β-lactams resistance among Enterobacteriaceae in Morocco
Antibiotic resistance center
Institut Pasteur du Maroc

Enterobacteriaceae (E. coli, Salmonella, ...)

S. aureus
Enterococcus spp.

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Global Theme

Antibiotics Resistance

- Epidemiology of multidrug-resistant bacteria (ESBL, Carbapenemases, MRSA, VanR etc.)
- Identification of mechanisms of resistance to betalactamins and quinolones
- Molecular typing of multidrug-resistant strains

Monitoring activity

- Monitoring the evolution of serotypes of salmonella
- Contribution to monitoring resistance to β-lactams & quinolones
- Contribution to the dissemination of surveillance data.
Antibiotics Resistance

- Antimicrobial resistance could become one of the leading causes of death in the world.

- On a global scale, microbial resistances are currently responsible for 700 000 deaths per year. (+ 10 M in 2050)

Universal problem
Discussion at the higher level

United Nations    WHO    USA- E.U - Africa    Maroc
β-lactam antibiotics have been widely used in the prevention and treatment of a variety of human bacterial infections.

Although they can be classified into penicillin, cephalosporin, carbapenem and monobactam subclasses, all have a chemical structure called a β-lactam ring and carry out bactericidal activity through binding to penicillin-binding proteins and inhibiting synthesis of the bacterial peptidoglycan cell wall.

β-lactamases are enzymes capable of destroying and inactivating β-lactam antibiotics.

Production of β-lactamases is one of the prime mechanisms for bacterial resistance to β-lactam antibiotics.
2004 et 2007

- 535 *E. coli* Strains : urinary tract infection

- 7 strains ESBL + (1,3%)

- 6/7 are CTX-M

- 6/6 CTX-M15

Diffusion du clone CTX-M15 en milieu communautaire !
Characterization of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates from the community in Morocco.

Barquiquia A, El Otmani F, Talmi M, Bourilat F, Haouzane F, Zerouali K, Timinouni M

Molecular Bacteriology Laboratory, Pasteur Institute of Morocco, Casablanca, Morocco.

2004 et 2009

- 767 E coli
- 36 K pneumoniae:

urinary tract

Casablanca, El Jadida et Settat

- 25/803 R to 3CG (3.1%)
- 12/803 ESBL + (1.5%)
- 11/12 CTX-M15
In 2010–2011

453 strains of K. p ECBU

5 Moroccan cities
- 34 (7.5%) ESBL +
- 91% multi-resistant

CTX-M 15 more common, followed by SHV-28 and SHV-12

- 41% carry the qnr gene
2009 et 2011

167 strains of urinary tract infection (private laboratories)
In 7 Moroccan geographical regions (Casablanca, El Jadida, Meknes, Beni Mellal, Settat, Rabat and Fez)

- 17 strains: Ert and / or Imp R (10.1%)
- 9/17: carbapenemase
  IMP-1 (n = 3), Oxa-48 (n = 6),
4/17: qnr
- 10/17: CTX-M15
1 strain of *K. pneumoniae* (2012)

49-year-old patient, urinary pvt

Non-hospitalized treatment with 3CG + Fq for 1st episode of urinary tract infection,

subsequently changed to Amikacin

Amp, AMC, 3CG, Aminoglycosides, Fq, TC, Sulfonamides: R

Amikacin: S

Presence of 3 types of Carbapenemases:

Oxa-48, NDM-1, VIM
Evolution of the prevalence of ESBLE

- **2004-2009**: 1.24%
- **2010-2015**: 5.66% (5X increase)

- **2010-2015**: 8.54%
Evolution of ESBL prevalence by species

**2004–2009**

- *E. coli* (1.58%)
- *K. pneumoniae* (1.21%)

**2010–2015**

- *E. coli* (5.10%)
- *K. pneumoniae* (10.53%)
- *E. cloacae, P. mirabilis, C. freundii, M. morganii, S. marcescens, P. vulgaris*
Molecular typing of ESBLs produced: High diversity

- **CTX-Mgp1**: 77.3%
  - CTX-M-15 (86.3%); CTX-M-1 (7.1%); CTX-M-28 (6.4%)

- **SHV**: 14.9%
  - SHV-12 (n=11); SHV-28 (n=7); SHV-36; SHV-5; SHV-27; SHV-110; SHV-125; SHV-148

- **TEM**: 8.34%
  - TEM-104 (n=8); TEM-20; TEM-3; TEM-176; TEM-198; TEM-70

- **PER**: 1.79%
  - PER-2 (n=3)

- **CTX-Mgrp9**: 1.79%
  - CTX-M-14 (n=2); CTX-M-27 (n=1)
The gene transfer experiments:

Two phenotypes have been identified:

- **ESBL**: 54kb and 125kb plasmid \((n = 14)\)

- **Carbapenemase + ESBL**: plasmid of 60kb \((n = 1)\) and 70kb \((n = 1)\)
  - *NDM*-1, *OXA*-48+ *CTX*-M-15

Different resistance associations have been transferred:

- **Aminoglycosides** (8–128µg/mL)
- **Cotrimoxazole**
- **Quinolone** (8–32µg/mL)
- **Tetracycline** (2–128µg/mL)
Genetic structures adjacent to the $\text{bla}_{\text{CTX-M}}$ genes

- 94.32% are ISEcp1
- 4.25% are IS26

**ISEcp1**

Characterized by a very high mobility capacity
confer to CTX-M a large capacity of diffusion
Would play a role in the expression of these enzymes

Indeed, it is no longer a question of transferring all of the plasmid on which it is located but only the sequence itself which contains the gene
Genetic environment of the $\text{bla}_{\text{CTX-M}}$ gene

**Tc30**

**Tc316**

**Tc20**

**Tc319**
Typing of ESBL E. coli by pulsed-field electrophoresis: clonal diffusion?

Dendrogram obtained using the DendroUPGMA utility after analyzing the pulsotypes of ESBL E. coli strains (2004-2009).
### Characteristics of ESBL-producing E. coli clones identified during the period 2004-2009

<table>
<thead>
<tr>
<th>Grp phyl</th>
<th>Code</th>
<th>Date</th>
<th>cities</th>
<th>Sexe/age</th>
<th>Antibiorésistance Profil</th>
<th>β-lactamases</th>
<th>PMQR genes</th>
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<td>CE2</td>
<td>B2/B23</td>
<td>E.c2</td>
<td>09/2006</td>
<td>Casablanca</td>
<td>F/71</td>
<td>NA , CIP, GM, TM, SXT</td>
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<td>CTX-M-15 ; OXA-1</td>
</tr>
</tbody>
</table>

#### Evolution of resistance patterns: Draws attention

- Illustrates perfectly the "increase" of community resistance.
- These strains had indeed acquired resistance determinants to other ATBs over the years.
ESBL genes carried by high molecular weight conjugative plasmids also harboring other resistance genes.

On the other hand, the ISEcp1-mediated blaCTX-M-15 transposition would be responsible for the epidemiological success of CTX-M-15.

Clonal dissemination exists within our community.
Conclusion

The spread of these multidrug-resistant strains constitutes a public health threat, significantly reducing the therapeutic alternatives for the treatment of community IUs.

Based on the information provided by these results

urgent intervention by all sectors of government and society as a whole to slow down the development of resistance and control the resulting health costs
Aknowledgments

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