ERFAN WORKING GROUP in SADC REGION

FOOD HYGIENE

PROPOSAL RESEARCH PROJECT

Baseline study on *Listeria monocytogenes*, *Salmonella* spp. and *Klebsiella pneumoniae* prevalence and AMR in RTE meat products and environmental samples in Botswana, Italy, Namibia, South Africa and Zambia

February 2022 up to January 2023

Sommario

Title

Baseline study on *Listeria monocytogenes*, *Salmonella* spp. and *Klebsiella pneumoniae* prevalence and AMR in RTE meat products and environmental samples in in Botswana, Namibia and Zambia.

Principal Investigator

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Background

Foodborne disease caused by Salmonella and Listeria monocytogenes are of great concern for consumers all over the world, causing loss caused by both the disease. The burden caused by bacterial foodborne diseases accounts for costs caused directly by morbidity, mortality, and indirectly caused by the squeal of the diseases.

Diarrhoea is the acute, most common symptom of foodborne illness, but other serious consequences include kidney and liver failure, brain and neural disorders, reactive arthritis. There is a strong need to strengthen surveillance systems for foodborne diseases. Surveillance data could be used for planning, implementing and evaluating public health policies.

Health Authorities need to develop a comprehensive strategy on strengthening foodborne disease surveillance. Among the most virulent foodborne diseases and foodborne pathogens causing disease are *Salmonella* spp. and *L. monocytogenes.*

Salmonella spp. causes salmonellosis, the most important foodborne disease since many years.

Available incidence data for salmonellosis in sub-Saharan Africa are scarce. National standardized data are required to better understand the nature and burden of disease in Namibia and to assess the burden of the disease in other SADC countries; in addition, the availability of isolates is of paramount importance to evaluate AMR of the causative agents (Florian Marks et al., 2017). As reported by Morpeth et al. (2009) invasive non-Typhoid Salmonella are endemic in sub-Saharan Africa, where it is a leading cause of bloodstream infection. Some host risk factors have been established, but little is known about environmental reservoirs and predominant modes of transmission, prevention strategies are underdeveloped.

L. monocytogenes is an important pathogenic bacterium which is transmitted through food, mainly Ready-to-Eat (RTE) foods. Food business operators (FBO) monitor the presence in food samples, as well as in food producing environment samples in order to ensure that food is safe for human consumption, along the entire food chain (from the production to the consumer). *L. monocytogenes* is usually transmitted through contaminated food, the diffusion is caused by the elimination with animal faeces and environmental contamination (soil, water), from animals to humans, usually.

The fatality rate ranges from 20 to 30% in high risk groups like pregnant women, unborn or newly delivered infants, and the elderly people as well as persons with severe underlying disease conditions like immune-suppression, AIDS, chronic disease like cirrhosis.

Listeriosis was recently diagnosed in South Africa, between 1 January 2017 and 14 March 2018. Data collection reported 978 laboratory-confirmed Listeriosis cases from all provinces, a strain of the ST6 was identified in 90% of the patients. The same ST6 sequence type was identified in a widely consumed ready-to-eat processed meat product called "Polony" and in the processing environment of the manufacturer of the implicated product. On 4 March 2018, the Ministry of Health, announced that this product was believed to be the source of the outbreak. The outcome of illness is known for 674 patients, of whom 183 (27%) of them died (WHO, 2018).

On the other hand, although *Listeria* and *Salmonella* in foods are a known food safety concern worldwide, *K. pneumoniae* could also be a source of food borne illnesses. A recent study has linked meat with some pathogenic *Klebsiella* spp. (Larsen, 2015) while *Salmonella* and *L. monocytogenes* have been isolated in street food RTE meat sold in Windhoek (Shiningeni *et al.* 2019).

K. pneumoniae is a common pathogen, causing serious illness in humans and animals, as septicemia, pneumonia, liver disease, linked to the virulence and AMR, epidemiology of K. pneumoniae is little known, and the role of food and environment is still under investigation.

Aims of the project

The aims of the project are listed below:

1. To perform a baseline study on prevalence of *Listeria monocytogenes (L. monocytogenes)*, *Salmonella* spp. and *Klebsiella pneumoniae (K. pneumoniae)* in food and environmental samples.
2. To build database of genomic data to support epidemiological surveillance for human transmission.
3. To determine MIC for isolated strains,
4. To define the ecological scenario of *K. pneumoniae* and presence of the clones in food and environmental samples.
5. To build competence on handling genomic data from Next Generation Sequencing based on online training.

Duration of the project

The maximum duration is fixed at 1 year, from February 2022 up to January 2023.

Participating countries and experts

Namibia

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Italy

Unit n. 6 Istituto Zooprofilatico Sperimentale dell'Abruzzo e del Molise (IZSAM)

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Methodology

Samples and sampling

The most commonly consumed RTE meat products produced in participating countries will be sampled, taking into account three (3) categories of samples. In detail 1) raw/processed RTE products; 2) raw fermented products and 3) environmental samples from food producing environment.

This is a baseline study, targeting at least one hundred and eighty (180) samples with a minimum of 60 samples of each sample category, considering the prevalence of the pathogen bacteria at a level of 5%.

Food samples will be randomly collected from producing factories or retail stores. A minimum amount of 100 g will be sampled and 50 g tested, 25 g will be used for *L. monocytogenes* analysis while other 25 g will be used for *Salmonella* and *K. pneumoniae* detection.

