Multidrug-Resistant *Salmonella enterica* in the Democratic Republic of the Congo (DRC)

Octavie Lunguya¹, Veerle Lejon², Sophie Bertrand³, Raymond Vanhoof³, Jan Verhaegen⁴, Anthony M. Smith⁵, Benedikt Ley², Karen H. Keddy⁵, Jean-Jacques Muyembe-Tamfum¹, Jan Jacobs²

¹National Institute for Biomedical Research, Kinshasa, DR of the Congo, ²Institute of Trop. Med. Antwerpen, Belgium, ³Scientific Institute of Public Health, Brussels, Belgium, ⁴University Hospital Leuven, Leuven, Belgium, ⁵University of the Witwatersrand, Johannesburg, Republic of South Africa
Burden of disease – Sub Saharan Africa

*Salmonella* Typhi:
- 77.4 / 100,000 (children + adults) (Buckle 2012)
- Case fatality rate of 1% (Crump 2004)

Non-typhi *Salmonella* (NTS):
- 175-388 / 100,000 (children) (Gordon 2012)
- 1800-9000 / 100,000 (non-ART, HIV+ adults) (Gordon 2012)
- Case fatality rate of 22%-25% (children + adults) (Gordon 2008)

=> No incidence data from Central Africa available
Reported resistance rates – Central Africa

**Salmonella Typhi**
- Low numbers of MDR, increasing to >50% since mid 90’s
- Very low rates of Fluorquinolone resistance
- No resistance to 3rd gen. Cephalosporin

*(Vlieghe, 2009)*

**Non-typhi Salmonella**
- High numbers of resistance to Ampicillin and Chloramphenicol
- Medium resistance to Cotrimoxazole and Fluorquinolones
- No resistance to 3rd gen. Cephalosporins
- Resistance to Cotrimoxazol + Fluorquinolones observed from 1999 onwards

*(Vlieghe, 2009)*
In 2004 / 2005 an outbreak of *Salmonella* Typhi was observed in Kinshasa.

Case fatality rates of >50% were observed (Muyembe-Tamfum, 2008).

All isolates evaluated (n=11) were MDR but susceptible to:
- Gentamicin
- Ciprofloxacin
- Cefotaxim

=> A project to assess current susceptibility status of *Salmonella* spp. in the DRC was implemented.
From **2007 to 2011** a prospective health care facility based passive survey at centers in **7/11 provinces**: 

- Inclusion criteria: suspicion of invasive bacteremia
- Standard demographic data was recorded
- Blood for culture was collected
  - Standard laboratory procedures + antisera testing performed at Institut National de Recherche Biomédicale, (Kinshasa, DRC)
  - Re-serotyping + AB susceptibility testing performed at the Institute of Tropical Medicine (Antwerpen, Belgium)
  - PFGE + molec. markers for fluoroquinolone resistance performed at the National Institute of Public Health (Brussels, Belgium)
Methods – Antimicrobial Susceptibility

• Antimicrobial susceptibility testing for ampicillin, cefotaxime, trimethoprim-sulphamethaxole (TMP-SMX) was performed using the Vitek II (bioMérieux)

• **MIC** for nalidixic acid, ciprofloxacin, chloramphenicol and azithromycin was determined using E-test macromethod (bioMérieux)

• **ESBL** testing was done with double disc diffusion method (CLSIM100S22)
Minimal inhibitory concentrations for **nalidixic acid** (MIC≥32 mg/l) and **chloramphenicol** (MIC≥16 mg/l) were determined according to CLS1M100S21

**Azithromycine** resistance: MIC>16mg/l (EUCAST v2.0)

Decreased ciprofloxacin susceptibility (**DCS**): MIC>0.064mg/l (EUCAST v2.0)

**Multi Drug resistance** (**MDR**): Resistance against first line antibiotics ampicillin, chloramphenicol, cotrimoxazol (TMP-SMX)
• **Pulsed field gel electrophoresis (PFGE)** was performed on a subset using *XbaI* as restriction enzyme according to PulseNet protocol.

