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

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Burden of disease – Sub Saharan Africa

***Salmonella* Typhi:**

- 77.4 / 100.000 (children + adults) (Buckle 2012)
- Case fatality rate of 1% (Crump2004)

Non-typhi *Salmonella* (NTS):

- 175-388 / 100.000 (children) (Gordon2012)
- 1800-9000 / 100.000 (non-ART, HIV^{+} adults) (Gordon2012)
- 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)

Trigger for Survey

- 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

Methods – Surveillance

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

- Anitibiotic susceptibility testing for **ampicilin, 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)

Definitions

- Minimal inhibitory concentrations for **nalidixic acid** (MIC≥32 mg/l) and **chloramphenicol** (MIC≥16 mg/l) were determined according to CLSIM100S21

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

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

- Multi Drug resistance (**MDR**): Resistance against first line antibiotics ampicillin, chloramphenicol, cotrimoxazol (TMP-SMX)

Methods – Molecular Analysis

- **Pulsed field gel electrophoresis (PFGE)** was performed on a subset using *Xbal* 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)

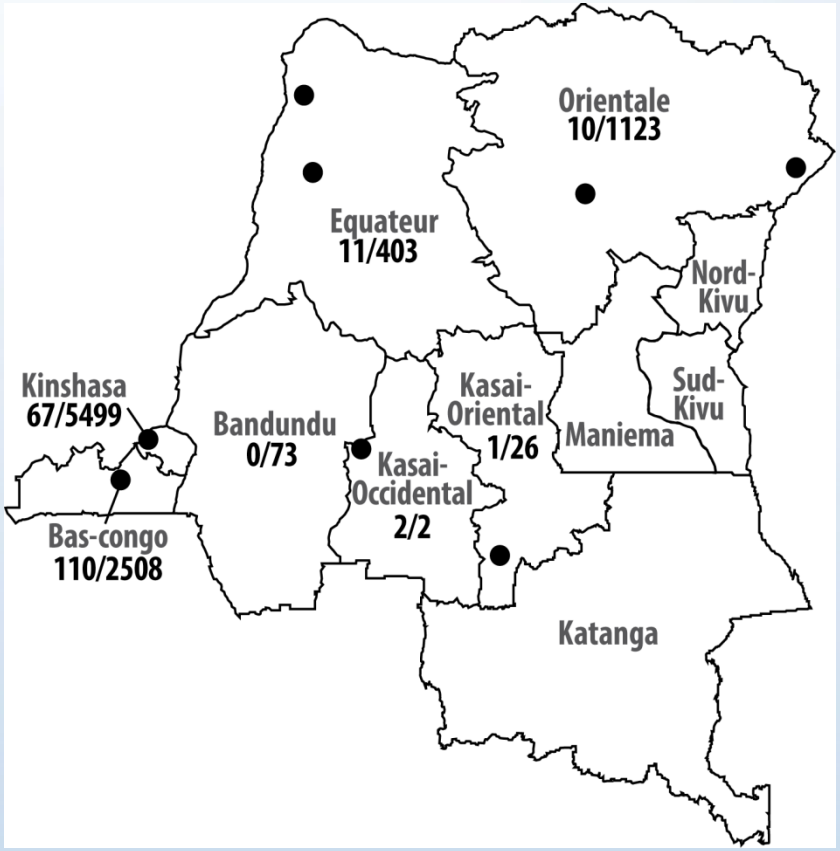
Results

A total of **9.634 blood** samples were collected in 7/11 provinces and cultured

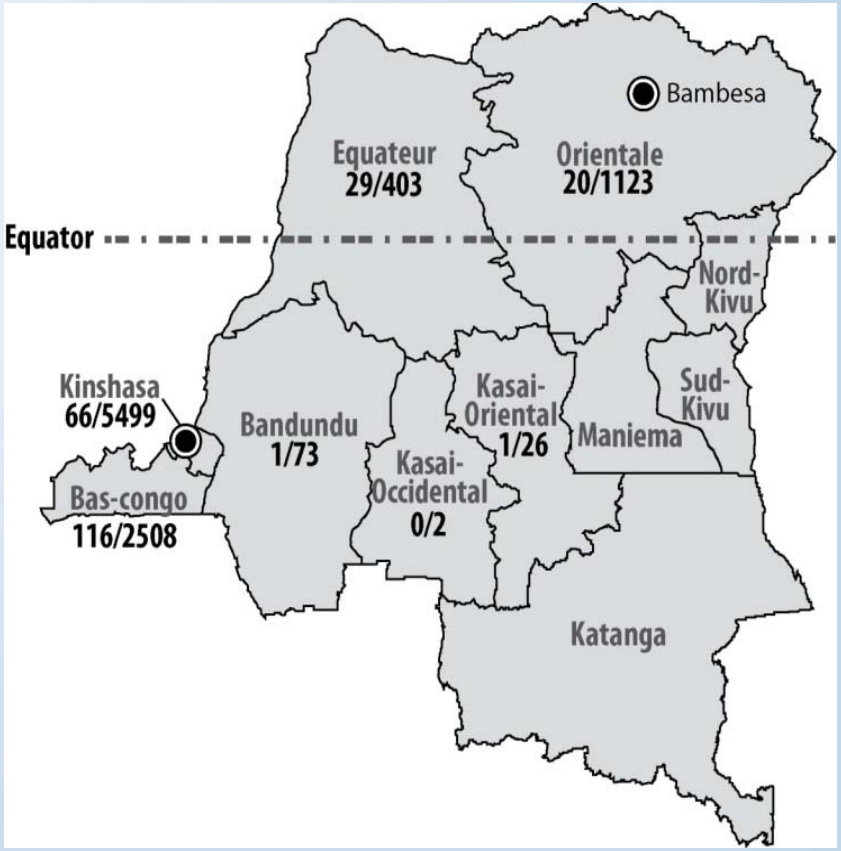
