Prevalence of Enterobacteriaceae producing carbapenemase (OXA-48) responsible for urinary tract infections in Casablanca.

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SUMMARY:

In recent years, we have witnessed the appearance of strains increasingly resistant to antibiotics. The large-scale spread of this resistance in the community today suggests that it will become a major public health problem shortly. Among the resistant strains are Enterobacteriaceae, which are responsible for numerous infections, particularly urinary tract infections. The present study focused on the phenotypic and genotypic characterization of uropathogenic enterobacteria isolated from the community environment. Our study included a total of 78 strains collected from UTIs during the year 2019. The confirmation of the species was performed by the Biomérieux VITEK system. The study of their antibiotic resistance profile was carried out by the method of diffusion on agar Mueller Hinton according to the recommendations of SFM-EUCAST 2019. The search for resistance genes $bla_{(NDM, KPC, OXA-48)}$ was performed by PCR. The results obtained showed that out of 78 samples of urinary enterobacteria, $E.coli$ species dominated by a percentage of 58%, followed by $K.pneumonia$ at 33%, and $E.cloacae$ 8%. The study of antibiotic resistance profile showed high resistance to penicillins 91% followed by cephalosporins 58%. Carbapenems have a low activity on the studied strains with resistance percentages of 41% and 33% for imipenem and ertapenem respectively. The carbapenems resistance study allowed the detection of the $bla_{OXA-48}$ gene in three strains among the studied strains.
**Introduction**

The emergence of enterobacteria responsible of urinary tract infections (UTIs), resistance to beta-lactams is increasingly reported worldwide and is a significant problem in health systems (K Jalalvand and al., 2020). Indeed, they have been able to develop different mechanisms of resistance to these treatments, including the synthesis of enzymes (β-lactamases) that efficiently and irreversibly catalyze the hydrolysis of the amide bond of the β-lactam ring giving a biologically inactive product (L Armand-Lefèvre. and al., 2017).

Nowadays, according to the Committee on Antibiogram Committee of the French Microbiology Society strains that are resistant to carbapenems are mostly not carbapenemase producers. When resistance is linked to the production of a carbapenemase, it is most frequently carbapenemases of the OXA-48, NDM, and KPC types. These carbapenemases are produced by various species of enterobacteria including *Klebsiella pneumonia, Escherichia coli*, and others (CASFM/EUCAST 2019).

Carbapenem-resistant Enterobacteriaceae (CRE) are multidrug-resistant gram-negative bacteria they are grouped into two categories (JD Lutgring, 2019).

Carbapenemases non-producing CRE (non- CP-CREs) generally isolates that express a beta-lactamase with low activity against carbapenems including AmpC or ESBL enzymes, in combination with a permeability defect (porin mutations) or overexpression of efflux pumps (K Van Loon And al., 2018 ; Y Teethaisong And al., 2018). Carbapenemase-producing CREs (CP-CREs), on the other hand, are enterobacteria that have acquired genes encoding carbapenemase enzymes that hydrolyze carbapenems, usually through plasmids (CA Moxon and S Paulus, 2016 ; MC Mills et J Lee, 2019).

Carbapenem resistance caused by carbapenemase production has been increasingly reported worldwide in enterobacteria (L Dortet and al., 2017). It is important to note that non- CP-CREs are less of a public health concern, as they have not been shown to have the same potential as CP-CREs for rapid spread (S Miller and RM Humphries, 2016). The widespread production of carbapenemases in Enterobacteriaceae was unknown until the early 2000s (E Temkin and al., 2014). The widespread use of carbapenems in the treatment of severe infections caused by extended-spectrum β-lactamase (ESBL)-producing pathogens are considered to be the main driver of the emergence of carbapenemases (C Duployez and al., 2016), these are frequently found on mobile genetic elements and they have the potential to be widespread worldwide (S Miller and RM Humphries, 2016). Multidrug-resistant gram-negative bacterial species, Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumanii are all carbapenemase-producing organisms (CPOs) (J Harting, 2019).

Carbapenemases belong to the Ambler classes A, B, and D. The most important type of class A carbapenemases are the KPC enzymes, while VIM, IMP, and (in particular) NDM in class B and OXA-48 (and related) in class D are the most relevant enzymes. While some enzymes are encoded by chromosomal genes, most carbapenemases are plasmid-mediated (with genes frequently localized in integrons), which promotes enzyme dissemination.