• **Screening** for chromosomal quinolone resistance determining regions (QRDR): *gyrA*, *gyrB*, *parC* genes (CEQ2000 DNA sequencer, Beckman Coulter)

• **Screening** for plasmid mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*) (Cavaco, 2009)
Results

A total of **9,634 blood** samples were collected in 7/11 provinces and cultured

- Positivity rate: 989 (10.3%, excluding contaminants)

- *Salmonella Typhi*: 201 (20.3%)

- NTS: 233 (23.6%)
  - 184 *Salmonella Typhimurium* (79%)
  - 42 *Salmonella Enteritidis* (18%)
  - 7 other *Salmonella* spp. (3%)
Results Survey

Salmonella Typhi

Non–typhi Salmonella
<table>
<thead>
<tr>
<th></th>
<th><em>Salmonella Typhi</em> (n=201)</th>
<th><em>non-typhi Salmonella</em> (n=233)</th>
<th><em>Salmonella Typhimurium</em> (n=184)</th>
<th><em>Salmonella Enteritidis</em> (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (%)</td>
<td>64.7</td>
<td>88.0</td>
<td>94.0</td>
<td>64.3</td>
</tr>
<tr>
<td>Chloramphenicol (%)</td>
<td>41.3</td>
<td>83.7</td>
<td>90.2</td>
<td>61.9</td>
</tr>
<tr>
<td>TMP-SMX (%)</td>
<td>57.7</td>
<td>88.0</td>
<td>94.0</td>
<td>64.3</td>
</tr>
<tr>
<td>MDR (%)</td>
<td>30.3</td>
<td>80.7</td>
<td>86.9</td>
<td>59.5</td>
</tr>
<tr>
<td>DCS (%)</td>
<td>15.4</td>
<td>4.3</td>
<td>4.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Nalidixic Acid (%)</td>
<td>15.4</td>
<td>4.3</td>
<td>4.9</td>
<td>0.0</td>
</tr>
<tr>
<td>MDR+DCS (%)</td>
<td>7.5</td>
<td>3.9</td>
<td>4.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Azithromycin (%)</td>
<td>1.0</td>
<td>3.0</td>
<td>3.3</td>
<td>0.0</td>
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<tr>
<td>Cefotaxime (%)</td>
<td>0.0</td>
<td>2.1</td>
<td>2.2</td>
<td>0.0</td>
</tr>
<tr>
<td>MDR+DCS+ESBL (%)</td>
<td>0.5</td>
<td>0.9</td>
<td>0.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Salmonella Typhi (n=31, all DCS):

- A total of 31 DCS associated point mutations:
  - gyrA: 83Ser > Tyr or Phe (n=22)
  - gyrA: 87Asp > Gly or Tyr or Asn (n=9)

- No qnrA and qnrB genes were detected
- No mutations in gyrB and parC genes
Mechanisms of DCS

Non-typhi *Salmonella* (n=10, all DCS):

- **gyrA**: 87Asp> Tyr (n=8)
- **gyrA**: 87Asp>Asn (n=2)
  - Also ESBL producers (all type SHV)
- No *qnrA*, *qnrB* and *qnrS* genes were detected
- No mutations in *gyrB* and *parC* genes
Pulsed Field Gel Electrophoresis

- **Salmonella Typhi** (n=185): 30 Profiles detected:
  - 132 (71%) shared one profile
    - Main profile over time and space
    - 41 (31%) were MDR
    - 23 (17%) were DCS
    - 11 (8%) were MDR + DCS

- **Salmonella Typhimurium** (n=34): 19 Profiles detected:
  - 7 (21%) shared one profile (T4)

- **Salmonella Enteritidis** (n=16): 10 Profiles detected:
  - 4 (25%) shared one profile (E5)
MDR and DCS were observed for *Salmonella Typhi*

- MDR was less frequent as had been reported in previous studies from the region
- DCS was more frequent as had been reported earlier from the region and was associated with point mutations in *gyrA*
- We possibly observed emerging azithromycine resistance
Very high rates of MDR were observed for NTS

Resistance to 3rd generation cephalosporins + ESBL in NTS is reported for the first time from the DRC

MDR rates in *Salmonella* Enteritidis were significantly lower than in *Salmonella* Typhimurium (p<0.01)
Conclusion

- The observed rates of MDR and DCS underline the importance of permanent antibiotic stewardship programs in the DRC.

- The appearance of strains resistant to 3\textsuperscript{rd} generation cephalosporins and azithromycin may be an indicator to spreading resistance against these drugs.

- Comprehensive surveillance systems and public health interventions targeting \textit{Salmonella} spp. are urgently needed to reduce the high burden of disease.

- Incidence studies on burden of disease are planned.
Thank you very much!