- Positivity rate: 989 (10.3%, excluding contaminants)
- *Salmonella* Typhi: 201 (20.3%)
- NTS: 233 (23.6%)
  - 184 *Salmonella* Typhimurium (79%)
  - 42 *Salmonella* Enteritidis (18%)
  - 7 other *Salmonella* spp. (3%)

Results Survey

Salmonella Typhi



Non – typhi *Salmonella*



Results – Antimicrobial Resistance (%)

	Salmonella Typhi (n=201)	non-typhi *Salmonella* (n=233)	*Salmonella* Typhimurium (n=184)	*Salmonella* Enteritidis (n=42)
Ampicillin (%)	64.7	88.0	94.0	64.3
Chloramphenicol (%)	41.3	83.7	90.2	61.9
TMP-SMX (%)	57.7	88.0	94.0	64.3
MDR (%)	**30.3**	**80.7**	**86.9**	**59.5**
DCS (%)	**15.4**	**4.3**	**4.9**	**0.0**
Nalidixic Acid (%)	15.4	4.3	4.9	0.0
MDR+DCS (%)	7.5	3.9	4.3	0.0
Azithromycin (%)	**1.0**	**3.0**	**3.3**	**0.0**
Cefotaxime (%)	**0.0**	**2.1**	**2.2**	**0.0**
MDR+DCS+ESBL (%)	0.5	0.9	0.5	0.0

Mechanisms of DCS

***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)

Discussion – *Salmonella* Typhi

MDR and DCS were observed for S***almonella* 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

Discussion non-typhi *Salmonella*

- 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 3rd generation cephalosporins and azithromycin may be an indicator to spreading resistance against these drugs
- Comprehensive surveillance systems and public health interventions targeting *Salmonella* spp. are urgently needed to reduce the high burden of disease.
- Incidence studies on burden of disease are planned.


Thank you
very much!