GENOMIC ANALYSIS OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM POULTRY AND OCCUPATIONAL FARM WORKERS IN UMGUNGUNDLOVU DISTRICT, SOUTH AFRICA

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Is Foodborne Disease (FBD) a Problem?

- The global foodborne diseases estimations conducted by World Health Organization (WHO) indicates that about 30% of the populations in the industrialized countries suffer from food borne diseases (WHO, 2015).

- The continent of Africa has been reported to have the highest burden of foodborne diseases (FDBs) per population with approximately 91 million related diseases and 137000 death per annum requiring an urgent stakeholder and policy direction to curb their escalation (FDBs) (WHO, 2015).

- Microbial contaminants, mainly bacteria, constitute the main cause of FDBs and is a risk factor for the zoonotic transmission of infections.

- Recently; various bacteria food outbreaks leading to large mortality rates has been reported threatening food safety hence the need for effective surveillance and monitoring systems for early detection of such incidences (CDC, 2018a, 2018b).
The Links in Antibiotic Resistance from the Farm to the Table

https://www.cdc.gov/onehealth/
INCEPTION OF WHO-AGISAR PROJECT

Fig.1: Examples of sampling considerations through the production to postharvest continuum

- **Cohort of animals on farm, in holding pens (sale yards or pre-slaughter), and post-slaughter**
  - Addresses what is on-farm, transport/holding exposures and what contaminates meat prior to retail
  - Helps estimate the impact of on-farm antimicrobial use
  - Cost may hinder ability to be geographically representative

- **Animals on farm**
  - Does not always reflect pathogens that will be recovered post-slaughter
  - Most direct indication of resistance arising from on-farm antimicrobial use
  - May not address resistance from historical use or from exogenous sources

- **Transport**
  - Reflects what is expected to contaminate retail meats
  - Bacteria on-farm plus bacteria from cross-contamination during transport and in slaughterhouse
  - Less indicative of current antimicrobial use on-farm
  - Cross-contamination by strains persisting in environment can confound analysis

- **Holding**
  - Caecal samples immediately post-holding may overlap with farm sampling
  - Carcass samples reflect cross-contamination in plant or poor carcass preparation
  - Addresses what has contaminated meat
  - May overlap with retail meat sampling
  - Same limitations as holding and post-slaughter

- **In-plant**
  - Packaging, transport to retail or further processing

- **Post-slaughter**
  - May reflect cross-contamination during handling, packaging, further processing and in-store handling/re-packaging
  - Same limitations as holding and post-slaughter

- **Retail meats**
  - Individual animal caecal contents immediately post-exsanguination
  - Carcass rinsates
  - Carcass swabs
  - Ground product
  - Meat juice
  - Lymph nodes

**Possible sample types**

- **Animals on farm**
  - Feecal
  - Litter
  - Environmental (e.g. dust, fluff, feed, water)

- **Holding**
  - Holding pen floor sample
  - Truck/crate swabs

- **Post-slaughter**
  - Ground product
  - Whole cuts

- **Retail meats**
  - Individual animal caecal contents immediately post-exsanguination
  - Carcass rinsates
  - Carcass swabs
  - Ground product
  - Meat juice
  - Lymph nodes
**Staphylococcus aureus**

- *Staphylococcus aureus* is associated with various infections in humans, including acute sepsis, respiratory infection, deep-seated infections, superficial infections and toxin-mediated infections that have resulted in major public health, economic and social problems worldwide (Amoako et al., 2016).

- Molecular epidemiological evidence suggests MRSA has evolved on multiple occasions from lineages of methicillin-susceptible *S. aureus* (MSSA) isolates.

- MRSA has gradually developed resistance towards all the main classes of antibiotics to which it was once susceptible. There is co-resistance between methicillin and to other antibiotics.

- The presence of *meca* is an absolute requirement for *S. aureus* to express methicillin resistance.
MRSA remains a prominent multi-drug resistant bacterial species, which presents a challenge to clinicians, as the introduction of new classes of antibiotics is usually followed by the emergence of resistant pathogenic forms.

MRSA strains harbor mobile genetic elements (MGEs), including plasmids, transposons, integrons, genomic islands, pathogenicity islands, bacteriophages, which comprise about 15-25% of the genome.

MGEs play a significant role in bacterial survival and adaptability because they encode many resistance and virulence genes, hence understanding their composition will broaden our knowledge on the genetic determinants of antibiotic resistance (Malachowa et al., 2010).

There are 3 main types of MRSA infections: Hospital-acquired (HA-MRSA), Community-associated (CA-MRSA), Livestock-associated (LA-MRSA).

