Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for the Identification of Bacteria Causing Urinary Tract Infections

Thesis

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Dedication to

The sole of my Parents

My loving& caring wife

My kids

My Dear sisters

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List of Abbreviations

Abb.	Full term
ASB	Asymptomatic bacteruria
CAUTI	Catheter associated UTI
CDC	The center for disease control and prevention
CFU	Colony-forming units
CLSI	Clinical and laboratory Standards Institute.
ELISA	Enzyme Linked immunosorbent assay
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
MDR	Multidrug resistance
MICS	Minimal inhibitory concentrations
MIS	Microbial identification system
MSU	Mid stream urine
NCCLS	National committee for Clinical laboratory Standards
NPV	Negative predictive values
PCR	Polymerase chain reaction
PPV	Positive predictive values

INTRODUCTION

Urinary tract infections (UTIs) are a severe public health problem. Many microorganisms cause UTIs. With the majority of cases are caused by *Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus*. The problem of high recurrence rates and antimicrobial resistance in urinary tract pathogens is increased. This lead to increase the economic burden of these infections ***(Flores-Mireles et al., 2015).***

Microbiological confirmation of a UTI takes 24–48 h. In the meantime, patients are usually given empirical antimicrobial therapy, sometimes unnecessarily or inadequately. Anticipation of clinically useful data is of the greatest importance, with both diagnostic and therapeutic consequences ***(Burillo et al., 2014).***

Traditional bacterial identification in the clinical microbiology laboratory is done by biochemical and phenotypic analysis, using both manual and automated systems, in addition to molecular methods. While some of these techniques are rapid, the majority depend on microbial growth and utilization of biochemicals, requiring hours to days for identification ***(Saffert et al., 2011).***

In the recent years several chromogenic media have been developed and commercialized, allowing for more specific direct differentiation of microorganisms on primary plates. The

Chromogenic agar offers simultaneous presumptive identification of gram Positive and gram negative bacteria and yeasts on a single medium by means of distinct colony colours produced by reactions of genus or species specific enzymes with a suitable chromogenic substrate ***(Sony et al., 2015).***

Standardized test systems such as API and VITEKH 2 (bioMe´rieux), or PHOENIX (BD Diagnostics), complemented by traditional culture and microscopy methods, have so far been used in routine labs for the rapid identification of clinical microorganisms. With the introduction of these methods, the average time needed for a reliable and validated identification ranged from 6 h to 18 h and in the last few years, sequence analysis of small-subunit rRNAs or selected genes by PCR methods has complemented the biochemical methods, additionally decreasing throughput time and becoming in several cases the gold standard ***(Benagli et al., 2011).***

Conventional methods of bacterial species identification are time consuming and labor intensive. Gram staining and automated reading systems of biochemical reactions such as Vitek 2 (bioMérieux, Marcy-l'Etoile, France) or BD PHOENIX (Becton Dickinson, Sparks, MD) as well as susceptibility testing still require 48–72 hours from sample collection to identification of the bacterial species. MALDI-TOF MS has been developed considerably in recent years and now constitutes a quantum leap in the identification of pathogenic microbes. The technology provides a very rapid, cost-effective,

easy-to-use and reliable method of bacterial (and fungal) identification to the genus and species level ***(Dierig et al., 2015).***

However, MALDI-TOF MS applications can go far beyond. Identification directly from clinical specimens such as urine or cerebrospinal fluid can make a timely difference in the management of patients with severe infections. In addition, the future potential has not been completely explored, and novel applications are developing, such as rapid typing in outbreak situations, early detection of antibiotic resistance and recognition of mixed infections employing advanced software algorithms ***(Mari et al., 2014 & Dierig et al., 2015).***

AIM OF THE WORK

- To evaluate MALDI-TOF MS for the rapid identification of clinically relevant urinary tract pathogens in urine samples to the species level and to compare the results with the routine conventional identification methods including UTI chrom agar and the BD PHOENIX.
- To detect the sensitivity and specificity of MALDI-TOF MS for bacterial isolates identification in comparison to PCR as gold standard test.

Chapter 1

URINARY TRACT INFECTION

Urinary tract infection (UTI) refers to bacterial or fungal infection of the bladder or the kidney, or both, in a patient regardless to the presence or absence of symptoms ***(Mandell et al., 2010).***

UTIs are upper or lower according to the anatomical site ***(Ezejiofor, 2016).***

Nosocomial UTI refers to UTI that is acquired in any institutional setting providing health care. The Center for Disease Control and Prevention (CDC) uses the term, health-care associated, instead of nosocomial that can also be used in reference to infection that are related to health care activities occurs anywhere, including the home ***(Chenoweth and Saint, 2016).***

UTI is the most common nosocomial infection (33%), followed by pneumonia (15%) then, surgical site infections (13%) ***(CDC, 2018).***

Symptomatic UTI

Symptomatic UTI commonly occur in sexually active women and more common in women than in men ***(Foxman et al., 2000).***

Asymptomatic bacteruria (ASB)

Defined as $\geq 10^5$ colony-forming units (CFUs)/mL without any symptoms or signs of urinary tract infection ***(Mouton et al., 2010).***

Pathogenesis of urinary tract infections

The ascent of microorganisms through the urethra is the main route of infection; other routes like hematogenous or lymphatic also present ***(Smelov et al., 2016).***

Most UTIs are caused by one organism with *Escherichia coli* as causative microorganism in 70–95% of cases ***(Czaja et al. 2007).***

In complicated UTIs the microbial spectrum is broader and include different species such as Pseudomonas, Staphylococcus, Serratia, Providencia, Enterococcus, and fungi ***(Nicolle, 2008).***

Few relatively uncommon microorganisms in urine, may cause hematogenous infection of the urinary tract like *Staphylococcus aureus*, Candida spp. and *Mycobacterium tuberculosis*. They all cause primary infections somewhere else in the body ***(Grabe et al., 2015).***

The most common type of healthcare-associated infection is UTIs. In 75% of UTIs it is related to urinary catheter. The prolonged use of urinary catheter is the main risk