High prevalence of multidrug resistant ESBL- and plasmid mediated AmpC- producing clinical isolates of *Escherichia coli* at Maputo Central Hospital (MCH), Mozambique

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Durban October 22, 2019
Structure of the presentation

- Introduction
- Aim and objectives
- Methods
- Results and discussion
- Limitations
- Conclusion and recommendation
- Acknowledgements
Introduction

• Antibiotic resistance is one of the main public health problems worldwide

• Epidemiological data of cephalosporin-resistant *Enterobacteriaceae* in Sub-Saharan África is still limited, and in particular in Mozambique
  • ESBL
  • AmpC

[Mandomando et al., 2010, Moon et al., 2013]
Research question

• What is the prevalence of ESBL- and plasmid mediated AmpC- production in clinical isolates of *Escherichia coli* at Maputo Central Hospital, Mozambique?
Study objectives

• Isolate and confirm clinical isolates of *E. coli* processed from urine and blood samples
• Ascertain their antibiotic susceptibility
• Phenotypic detection of ESBL- and/or increased AmpC-production
• Identification of ESBL-/ pAmpC-encoding genes by PCR
Methods

Ethical considerations

• This study was approved by the Biomedical Research Ethics Committee of the UKZN (BE 030/16) and the Institutional Bioethics Committee for Health-CIBS-ISCISA (TFMCSCSCE 02/15).
Methods

• **Study period:** August – November 2015, 3 months

• **Strain inclusion criteria:**
  – *E. coli* from urine and blood cultures from in- and outpatients

• **Criteria for ESBL- and AmpC-testing:**
  – ESBL: Reduced susceptibility (I or R) to cefotaxime (CTX) or ceftazidime (CAZ) by disc diffusion (EUCAST criteria)
  – AmpC: …. AND reduced susceptibility to cefoxitin

• **Phenotypic test for ESBL:** Combined disc method

• **Molecular analysis:**
  – ESBL CTX-M PCR and amplicon sequencing
  – TEM, SHV and pAmpC PCRs
  – ERIC PCR for clonal relatedness between strains
Results and discussion
Total of samples and *E. coli* from blood and urine

**n=3326**

- **Blood + Culture (n=1078; 32.4%)**
  - **Urine**
    - **n=823; 24.7%**
      - **E. coli /urine**
        - **n=199/823; 24.1%**
    - **Blood**
      - **n=255; 7.7%**
        - **E. coli /blood**
          - **n=31/255; 12.1%**

- **n=1841; 55.4% In-patient**

- **n=1485; 44.6% Out-patient**
ESBL and AmpC detection

n= 230
E. coli

75/230 (32.6%)
E. coli  CTX/CAZ I/R
ESBL +

25/75 (33%)
AmpC +

140/230; (60.8%)
Inpatient

90/230; (39.1%)
Outpatient

17/31 (55%)
ESBL +; blood

58/199 (29%)
ESBL +; urine
Multidrug resistance (MDR)

<table>
<thead>
<tr>
<th>Sample</th>
<th>CIP (%)</th>
<th>GEN (%)</th>
<th>SXT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>12/17 (70, 6%)</td>
<td>8/17 (47, 1%)</td>
<td>17/17 (100%)</td>
</tr>
<tr>
<td>Urine</td>
<td>40/58 (68, 9%)</td>
<td>27/58 (46, 5%)</td>
<td>55/58 (94, 8%)</td>
</tr>
</tbody>
</table>

Abbreviations: CIP- Ciprofloxacin; GEN- Gentamicin; SXT - Trimethoprim-sulfamethoxazole

A large proportion of the ESBL-producing strains also expressed resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole
**ESBL and pAmpC genes**

<table>
<thead>
<tr>
<th>Genes</th>
<th>ESBL</th>
<th>Sequence</th>
<th>AmpC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>blaCTX-M</em></td>
<td>58/75 (77 %)</td>
<td>CTX-M-15 (n= 24); CTX-M-28 (n=2);</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX-M- 117 (n=1); CTX-M -36 (n=1);</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CTX-M – 164 (n=1);</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CTX-M – no typing (n= 29)</td>
<td></td>
</tr>
<tr>
<td><em>blaSHV</em></td>
<td>39/75 (52 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>blaTEM</em></td>
<td>1/75 (0,1 %)</td>
<td></td>
<td></td>
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<tr>
<td><em>blaMOX</em></td>
<td></td>
<td>22/75 (29 %)</td>
<td></td>
</tr>
<tr>
<td><em>blaFOX</em></td>
<td></td>
<td>24/75 (35 %)</td>
<td></td>
</tr>
<tr>
<td><em>blaDHA</em></td>
<td></td>
<td>13/75 (17%)</td>
<td></td>
</tr>
<tr>
<td><em>blaCMY</em></td>
<td></td>
<td>1/75 (0,1%)</td>
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</tbody>
</table>

CTX-Ms were the most dominant ESBL; FOX and MOX were the most dominant pAmpCs
Genes encoding ESBL and AmpC

$\text{Bla}_{\text{CTX-M}}$ was the most dominant ESBL-type, with $\text{CTX-M-15}$ as the major subtype.

[Umaer and Sundsfjord 2011, Laxminarayn et al., 2013].

$\text{Bla}_{\text{FOX}}$ and $\text{bla}_{\text{MOX}}$ were the most prevalent pAmpC genes. These observations contrast the worldwide observations of $\text{bla}_{\text{CMY}}$ as the most prevalent in $E. \text{coli}$ populations.

[Cherif et al., 2016, Egorova et al., 2014].
ERIC – PCR Result

- No dominant clone of ESBL-/pAmpC positive *E. coli*.
- Several clusters with clonal relatedness indicating minor outbreaks between patients at specific departments.
Limitations

Limited resources precluded the final identification of some $bla_{CTX-M}$ and all $bla_{SHV}$ genotypes as well as more extensive comparative genetic analysis of strain relatedness.
Conclusion

• We have observed a high prevalence of MDR ESBL- and/or pAmpC-producing clinical *E. coli* isolates with *bla*$_{CTX-M}$ and *bla*$_{FOX/bla_MOX}$ as the major types, respectively.

• ERIC-PCR revealed genetic diversity and some clusters indicating within-hospital spread.
Recommendations

• Enhance bacterial culture and susceptibility testing to choose the correct antimicrobial treatment
• Improved infection control measures within the hospital at all level
• More studies should be done in order to minimize this problem
Acknowledgments

• Norwegian Agency for Development Cooperation for funding under the NORHED-program

• Microbiology Laboratory of MCH for providing isolates

• UKZN for providing space and reagents in the accomplishment of the molecular diagnosis

• Co-authors for good collaboration and support
  – MCH, UKZN, UiT and UNN