INTRODUCTION

Carbapenemases have led to the ultimate evolution of resistance in *Enterobacteriaceae*, leaving virtually very few efficient antibiotics left for treating related infections. The most clinically significant carbapenemases in *Enterobacteriaceae* are Ambler class A enzymes, including KPC, IMI, and SME enzymes, metallo-β-lactamases (MBL) from the VIM, IMP, and NDM types and OXA-48-like enzymes ***(Dortet et al., 2014)***.

The level of resistance to carbapenems conferred by those carbapenemase may vary significantly, making their detection difficult when based on in vitro susceptibility values ***(Girlich et al., 2013).***

The OXA-48 carbapenemase was first discovered in various *Enterobacteriaceae* species isolated in Turkey and other countries in the Middle East. Within years, several sporadic cases of OXA-48 producing *Enterobacteriaceae* infections have been reported across Europe. Most of these cases were related to patients with previous exposure to health care facilities in the Middle East and Northern Africa ***(Adler et al., 2013)***.

The enzyme hydrolyses penicillins but has a weak activity against carbapenems or extended-spectrum cephalosporins (third generation cephalosporins,

aztreonam). However, its frequent association with ESBL (notably CTX-M-15 enzyme) increases the level of resistance to carbapenem. Its activity is not inhibited by EDTA or clavulanic acid, tazobactam, and sulbactam, whereas its activity may be inhibited by NaCl in vitro. Its high level of resistance to temocillin is interesting to detect this enzyme ***(Djahmi et al., 2014)***.

The partially unnoticed spread of OXA-48-type carbapenemase producers is usually assigned to low MICs of carbapenems that OXA-48-producing isolates often display. The minimal inhibitory concentrations (MICs) of carbapenems against OXA-48-type producers range between 0.5 and ≥64 mg/L for ertapenem, 1 and ≥64 mg/L for imipenem, and 1 and ≥64 mg/L for meropenem ***(Studentova et al., 2015).***

The OXA-48-type producers with low MICs, categorized as susceptible to carbapenems by the European Committee on Antimicrobial Susceptibilities Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines, are very difficult to detect and may spread unnoticed, evolving then to resistance phenotypes ***(Studentova et al., 2015)***.

Therefore, selective isolation and sensitive detection of OXA- 48-type producers remains a big challenge for current microbiological diagnostics ***(Djahmi et al., 2014)***.

ChromID OXA-48, a new chromogenic medium developed for the specific detection of *Enterobacteriaceae* producing OXA-48 carbapenemase (OXACPE) by a coloration of the colonies. The evaluation of chromID OXA 48 shows that this ready-to-use chromogenic medium is both sensitive and highly specific for OXACPE detection and should therefore facilitate infection control and outbreaks prevention ***(Devinge et al., 2013)***.

Aim of the Work

This study aims to evaluate chromID OXA-48 (bioMérieux), a new chromogenic medium specifically designed for the screening of OXA-48 producing *Enterobacteriaceae* compared to blaOXA-48 gene amplification by polymerase chain reaction (PCR).

Introduction:

Antibacterial resistance is a serious public health problem, and the prevalence of multidrug- and pandrug-resistant organisms in tertiary medical institutions has compounded concerns for patients. In recent years, the proportion of antibacterial resistance has been increasing annually in community-acquired infections such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella*, vancomycin-resistant *Enterococci*, and penicillin-resistant *Streptococcus pneumoniae* ***(Xiao et al., 2015).***

Bacteria belonging to *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Citrobacter* spp, *Serratia* spp, *Proteus* spp, and *Morganella*, are all important human pathogens. They cause a wide array of diseases including urinary tract, respiratory tract, bloodstream, intra-abdominal, and skin and soft tissue infections. Treatment of infections caused by these bacteria has become challenging particularly those with increasing resistance to extended spectrum β-lactams due to expression of extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase ***(Wang et al., 2015).***