The vectors for the dissemination of carbapenemases (mainly KPC, VIM, IMP, NDM-1, NDM-4, and NDM-5) are plasmids, which can easily be transferred between bacteria. These plasmids can also carry other resistance genes that can spread to other bacteria. This poses a significant challenge for healthcare systems worldwide, as it makes it difficult to control the spread of multidrug-resistant bacteria.
or OXA) can be either the strains or the genes themselves. Most of the genes coding for these enzymes have been isolated on mobile genetic structures (transposons, plasmids) and/or integrons, giving them an important dissemination power (N.Grall and al, 2011) (L Martínez and J González, 2014).

The objective of the present study was to investigate the prevalence of CPE community strains responsible for urinary tract infection isolated from bacteriology laboratory in Institut Pasteur of Morocco.

Material and methods

Bacterial strains: This is a retrospective study for statistical purposes (Excel), a collection of strains multi-resistant enterobacteria (n=78). The strains are collected in a conservation agar, they were identified in the bacteriology laboratory of the Pasteur Institute of Morocco using an automated system (VITEK bioMérieux). All strains are accompanied with their clinical information (Date, Sex, Age, Origin of the strain, Name of the species, Antibiogram).

Antimicrobial susceptibility testing: The antibiogram was performed by diffusion method on MH agar (Mueller Hinton) to confirm the resistance profile of the isolated strains, according to the recommendations of the French Society of Microbiology CASFM/EUCAST 2019 (European Committee on Antimicrobial Susceptibility Testing). Strains with reduced susceptibility to Imipenem (inhibition zone < 23 mm) or Ertapenem (zone < 22 mm) on MH agar were tested for their ability to synthesize carbapenemases.

Extraction of DNA template for PCR: Preparation of DNA template for PCR. DNA templates for PCR were generated by suspending five colonies of an overnight growth of Enterobacteriaceae isolates on Luria–Bertani agar (Bio-Rad) in 500 ml DNase- and RNase-free water (Invitrogen). The suspension was boiled at 100uC for 10 min in a thermal block (Polystat 5; Bioblock Scientific), then centrifuged at 19 000 g for 5 min. An aliquot of 1 ml of the supernatant was used as DNA template for PCR.

Detection of Carbapenemase genes: Carbapenemase genes detection has been studied by the Polymerase Chain Reaction (PCR). Each strain with reduced susceptibility to carbapenems was tested using specific primers coding for the genes bla(KPC, OXA48, NDM) as previously described by Barguiga A and al (2015). The amplification is performed according to the specific program. The amplified genes (Table 1) are revealed using a 1.5% agarose gel electrophoresis.

Table 1. PCR primers used for the detection resistance (A Barguigua and al., 2015)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Primers Sequence (5’-3’)</th>
<th>Gene size</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA48</td>
<td>OXA48(+)</td>
<td>TGGGTGGCATCGATTATCGG</td>
<td>744 pb</td>
</tr>
<tr>
<td>OXA48</td>
<td>OXA48(-)</td>
<td>GAGCACTTCTTTTGTGATGGC</td>
<td></td>
</tr>
<tr>
<td>NDM</td>
<td>NDM(+)</td>
<td>AATGGAATTGCAAATATTATGC</td>
<td>489 pb</td>
</tr>
<tr>
<td>NDM</td>
<td>NDM(-)</td>
<td>CGAAAGTCAGGCTGTGTTGC</td>
<td></td>
</tr>
<tr>
<td>KPC</td>
<td>KPC(+)</td>
<td>ATGTCACTGTATCGCGTTAC</td>
<td>881 pb</td>
</tr>
<tr>
<td>KPC</td>
<td>KPC(-)</td>
<td>TTACTGCCGTTGACGCCA</td>
<td></td>
</tr>
</tbody>
</table>

Results

In the studied strains collection, the prevalence of urinary tract infections is significantly higher in women than in men with percentages of 73% and
27% respectively. This predominance is explained by the anatomy of the female urinary tract. Repair by age has shown that the patients with the most UTIs are the elderly with a percentage of 83%, and only 17% for children under 15 years old, so this type of infection affects more adults than children.

The strains selected for this study were resistant to ertapenem as an indicator in carbapenem-producing Enterobacteria (CP-CRE). Among the 78 bacterial strains studied three bacterial species were identified, they are distributed as follows: E.coli strains represents 58%, K. pneumonia 33%, 2 E.cloacae 8%.