However, there are recent reports of a blurring epidemiology attributable to its progressive clonal expansion, adaptability and transmission between the various types has made the traditional definitions indistinct (Bal et al., 2016).
RELEVANCE OF THE PROJECT IN THE CONTEXT OF SOUTH AFRICA

- Poultry is the most consumed meat in South Africa with a consumption amounted to **2.152 million tonnes** according to SAPA's calculations (Viljoen, 2017).

- South Africa consumes **13 times the average** per capita poultry consumption in sub-Saharan Africa and almost **3 times the average** per capita world poultry consumption.

- Furthermore; Its consumption in 2016 accounted for **60% of total animal protein** consumption in the country, dwarfing the total consumption of other food animals (Whitfield, 2018).

- However, domestic production has been unable to keep up with rising domestic demand, necessitating supplementing the shortfall with imports from other world producers (Mkentane, 2017).

- Owing to its intensive nature and high demand, different antimicrobial agents such as penicillin, erythromycin, tetracycline, sulphonamides are extensively used as growth promoters as well as for treating infections in the country, which leads to the development of drug resistant strains (Boeckel et al., 2015; Gouws and Hoffman, 2018).
RELEVANCE OF THE PROJECT IN THE CONTEXT OF SOUTH AFRICA

- Nonetheless, there is a shortage of data on the microbial quality and safety in poultry farm production, particularly in relation to bacteria contamination in the country.

- Most of these reports are solely from clinical settings, leaving a major data gap in the antibiotic resistance levels from the food animals, food products and environmental sectors.

- In order to fill this gap, the resistance data from environmental and animal sectors need to be upped in order to obtain a holistic view of the actual state of antibiotic resistance in South Africa.

- This will offer valuable insights into their spread, evolution and molecular epidemiology of these organisms thus, necessitating an urgent incorporation of this concept into current projects.

**Abstract:** The interest about *Staphylococcus aureus* (S. aureus) and methicillin resistant *S. aureus* (MRSA) has bloomed in recent years. The spread of different drug-resistant related to livestock animals, mostly CC398, and the recent description of the new drug-resistant strain (CC113) may give momentum to the debate. The resistance and population structure of this microorganism all over the world. Nowadays, there are several descriptions about...
Antibiotic Resistance in Food Animals in Africa: A Systematic Review and Meta-Analysis

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Objectives: This study critically reviewed the published literature and performed a meta-analysis to determine the overall burden of antibiotic-resistant bacteria in food animals in Africa.

Methods: English and French published articles indexed in EBSCOhost, PubMed, Web of Science, and African Journals Online were retrieved, with searches being conducted up to August, 2015. Data were pooled and meta-analysis performed using a random-effects model, and the results are described as event rates.

Results: According to the predefined inclusion and exclusion criteria, 17 articles out of the 853 retrieved were included. These studies were conducted in Nigeria, South Africa, Egypt, and Senegal. The pooled estimates showed high level of antibiotic resistance (ABR) (OR: 0.68; p<0.001) and multiresistance (MDR) (OR: 0.48; p<0.001). The analysis yielded some interesting findings on the burden of ABR that could be useful in developing measures to contain this threat in the food-animal continuum in Africa.

Keywords: antibiotic resistance, food-borne infection, zoonosis, food animals, One Health approach

Results:
1. High level of antibiotic resistance (ABR).
2. High level of multi-drug resistance (MDR).

Conclusions:
1. ABR is prevalent and poses a serious threat to food safety in Africa.
2. Limited available data with regards to ABR research in Africa.

Recommendations:
1. One Health Approach and advocated recommendation by the WHO, OIE and FAO be followed to restrict antibiotics use.
2. Sound sampling and laboratory analysis schemes be used for investigating ABR.
3. Co-operation and good communication between various sectors (agriculture, veterinary, and public health etc) are required.
PROJECT AIM

• To investigate the **prevalence, antibiotic resistome, mobile genetic support (MGEs) and genetic lineages** of circulating **MRSA** isolated from poultry and occupationally exposed workers in an intensive poultry production system in uMgungundlovu, South Africa using whole genome sequencing (**WGS**) across the “farm to fork” continuum.
METHODS

1. ETHICAL APPROVAL

2. FARM TO FORK APPROACH
   - SAMPLING

3. ISOLATION OF MRSA

4. ANTIBIOTIC SUSCEPTIBILITY TESTING
WGS and Bioinformatic analysis

The use of whole genome sequencing (WGS), bioinformatic analysis as well as other complementary approaches to ascertain:

- resistance profile (as compared to the MICs and standard phenotypic assays).
- resistance mechanisms (known and novel resistome).
- novel mutations (via comparative genomics).
- genetic support (plasmids, integrons, transposons, insertion sequences).
- evolutionary relationship (in-silico prediction of MLST, Clonal complex, and SNP-based phylogeny to predict the association of these isolates).