Beta-lactamases are produced by various Gram-positive and Gram-negative microbial species. Some of

them are exocellular, i.e. are secreted out of the microbial cell (staphylococcal penicillinases) whereas others, especially in Gram-negative bacteria, are located in the periplasmic space and are available only after cell wall breakdown. In a number of bacterial species they are inducible, while in others they are constitutively synthesized. Enzyme inactivation is of special interest due to its clinical significance, ecological aspects and evolutionary development ***(Urumova., 2015).***

Resistance to β-lactam antibiotics due to ESBLs has become a common problem worldwide. The prevalence of this resistance mechanism has increased rapidly, even in countries known for prudent antibiotic use ***(Reuland et al., 2016).***

In addition to ESBLs, resistance to extended-spectrum β-lactams in *Enterobacteriaceae* is increasingly mediated by other plasmid and chromosomally encoded enzymes, such as carbapenemases and AmpC cephalosporinases ***(Logan et al., 2014).***

Carbapenems are last-line antimicrobial substances with a broad spectrum and high efficacy. Originally, they were developed from thienamycin, a substance that is produced by *Streptomyces cattleya*. As all beta-lactam antibiotics, carbapenems inhibit the D-alanyl-D-alanine

carboxypeptidase and therefore they interfere with the cell wall synthesis. They are stable against penicillin and cephalosporin hydrolyzing enzymes that are commonly encountered in human pathogens ***(Braun et al., 2014).***

Carbapenems includes imipenem, meropenem, ertapenem ***(Bialvaei et al., 2015).*** Carbapenems are often considered as a last therapeutic choice for the treatment of infections due to multidrug-resistant Gram-negative rods. Emergence of carbapenem-resistant *Enterobacteriaceae* is increasingly reported worldwide and is becoming an important issue in health care systems ***(Azimi et al., 2014).***

Resistance to carbapenems is mediated mostly by two main mechanisms: (i) production of a β-lactamase (depressed cephalosporinase or ESBL) with nonsignificant carbapenemase activity combined with decreased permeability due to porin loss or alteration; (ii) production of a carbapenem-hydrolyzing β-lactamase ***(Djahmi et al., 2014).***

Carbapenemases:

Carbapenem resistant *Enterobacteriaceae*(CRE) can cause a number of serious infection types (such as intra-abdominal infections, pneumonia, urinary tract infections, and device-associated infections) or asymptomatic colonization. Each year approximately 600 deaths result from CRE infections. Infections caused by CRE are

extremely concerning, as CRE mortality rates are high and range from 18% to 60% depending on therapy. This may be due to delayed time to active therapy, pharmacologic limitations of available treatment options, and that patients with CRE infections tend to be critically ill ***(Morrill et al., 2015).***

Inevitably, the first reports of carbapenemases specific carbapenem hydrolyzing β-lactamases-were reported in the early 1990s (in *P. aeruginosa* and *Serratia marcescens* strains), and those in *K. pneumoniae* were first reported in 1999 ***(Rapp and Urban, 2012).***

K. pneumoniae is known to be the most common species among CRE, thereafter called carbapenem-resistant *K. pneumoniae* (CRKP). CRKP has been reported in many countries and their molecular epidemiology based on multilocus sequence typing (MLST) demonstrates that various sequence types (STs) are widespread. For example, *K. pneumoniae ST258* is most commonly associated with carbapenem resistance in the United States and Greece ***(Netikul and Kiratisin, 2015).***

Carbapenemase enzymes are encoded by bla genes carried on mobile elements (e.g. plasmids and/or integrons) that facilitate their horizontal spread among different Gram-negative species. Beta-lactamase enzymes with hydrolytic

activity against carbapenems have been identified in each of the four Ambler molecular classes, though those of class A, B, and D have major epidemiological impact ***(Levy et al., 2013).***

Classification of Carbapenemases:

Ambler molecular class A (KPC), class B (VIM, IMP, NDM), and class D (OXA-48) types. These are mostly found in *K. pneumoniae* isolates and are also frequently associated with serious nosocomial infections and outbreaks. Prior to the first report of NDM-1 (New Delhi metallo-beta-lactamase) in 2009, the VIM (Verona integron-encoded MBLs) and IMP (active on imipenem) types were the common metallo-beta-lactamases identified in *Enterobacteriaceae* ***(Shibl et al., 2013).***

Class A Carbapenemase:

Class A carbapenemases include the IMI/NMC, SME, KPC and GES enzymes that confer resistance to carbapenems at various levels, from reduced susceptibility to full resistance. SME, NMC and IMI enzymes are usually chromosomally encoded, whereas KPC and GES enzymes are plasmid-encoded. SME enzymes are usually restricted to *Serratia marcescens*, whereas IMI and NMC enzymes are sporadically detected in *Enterobacter cloacae*. The fact that these latter three types of enzyme are chromosomally

encoded probably explains why they are rarely reported worldwide. Conversely, the genes for KPC enzymes are found on transferable plasmids, and are highly prevalent, mainly in *K. pneumoniae*, but have also been detected in other *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* ***(Diene and Rolain., 2014).***

All effectively hydrolyze carbapenems and are partially inhibited by clavulanic acid. *KPCs* (acronym for *K. pneumoniae* carbapenemase) are the most frequently encountered enzymes in this group. Since the first report of this enzyme in 1996 isolated from a clinical *Klebsiella pneumoniae* strain in North Carolina, USA, the KPC producers had spread around the world and are becoming a major clinical and public health concern ***(Djahmi et al., 2014)***.

Klebsiella pneumoniae carbapenemase-production can confer variable levels of carbapenem resistance with reported minimum inhibitory concentrations (MICs) ranging from susceptible to ≥16mg/ml. Analysis of isolates displaying high-level carbapenem resistance demonstrated that increased phenotypic resistance may be due to increased blaKPC gene copy number or the loss of an outer membrane porin, Omp*K*35 and/ or OmpK36. The highest level of imipenem resistance was seen with isolates lacking both porins and with augmented KPC enzyme production ***(Patel and Bonomo, 2013).***

Class B Carbapenemase:

Metallo-β-lactamases (MBLs) belong to molecular class B β-lactamases and are dependent on zinc for the hydrolysis of β-lactam antibiotics. With the exception of poor hydrolysis of monobactams (aztreonam), like KPCs, they are mostly able to breakdown all β-lactams. Due to the co-existence of other mechanisms of resistance, such as ESBLs, aztreonam is very rarely a treatment option. Like KPCs, MBLs may also cause low-grade reduced susceptibility to carbapenems in some cases ***(Tängdén and Giske , 2015).***

Class B metallo-β-lactamases (MBLs) are mostly of the Verona integron–encoded metallo-β-lactamase (VIM) and IMP types and, more recently, of the New Delhi metallo- β-lactamase-1 (NDM-1) type. The first acquired MBL, IMP-1, was reported in *Serratia marcescens* in Japan in 1991. Since then, MBLs have been described worldwide. Endemicity of VIM- and IMP-type enzymes has been reported in Greece, Taiwan, and Japan, although outbreaks and single reports of VIM and IMP producers have been reported in many other countries ***(Nordman et al., 2011).***

The first two reported cases of infections due to VIM-harboring GNB were in France (VIM-2) in 1996 and in Italy in 1997. In both countries, the VIM-harboring

pathogen was *P. aeruginosa*. Since then, genes encoding for various VIM enzyme variants have been found in *Pseudomonas* spp., many *Enterobacteriaceae* spp. (mainly *K. pneumoniae, E. coli, E. cloacae* and *Proteus mirabilis*), and *A. baumannii* in a few European countries (principally VIM-1, VIM-2 and VIM-5), Turkey (VIM-5), Tunisia, the USA, Korea (VIM-2), Japan (VIM-2), Taiwan (VIM-3) and some countries in South America (mainly VIM-2) ***(Jean et al., 2015).***