The resistance profile to antibiotics differs depending on the bacterial species. For E.coli the resistance profile is high for penicillins such as Amoxicillin + Clavulanic Acid (AMC) 89%, Ampicillin (AMP), and Ticarcillin (TIC) 79%, in addition to a decreased resistance for carbapenems Ertapenem (ETP) and Imipenem (IPM) 3%. While for K. pneumonia there is total resistance 100% for AMP, TIC, and cephalosporins such as Cefotaxim (COX), and Ceftazidim (CZD), nevertheless no resistance to Imipenem (IPM) and Ertapenem (ETP).

The resistance profile of the CREs showed that a total of 19 strains are resistant to carbapenems, and beta-lactams. The frequency analysis of strains resistant to beta-lactams showed a total resistance to meropenem (MEM) (100%), but a lower resistance to Imipenem (IPM) (41%), and Ertapenem (ETP) (33%).

The genes encoding resistance to carbapenemes was carried out for 12 strains with a phenotypic profile of resistance to ertapenem. The results obtained showed that out of a total of 78 strains studied, three strains presented the blaOXA-48 gene (strains producing carbapenemase of type OXA), they belong respectively to the species Klebsiella pneumoniae (two strains) and Escherichia coli (one strain). However, the blaNDM and blaKPC genes were not detected in these strains.

**Discussion**

In recent years, we have seen the emergence of strains increasingly resistant to ATB, raising fears of an epidemic and endemic situations and therapeutic dead end. This essentially hospital-based phenomenon, now spreading on a large scale in the community, this resistance portends a major public health problem shortly. Among the resistant strains are the Enterobacteriaceae, responsible for many potentially severe infections and for prescriptions of broad-spectrum bacterial ATB, which threaten the future activity of last-line molecules (M Sbiti and al., 2017) (S Smaoui e and al., 2015). UI is one of the most common infections among Enterobacteriaceae, very common in the community and hospital settings, and imposes a considerable economic burden on society. Among the enterobacteria most involved in this type of infection are E.coli and K. pneumonia (Tagajdid and al., 2010). Our study consists of studying the resistance profile to Carbapenems of Enterobacteriaceae isolated from UI of community origin.

The distribution of the Enterobacteriaceae strains studied showed that 91% belong to E. coli and K. pneumoniae species, this result is consistent with that found in a study by K Jalalvand and al., 2020, where they detected E.coli and K. pneumonia species with a percentage of 96.5% from various clinical samples, including uropathogenic strains. Furthermore, E.coli was the predominant species (58%) in the population studied, M Moutachakkir
and al., 2015 report that *E. coli* is responsible for 60 to 80% of UIs in the community. The ascending pathophysiology of UI, as well as the strong colonization of the perineum by Enterobacteriaceae of digestive origin, associated with specific uropathogenicity factors such as bacterial adhesins capable of binding to the urinary epithelium, explain this predominance.

The influence of sex on urinary tract infections has shown that the female sex is more sensitive than the male; the infections percentages recorded are 73% and 27% respectively. Our results are close to those of KJ Mbuya and al., 2020, who reported that the female sex is most affected by urinary tract infections. This female predominance is due to the anatomical nature, along with pregnancy, sexual intercourse promotes the development of urethral bacteria in the bladder. On the other hand, the effect of prostatic secretions in men provides additional protection (KJ Mbuya and al., 2020). Still, according to Bergogne 2008, women count more thanks to the number of ECBUs required (pregnant, menopausal, etc.). In contrast, our study found that UI risks increase with age, which is consistent with the data. According to the mentioned literature, the most affected site in the elderly is the urinary tract site and, therefore, urinary tract infections account for about 35% of infections in the elderly.

Our study shows that 83% of patients are adults, these results are close to those of Barrier 2014 and W Aries et al 2004. The best explanation we can provide is that the elderly are the most affected by UTIs. Cause of several factors: Lack of immunity with age, urinary incontinence, a marker of abnormal genitourinary emptying, which promotes bacteriuria, the decrease in functional autonomy is strongly associated with the occurrence of UI.

