety a *Staphylococcus aureus contamination of animal-derived foods in Nigeria Food* Safety and Risk2023;10(6) 2- 3.

7 Williams L. L., Ajayi O. A., Johnson J., Carter B. and Khartiwada J. *Molecular typing and cytotoxicity testing of Staphylococcus aureus isolated from meat, milk and clinical sources.* African Journal of Microbiology Research. 2014;8 (2): 1282 - 1291.

8 Ramesh N. B., Bakhya G. S., Rekha L., Karthiga P. *Molecular analysis of Coagulase (coa) gene polymorphism in clinical isolates of Staphylococcus aureus by PCR-RFLP*. International Journal of Innovative Research in Science Engineering and Technology. 2014; 1(3): 8163 – 8168.

9 NF V 08-057-1. *Microbiologie des aliments - Méthode de routine pour le dénombrement des staphylocoques à coagulase positive par comptage des colonies à 37 °C* : technique avec confirmation des colonies. Ministère de l'alimentation, de l'agriculture et de la pêche. 2004 ;1 - 18.

10 NF ISO 6579. *Microbiologie des aliments - Méthode horizontale pour la recherche des Salmonella spp*. Association Française de Normalisation, Paris. 2002; 1- 27.

11 Zhao H.K., Wang W.J., Zhao P., Zhang H.B., Kong N., Zhou M.D. and Zhang H. *Simultaneous Detection of Salmonella, Listeria Monocytogenes and Shigella in Poultry Samples by Triplex PCR*. Journal of Bacteriology and Mycology. 2014;1(1) : 1 -5.

12 Kav K., Ramazan C., and Mustafa A. *Characterization of Staphylococcus aureus Isolates from White-Brined Urfa Cheese, Turkey*. Journal of Food Protection. 2011;74(11): 1788 - 1796.

13 Stella I. S., Muinah A. F., Adedamilola T., Joseph A., Tina F., Mary E. A., Emmanuel A. O., Margaret I., Moses B. and Peter O. *Molecular Detection of Some Virulence Genes in Salmonella Spp Isolated from Food Samples in Lagos, Nigeria*. Animal and Veterinary Sciences. 2015;3 (1) : 22 - 27.

14 Nwiyi P. O., Soyoola M., and Oguoma I. O. *Detection of Virulence Genes in Salmonella Isolated From Chicken and Chicken Waste Water.* Global Advanced Research Journal of Microbiology. 2015;4(11) : 125 -129.

15 Fasanmi, G. O., Olukole, S. G., and Kehinde, O. O. *Microbial studies of table scrapings from meat stalls in Ibadan Metropolis, Nigeria: Implications on meat hygiene.* African Journal of Biotechnology. 2010;9 (21) : 3158 - 3162.

16 Anbessa D. *Evaluation of home slaughtered meat quality used for human consumption at household and food seller house in Jimma.* World Journal of Medicine and Medical Science Research.2013; 1 (3) : 038 - 043.

17 Abdelhaziz N.M. *Detection of Salmonella species in chicken carcasses and bovine meat using genus specific primer belong to invA gene in Sohag city, Egypt.* Veterinary World. 2016;9 (10): 1125 -1128.

18 Bagumire A., Roland T., and Karumuna R. *Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda*. African Journal of Food Science. 2017; 11(6) : 160 -170.

19 Hassan M., Ebrahim R., and Elahe T. *Detection of some virulence factors in Staphylococcus aureus isolated from clinical and subclinical bovine mastitis in Iran.* African Journal of Biotechnology. 2010;9(25) : 3753 - 3758.

20 Marwa I.A.E., and Mahmoud M. B. *Association between agr Alleles and Toxin Gene Profiles of S. aureus Isolates from Human and Animal Sources in Egypt* International Journal of Advanced Research. 2013;1(8) : 133 - 144.

21 Beshiru A, Igbinosa IH, Igbinosa EO. *Characterization of enterotoxigenic Staphylococcus aureus from ready-to-eat seafood* (RTES). Food Safety and Risk. 2021; 10 : 6 11- 14.

22 Firew T., Gulelat D., Ketema B. and Haile A. *Microbiological quality and safety of street vended raw meat in Jijiga town of Somali Regional State, southeast Ethiopia*. African Journal of Microbiology Research. 2014;8(48): 3867- 3874.

23 Nicoline F. T., Eunice S., Roland N. N., and Pascal O. B. *Detection of Pathogenic Escherichia coli and Staphylococcus aureus from Cattle and Pigs Slaughtered in Abattoirs in Vhembe District, South Africa.* Scientific World Journal. 2015;1 : 1 - 8.