To offer insights into the molecular epidemiology of these resistant isolates in KZN, South Africa.
RESULTS

A

- Total
- Retail products
- Post-slaughter
- Holding/Transport
- Human samples
- Animal on the farm

NUMBER OF MRSA

B

- Litter (L)
- Faecal (F)
- Human (Nasal swabs)
- Truck swabs (TR)
- Crate swabs (CR)
- Caecal content (Ce)
- Neck sample (Ne)
- Thigh sample (Th)
- Whole meat samples
- Total

SAMPLES
Twelve (12/145) isolates were MRSA and included nine from poultry (9/120; 7.5%) and three from poultry workers (3/25; 7.5%) across the poultry food-chain.

All the MRSA isolates harbored the \textit{mecA} carrying SCCmec MGE mediating resistance to beta-lactams.

\textbf{Figure 1:} A schematic representation of the backbone structure of SCC\textit{mec\_type\_IVd (2B)} elements in the MRSA isolates comprising of methicillin resistance determinant (\textit{meca}), methicillin resistance regulatory sensor-transducer (\textit{mecR1}), associated Insertion sequences (\textit{IS1272} and \textit{IS431}) and cassette chromosome recombinase gene (\textit{ccr}) allotype (\textit{ccrB2-ccrA2}).
WGS revealed the dissemination of a MRSA clone across the poultry food chain in KwaZulu Natal province, South Africa.

MRSA isolates were predominated by closely related isolates of the MDR clone **ST612-CC8-t1257-SCCmec-IVd (2B)** previously associated with humans, most often in South Africa.

The antimicrobial resistome was conserved in **ST612** isolates and all resistome predicted the (multi-resistant) AST phenotypes.

Mutations in a chromosome-borne DNA gyrase (**gyrA-S84L**), topoisomerase IV (**parC-S80Y, parE-D434N**) and rpoB (**H481N**) mediated fluoroquinolone and rifampicin resistance, respectively.
Insertion Sequences and Prophages

- The ST612 clone also harboured other MGEs namely plasmid replicons based on Rep 7 and 20; insertion sequences (IS1182) and prophages (phi2958PVL).
Whole genome phylogenetic branch and metadata
Evolutionary and Phylogenomic tree of MRSA isolates from the “Farm to Fork” continuum
DISCUSSION

- The findings revealed multiple AMR encoding MGEs in a local multidrug resistant MRSA clone; **ST612-CC8-t1257-SCCmec_IVd (2B)** that was circulating between humans and animals along the ‘farm to table’ continuum.

- This could probably be underestimated considering the absence of data on the molecular epidemiology of MRSA in food animals in the country.

- This requires the establishment of appropriate control measures in order to curb further spread, in the South African food chain, and ameliorate this additional threat to our antimicrobial armamentarium.

- We, therefore, hope that the findings of the present study would be a crucial step towards highlighting the need to develop a more comprehensive surveillance and reporting systems for multidrug-resistant (MDR) organisms in Africa to help understand the molecular epidemiology.
CONCLUSION

- Adoption of the ‘One Health’ approach for tackling AMR.

- The need for comprehensive surveillance and monitoring schemes.

- An urgent review of the legislation and regulation governing the use of veterinary products in the country.

- The use high throughput technologies such as whole genome sequencing (WGS) in tandem with traditional techniques to give valuable insights into the spread, evolution and epidemiology of MRSA.
REFERENCES


WHO (FUNDERS)
Genomic analysis of methicillin-resistant *Staphylococcus aureus* isolated from poultry and occupational farm workers in Umgungundlovu District, South Africa

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**HIGHLIGHTS**

- MDR MSRA along the poultry food chain in South Africa was studied using WGS.
- An MRSA clone, ST612-CC8-t1257-SCCmec_tVd (2B), dominated the food chain.
- This predominant clone has been associated to humans in South Africa.
- The clone showed a conserved antibiotic resistance, confirming its MDR phenotypes.
- Presence of the human-linked clone in poultry highlights a complex spread
Article

Genome Mining and Comparative Pathogenomic Analysis of An Endemic Methicillin-Resistant Staphylococcus Aureus (MRSA) Clone, ST612-CC8-t1257-SCCmec_IVd(2B), Isolated in South Africa

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Abstract: This study undertook genome mining and comparative genomics to gain genetic insights into the dominance of the methicillin-resistant Staphylococcus aureus (MRSA) endemic clone