Urinary stasis due to incomplete bladder emptying with bladder residue after voiding, in particular related to a urethral or prostatic obstruction or a bladder diverticulum. Co-morbidities: diabetes, neurological and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

The study of the sensitivity of Enterobacteriaceae to beta-lactams, in particular carbapenems, revealed variable rates of resistance to the ATBs tested, the most affected family being that of penicillins (91%) followed by cephalosporins (58%). However, the percentage of resistance to Meropenem is 100%. In the presence of Imipenem, this resistance drops to a rate of 41% of the strains studied, with a resistance rate of 33% to ETP. the study by K Jalalvand and al., 2020, Resistance rates to imipenem and meropenem among isolates were 90% (97/108) and 96.4% (104/108), respectively. On the other hand, SH Hashemi et al., 2013 found higher rates of resistance of the order of 19% for IMP, which shows that resistance to carbapenems increases more rapidly and that the uncontrolled use of this antibiotic puts in guard against increased resistance shortly. The phenotypic analysis of the antibiogram of the enterobacteria studied resistant to ETP, can probably be linked to the existence of the producing strains of carbapenemas, The molecular research of the carbapenemas genes carried out in this work, remains necessary to confirm this phenotypic suspicion. We continued our study on a genotypic level by searching for genes for resistance to carbapenems. The PCR research carried out in this work followed by electrophoresis showed the presence of the *bla*OXA-48 gene in three strains among the 78 strains studied, two strains of *K. pneumonia* and one strain of *E. coli*. However, the *bla*NDM and *bla*KPC genes were not detected in any of these strains. These results
are in agreement with those reported by others studies such as the study in Rabat conducted by Benouda and al., 2010, Baroud, M., and al., 2013. Therefore carbapenem resistance encoding genes such as blaOXA-48 are combined with permeability defects (El-Herte RJ and al., 2012). Barguigua and al., 2011 also showed signs of emergence in the Moroccan community environment of Enterobacteriaceae producing carbapenemas expressing the blaOXA-48 genes. Any emergence of resistance to carbapenems is a serious concern; its necessary to take into account its possible dispersion in the community. This correlates with the study by K Jalalvand et al. 2020, which showed that the blaOXA-48 gene is one of the most common carbapenem-resistant genes in Iran. From a general point of view and the results obtained in this study, we can therefore think that the resistance profile obtained by the antibiogram method may be due to other resistance mechanisms that we have had not targeted in our research. Indeed, the emergence of resistant bacteria in community settings has increasingly become a public health impact. The high rates of resistance towards the different families of ATB tested are worrying and alarming, which makes monitoring of these multi-resistant bacteria essential, to detect new multi-resistant bacteria as well as their emergence in community environments, this multiresistance will remain a major problem for the coming decades. The percentages of resistance to the antibiotics tested are higher for the majority of the strains studied with regard to β-lactams, with resistance less observed compared to carbapenems, which makes it necessary to monitor their uses to keep their powerful activities as molecules of last resort. The genotypic study concerning the search for genes for resistance to antibiotics showed that the strains of enterobacteria studied have genes for resistance to carbapenems. This resistance is the consequence of selection pressure, due to the widespread use of these sometimes abusive and uncontrolled antibiotics, causing the spread of these multi-resistant bacteria involved in many pathologies, including UI. The reasoned use of antibiotics, compliance with good pharmacy practice for the delivery of drugs, are key actions to slow the emergence and dissemination of these multidrug-resistant bacteria. Several perspectives can be considered to follow this work: Extend the sampling in time and space, search for and detect other carbapenemas: VIM, SIM, GES, IMP, have an interesting collection to study the transferability of carbapenemas genes by conjugation, research the coexistence of other resistance mechanisms.

Conflicts of Interest
There are no competing interests.

Conclusion
As part of the study of resistance profiles of uropathogenic Enterobacteriaceae in community settings, the results obtained in this direction confirm the the increased infectious risk of these multiresistant bacteria as well as their emergence in community environments, this multiresistance will remain a major problem for the coming decades. The percentages of resistance to the antibiotics tested are higher for the majority of the strains studied with regard to β-lactams, with resistance less observed compared to carbapenems, which makes it necessary to monitor their uses to keep their powerful activities as molecules of last resort. The genotypic study concerning the search for genes for resistance to antibiotics showed that the strains of enterobacteria studied have genes for resistance to carbapenems. This resistance is the consequence of selection pressure, due to the widespread use of these sometimes abusive and uncontrolled antibiotics, causing the spread of these multi-resistant bacteria involved in many pathologies, including UI. The reasoned use of antibiotics, compliance with good pharmacy practice for the delivery of drugs, are key actions to slow the emergence and dissemination of these multidrug-resistant bacteria. Several perspectives can be considered to follow this work: Extend the sampling in time and space, search for and detect other carbapenemas: VIM, SIM, GES, IMP, have an interesting collection to study the transferability of carbapenemas genes by conjugation, research the coexistence of other resistance mechanisms.

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Reference


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