NDM stands it is a class B β-lactamase and is capable of hydrolyzing penicillins, cephalosporins, and carbapenems, but unlike KPC, it does not hydrolyze aztreonam. NDM-producing *K. pneumoniae* and *E. coli* were first identified in an Indian patient residing in Sweden who had hospitalization for wound infections in New Delhi before returning to Sweden in early 2008. It was soon reported that NDM-producing *Enterobacteriaceae* were present in Indian hospitals as early as 2006 ***(Doi and Paterson, 2015).***

Class C Carbapenemase:

These enzymes were initially determined by chromosomal genes in a number of intestinal and other Gram-negative bacteria. The expression of chromosomal AmpC genes is constitutively weak but could be induced in

the presence of beta-lactams. These enzymes hydrolyze cephamycins and cephalosporins from I, II and III generations. They are not inhibited by beta-lactamase inhibitors. All are resistant to all beta-lactams except carbapenems and fourth generation cephalosporins ***(Urumova, 2015).***

Class D Carbapenemase:

Class D β-lactamases, also named OXAs for oxacillinases include 232 enzymes with few variants, possessing the same carbapenemase activity. Initially OXA β -lactamases were reported from *P. aeruginosa* but until now, these carbapenemases have been detected in many other Gram-negative bacteria, including *Enterobacteriaceae* ***(Djahmi et al., 2014)***.

They are broadly classified into narrow- and extended-spectrum enzymes based upon the conferred resistance profile against β-lactam antibiotics. The OXA-2 and OXA-10 β-lactamases exemplify the narrow-spectrum enzymes capable of producing resistance to penicillins and some early cephalosporins. Both enzymes, however, can extend their substrate profile to produce resistance to expanded-spectrum cephalosporins, such as ceftazidime, by accumulating one to several amino acid substitutions ***(Antunes et al., 2014).***

The enzymes (e.g., OXA-13 and -17) seem to arise when a small number of substitutions occur in the background of a narrow-spectrum parental enzyme (most often OXA-10). Carbapenem-hydrolyzing class D β-lactamases (CHDL) such as OXA-23, OXA-24/40, and OXA-48 provide resistance to carbapenems such as imipenem, meropenem, and doripenem through a weak hydrolytic activity toward those antibiotics ***(Kaitany et al., 2013).***

Genes encoding oxacillinases are usually embedded as gene cassettes into class 1 integrons, but other potentially mobile genetic vehicles have also been described, including insertion sequences (ISs). Among class D β-lactamases, some enzymes confer the ability to hydrolyze carbapenems and have mostly been identified in *Acinetobacter* spp. but rarely in *Enterobacteriaceae* ***(Poirel et al., 2011).***

Class D β -lactamases are not inhibited by clavulanic acid, tazobactam and sulbactam (apart from very few exceptions), whereas their activity may be in vitro inhibited by NaCl. Some of those class D β -lactamases hydrolyse carbapenems and are therefore defined as carbapenem-hydrolysing class D β-lactamases (CHDLs) ***(Poirel et al., 2012).***

CHDLs are most problematic clinically, as they produce resistance to the antibiotics of last resort, carbapenems, thus severely limiting therapeutic options. Based on their amino acid sequence identity, CHDLs have been subdivided into several subgroups. Enzymes belonging to the OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143 subgroups are of major clinical importance due to their wide dissemination in bacterial pathogens ***(Antunes et al., 2014).***

Of the Class D carbapenemase, OXA-48 is of major concern due to its: difficulty in detection, association with treatment failure and high dissemination rate due to transferable plasmid ***(Bakthavatchalam et al., 2016).***

OXA-48 Group:

The class D OXA-48-type carbapenemases have become increasingly prevalent among the carbapenem-non susceptible *Enterobacteriaceae* in regions of North Africa, the Middle East, and Turkey and subsequently have disseminated and caused outbreaks in several European countries as well as sporadically in South and North America, Israel, and India. Notably, OXA-48-type carbapenemases are spread in *K. pneumoniae* but also in *Escherichia coli* and other *Enterobacteriaceae* species ***(Tsakris et al., 2015).***