The origin of the product will also be noted as beef, poultry, pork and lamb products.

Environmental samples will be taken from food producing plants or from retail shops, at least 10 set of samples will be taken and tested for *L. monocytogenes*, *Salmonella* and *Klebsiella pneumoniae* detection, consisting of at least five sponges (gauzes or tissue fabric). The site of sampling in food producing plants could be drains, cleaning equipment, basins, deboning boards, vats for meat. In retail shops drains, cleaning equipment, basins, slicing and mincing machines and scales.

Sampling site

Botswana: The samples will be taken in the South East District, at least in two food producing plants to be identified in and around Gaborone. Seasonal aspect will be considered.

Namibia: The samples will be taken in the Northern Communal Areas (NCA), at two facilities in Oshakati and Ondangwa. The other four facilities selected for sampling are located in different areas of Windhoek. Seasonal aspect will be considered.

Zambia: Seasonal aspect will be considered taking into account the hot and cold seasons. In Zambia the months of June to August as cold season, and October to December as hot season. Samples will be collected from 3 districts, i.e. Lusaka, Choma and Ndola which are suburban areas where ready to eat meat products are highly consumed.

South Africa: The samples will be taken in Free State and Limpopo provinces, at two facilities in Bloemfontein and Polokwane respectively. Seasonal aspect will be considered.

Laboratory analysis

Listeria monocytogenes

The isolation of *L. monocytogenes* will be done according to ISO 11290-1:2017. To test environmental sponges the same method should be used using the bag containing the sponge as first container for enrichment, adding to the sponge 225 ml of half strength Fraser broth.

During the isolation procedure 1 ml of full strength Fraser will be sampled and dispensed in a 1.5 ml Eppendorf vial for PCR, identified and sealed. Five suspect colonies will be selected from the agar plates and dispensed into cryotubes and stored at -80 °C. Both vials and cryotubes will be sent to IZSAM, Teramo, Italy for molecular characterization and NGS.

Salmonella spp.

Detection of *Salmonella* spp. will be done according to ISO 6579-1:2017. Five suspect colonies from each positive sample will be selected from the agar plates and dispensed into cryotubes and stored at -80 °C. Cryotubes will be sent to IZSAM, Teramo, Italy for molecular characterization and NGS.

Klebsiella pneumoniae

The isolation and identification of *Klebsiella* spp. will be done according to the method used for isolation from clinical samples, using BPW (the same enrichment step used for *Salmonella* spp. detection), incubated for 24 hours at 37°C followed by streaking onto Simmons Citrate with 10 % Inositol (SCAI) agar and incubation at 44°C for 48 hours.

Presumptive colonies will be subcultured on SCAI agar and the plates will again be incubated at 37 °C for 48 hours. Suspected colonies are yellow on SCAI agar.

Five suspected colonies will be sampled and dispensed into cryotubes and stored at minus 80 °C before being sent to IZSAM, Teramo, Italy for species confirmation, molecular characterization, including NGS and identification of virulence-associated genes.

	Botswana	Namibia	Zambia	South Africa	Italy TE	Italy FG
Listeria monocytogenes	ISO 11290-1:2017	ISO 11290-1:2017	ISO 11290-1:2017	One-broth method and/or ISO 11290-1:2017		
Salmonella spp.	ISO 6579-1:2017	ISO 6579-1:2017	ISO 6579-1:2017	ISO 6579-1:2017		
Klebsiella pneumoniae	In house method	In house method	In house method	In house method		
Species confirmation	To be filled if applicable	To be filled if applicable	To be filled if applicable	*To be filled if applicable	Maldi TOF	Maldi TOF
Molecular characterization	To be filled if applicable	To be filled if applicable	To be filled if applicable	*To be filled if applicable	PCR	
AMR	To be filled if applicable	To be filled if applicable	To be filled if applicable	*To be filled if applicable	MIC microdution method Kirby Bauer	
NGS					Illumina	
Biofilm					Microwell method	

- South Africa has capacity to species confirmation, molecular characterization, AMR and NGS should a need arise

Species confirmation, molecular characterization, MIC determination and NGS

1. Species confirmation will be performed using MALDI-TOF method or validated tests (e.g. Vitek).

2. AMR for MIC determination could be done in Italy to detect the sensibility to antimicrobial on a selection of isolated strains. AMR will also be determined by disc diffusion test and/or Vitek 2 analysis.
3. NGS could be done on a selected number of confirmed colonies.
4. Biofilm production in vitro could be done on a selected number of confirmed colonies of *L. monocytogenes.*

Note. If the laboratory is unable to adopt the method reported in the project, to test the samples validated or internationally recognized methods should be used.

Expected Results

The expected results are listed below:

1. Results (Figure) related to prevalence of *L. monocytogenes*, *Salmonella spp.* and *K. pneumoniae* in food and environmental samples.
2. Molecular characterization and NGS for *L. monocytogenes* to allow determination of ST, CC and cgMLST; for *Salmonella* spp. serotyping and NGS and for *K. pneumoniae* NGS, MIC determination and NGS of MDR strains.
3. Genomic data will be used to build the first genomic database in the participating countries, handled by IZSAM in the framework of GENPAT activities, and used to generate the studies of foodborne diseases circulating in the participating countries.
4. Ability to handle genomic data, download and build clustering studies, virulence and AMR studies.
5. MIC for a selection of isolated strains of *L. monocytogenes*, *Salmonella* spp. and *K. pneumoniae.*

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