Detection of Carbapenemases genes responsible for carbapenem resistant in commonest bacteria causing Gestational Pyelonephritis

Thesis

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Contents

List of Abbreviations

AMC	:	Amoxicillin-Calvulanic acid.
AMR	:	Antimicrobial resistance
ARDB	:	Antibiotic Resistance Database
ARGs	:	Antibiotic resistance genes
AST	:	Antimicrobial susceptibility testing
C	:	Chloramphenicol
CARD	:	Comprehensive Antibiotic Resistance Database
CAZ	:	Ceftazidime
CHDLs	:	Carbapenem-hydrolysing class D-lactamases
CDC	:	Center of Disease Control and prevention
CIP	:	Ciprofloxacin
CLSI	:	Clinical and Laboratory Standards Institute
CP-CRE	:	Carbapenem-resistant Enterobacteriaceae
CPD	:	Cefpodoxime
CPE	:	Carbapenemase-producing Enterobacteriaceae
CPM	:	Cefepime
CPO	:	Carbapenemase-producing organisms
CPR	:	Called candidate phyla radiation
CRE	:	Carbapenem-resistant Enterobacteriaceae
CRO	:	Carbapenem-resistant organisms

List of Abbreviations (Cont.)

CTX	:	Cefotaxime
DDT	:	Disk Diffusion Test
ESBL	:	Extended spectrum β-lactamase
ESBL-E	:	Extended-spectrum β-lactamase-producing Enterobacteriaceae
ESBLs	:	Extended spectrum β-lactamases
FDA	:	Food and Drug Administration
GEN	:	Gentamycin
HMM	:	High molecular mass
LMM	:	Low molecular mass
MDR	:	multi drugs resistance
NA	:	Naldixic acid
NGS	:	Next-generation sequencing
ORFs	:	Open reading frames
PCR	:	Polymerase chain reaction
PDR	:	Pandrug-resistant
SXT	:	Cotrimoxazole
TE	:	Tetracycline
TSI	:	Triple sugar iron agar medium
UTI	:	Urinary tract infection
UTIs	:	Urinary tract infections
XDR	:	Extensively-drug-resistant

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Introduction and aim of the work

Multi drug resistance is now emerging worldwide at an alarming rate among gram negative bacteria, causing both community and hospital -acquired infections ***(Schwaber et al., 2006).*** One of the most important emerging resistance traits in *Enterobacteriaceae* family members corresponds to resistance to carpabenem antibiotics, which is mainly associated with production of carpabenemases enzymes ***(Pitout and Laupland, 2008 and Coque et al., 2011).***

Most enzymes mediates resistance are typically plasmid-mediated enzymes that hydrolyze the antibacterial agents ***(Pfaller and Segreti, 2006).***

In the context of world wide spread of multidrug resistance, enzymes producers that are mostly *E.coli* and *Klebsiella pneumoniae* species are not only found as source of hospital but also of community acquired infections ***(Coque et al., 2008; Pitout and Laupland 2008 and Poirel et al., 2012).***

A variety of enzymes, had been mainly reported in members of *Enterobacteriaceae* family ***(Poirel et al., 2012).***

Current techniques for detecting enzymes producers are based on the determination of susceptibility to antimicrobial agent.

The disk diffusion test and the E-test were proposed for that purpose with 80% to 90% sensitivities and specificities ***(Drieux et al., 2008 and Gazin et al., 2012).*** Based on the same principle, automated methods for bacterial identification and susceptibility testing are also used for the

detection of resistant bacteria with a sensitivity range 80-90% and 50-80% specificity ***(Drieux et al., 2008).***

The previous methods require overnight growth, meaning that up to 24 to 48 hrs can elapse before enzymes production is detected once the isolate had grown ***(Drieux et al., 2008 and Gazin et al., 2012).*** This may conduce to a delay in the initiation of appropriate antibiotic therapy ***(Schwaber et al., 2006).***

Molecular detection of genes by PCR, hybridization, and sequencing is an alternative but remains costly and requires a certain degree of expertise that is not accessible to non-specialized laboratories, as PCR-based techniques require isolation of the organism from clinical specimens, the results cannot be obtained until 48hrs after obtaining the pathological samples ***(Gazin et al., 2012).***

Carbapenemases-producing Enterobacteriaceae are increasingly reported worldwide ***(****www.CDC.gov****)*** Urinary tract infection (UTI) is a bacterial infection commonly occurring during pregnancy. The incidence of UTI in pregnant women depends on parity and socioeconomic status and can be as high as 8%. ***(Nordman P et al., 2014)***. About 20%-40% of pregnant women with untreated bacteriuria develop gestational pyelonephritis ***(Nicole et al., 2009)***.

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* and considered as category B (page.31) during pregnancy according to FDA ***(Schwarz S. et al., 2016).***

As all beta-lactam antibiotics, carbapenems inhibit the D-alanyl-D-alanine carboxypeptidase and therefore they

interfere with the cell wall synthesis ***(Del Pozo JL et al., 2007).***

Antimicrobial resistance against carbapenems in Enterobacteriacae, and occasionally also in other gram-negative bacteria can be caused by specific enzymes named ***carbapenemases (Doumith M. et al., 2009)***.

Several carbapenemases have been known already for more than 20 years. but have been restricted to certain species and geographic regions. Therefore, molecular methods are additionally required for an exact, fast and sensitive determination of carbapenemases genes.Recently, several PCRs and real-time PCRs have been established ***(Nordmann P. et al., 2011).***

Pyelonephritis in pregnant women has been associated with increased risk of preterm labour, low birth weight, poor child health during early infancy, maternal adult respiratory distress syndrome, and possible renal failure.E. coli is the main etiologic agent in urinary tract infection (UTI) and Pyelonephritis ***(Kazemier et al., 2015).***

Aim of the Work

The aim of this study is to detect carbapenemases encoding genes responsible for carbapenem resistant in commonest bacteria causing gestational Pyelonephritis.

Antimicrobial Resistance

Antimicrobial resistance (AMR) is a serious threat to health and the inappropriate use of antibiotics is central to the development of antibiotic resistance (www.CDC.gov). The UK five year AMR strategy recommends the strengthening of AMR surveillance to inform local prescribing and enables the monitoring of the impact of interventions aimed at reducing the burden of antibiotic resistance ***(AMR strategy UK. 2015).***

Antimicrobial susceptibility data from diagnostic microbiology laboratories can be used for surveillance to monitor trends in AMR ***(Ironmonger D et al., 2013).***

Study in 2004 found a wide range for sampling urine specimens, from 29 to 266 urine samples/1000 patients/ year ***(McNulty CA.et al., 2004).***

A Welsh study in 2006 found a similar range, with sampling rates varying from 0.6 to 237.3 urine samples/ 1000 patient/year ***(Hillier S. et al., 2006),*** suggesting substantial variability in local sampling policies. Studies in England and the United States have shown urine is the most frequent specimen sent for microbiological examination from non-hospitalised patients and urinary tract infection (UTI) is one of the most common diagnoses that results in antibiotic prescribing ***(Petersen I 2007, Shapiro DJ, 2014)***.

In England in 2009, there was a fivefold difference in antibiotic prescribing volume between general practices ***(Wang KY et al., 2009)***, with 74 % of antibiotic prescribing occurring in community settings in 2014 ***(Public Health England 2015).***

There is a positive linear relationship between trends in antibiotic consumption and resistance ***(Bell BG et al., 2014, Costelloe C, et al., 2010)***.

National guidance for the management of infections and prescribing in the community has not reduced the variation in antibiotic prescribing across general practices in the UK, particularly in the management of upper respiratory and urinary tract infections ***(Hawker JI et al., 2014)***.

Causes of Bacterial Resistance:

1. Overuse:

The overuse of antibiotics is considered the most important cause for the evolution of resistance, confirmed by several epidemiological studies that have demonstrated a direct relationship between antibiotic consumption and the emergence of resistant bacteria strains. Despite warnings regarding overuse, antibiotics are overprescribed worldwide, In many countries, antibiotic use is unregulated and available over the counter without a prescription ***(Golkar et al., 2014)***.

This lack of regulation results in accessible, plentiful and cheap antibiotics which promotes overuse ***(Michael et al., 2014).***

2. Inappropriate Prescribing:

Incorrect prescription of antibiotics widely contributes to the evolution of resistant bacteria, & exposes patients to potential complications of antibiotic therapy ***(Lushniak, 2014)***. Studies had been shown that treatment indication, choice of agent, or duration of antibiotic therapy is incorrect in 30% to 50% of cases ***(CDC, 2013).*** Sub-therapeutic antibiotic concentrations can lead to the development of

antibiotic resistance by supporting genetic alterations, such as changes in gene expression, horizontal gene transfer and mutagenesis ***(Viswanathan, 2014)***

3. Extensive agricultural use:

The antibiotics used in live stock are ingested by humans when they consume food. The transfer of resistant bacteria to humans by farm animals was first noted more than 35 years ago, when high rates of antibiotic resistance were found in the intestinal flora of both farm animals and farmers. Molecular detection methods demonstrated that resistant bacteria in farm animals reach consumers through meat products ***(Bartlett, 2013).***

This occurs through the following sequence of events:

1. Antibiotic use in food-producing animals kills or suppresses susceptible bacteria, allowing antibiotic-resistant bacteria to thrive.

2. Resistant bacteria are transmitted to humans through the food supply.

3. These bacteria can cause infections in humans that may lead to adverse health consequences ***(CDC, 2013)***.

Antibacterial products sold for hygienic or cleaning purposes may also contribute to this problem, since they may limit the development of immunities to environmental antigens in both children and adults ***(Michael et al., 201).***

4. ***Availability of few new antibiotics:***

The development of new antibiotics by the pharmaceutical industry had been hindered due to economic and regulatory obstacles, impeding a strategy that had been

effective at combating resistant bacteria in the past. Of the 18 largest pharmaceutical companies, 15 abandoned the antibiotic field, reducing the number and diversity of research teams ***(Bartlett, 2013).***

In addition, microbiologists recommended restraining antibiotic use. Therefore, once a new antibiotic is marketed, physicians often hold this new agent & reserve it only for the worst cases for fear of evolution of drug resistance. So, new antibiotics are often treated as "last-line" drugs to combat serious illnesses.

When new agents are eventually used, the emergence of resistance is nearly inevitable, yet the timeline for the development of resistance is unpredictable ***(Gould and Bal, 2013)***

5. ***Regulatory barriers:***

Between 1983 and 2007, a substantial reduction occurred in the number of new antibiotic approvals. Changes in standards for clinical trial design made by the U.S. Food and Drug Administration (FDA) during the past two decades have made antibiotic clinical trials particularly challenging. This requires a large sample population and consequently high costs, making the development of antibiotics uneconomical and unattractive ***(Wright, 2014).***

Mechanisms of Antibiotic Resistance:

Resistance of bacteria to antibiotics often results from an inheritable resistance *(vertical transmission*), but it also occurs through horizontal gene transfer (acquired resistance). Horizontal transfer is more likely to happen in locations of frequent antibiotic use. Cross-resistance to several antibiotics may occur when a resistance mechanism encoded by a single