IN VITRO ASSESSMENT OF COMBINATION OF BOTH LINEZOLID AND ERTAPENEM AS HIGHLY SYNERGESTIC EFFECT AGAINST MRSA

Thesis

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بسم الله الرحمن الرحيم

فَالُوا سُبْحَانكَ لاَ علِمَ لَنَا إِلاّ مَا

عَلّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيم

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List of Abbreviations

(CA-MRSA)	Community-Acquired MRSA
(CCOHS)	Canadian Centre for Occupational Health and Safety
(CDC)	Centers for Disease Control and Prevention
(CLSI)	Clinical and Laboratory Standards Institute
(CONS)	Coagula se negative staphylococci
(cSSSIs)	Complicated skin and skin-structure infections
(DNase)	Deoxyribonuclease test
(ETP)	Ertapenem
(ICU)	Intensive care unit
(Luk)	Leukocidin
(LZD)	Linezolid
(LRS)	Linezolid resistant Staphylococcus
(MASO)	Mannitol salt agar with oxacillin
(MDRSP)	Multidrugresistant Streptococcus pneumoniae
(MIC)	Minimum inhibitory concentration
(MRSA)	Methicillin resistant staphylococcus aureus
(MSSA)	Methicillin-sensitive S. aureus
(ORSAB)	Oxacillin resistant screening agar base test
(PBP2)	Penicillin-binding protein PBP2'
(PCR)	Polymerase chain reaction
(PRSP)	Penicillin-resistant S. pneumoniae
(PVL)	Valentine leucocidin
(SaG)	S.aureus endo B-N-acetylglucosaminidase
(SA-RVS)	S. aureus with reduced vancomycin susceptibility
(SSI)	Surgical site infections
(Staph.)	Staphylococcus

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) became the leading nosocomial pathogen worldwide and seems to have spread into the community ***(Kluytmans and Struelens, 2009)***. The prevalence of CA-MRSA continued to rise; a meta-analysis published in 2003 reported a pooled CA-MRSA prevalence rate of 30.2% in retrospective studies and a colonisation rate of 1.3% ***(George and Lalitagauri, 2010)***.

The reservoir of MRSA is the infected and colonized patients and the major mode of transmission is direct or indirect contact involving the hands of healthcare workers ***(Luft and Dettenkofer, 2010).*** Thus, active surveillance and timely identification of MRSA colonization of patients is an important infection control activity that helps to prevent nosocomial spread and is cost effective ***(Wernitz et al., 2005).***

There are several methods available to laboratories for detecting methicillin resistance. These include oxacillin disk test, automated susceptibility testing systems, and oxacillin agar screen plate. In addition, the cefoxitin disk test was recently recommended by the Clinical and Laboratory Standards Institute for prediction of mec A–mediated resistance ***(CLSI, 2007)***.

More rapid alternative methods for the detection of MRSA have been developed including the use of chromogenic media, which have been shown to be comparable or superior in sensitivity and specificity to traditional selective media ***(Philippe et***

al., 2008). The use of mannitol salt agar supplemented with oxacillin and the use of the oxacillin resistance screening agar (ORSA) are two of the most commonly used culured methods ***(Compernolle et al., 2007).***

Molecular techniques for the detection of mec A gene are viewed as the gold standard for determining MRSA. Polymerase chain reaction (PCR) for amplification of the mec A gene can be performed within few hours, providing same day results. However, these methods have certain disadvantages, including the need to batch clinical specimens, greater technical demands than culture, expensive reagents and the need for specialized laboratory equipment ***(Swenson, and Lonsway et al., 2007).***

Methods to reduce nosocomial transmission of methicillin-resistant Staphylococcus aureus (MRSA) include contact isolation precautions, measures to decolonise MRSA carriers, staff education, screening for contacts, and screening of risk groups for MRSA carriage at hospital admission. Microbiological screening at the point of hospital admission is the only opportunity to detect MRSA patients early enough to enable the implementation of timely contact isolation precautions that will reduce the subsequent nosocomial spread of this multiresistant organism ***(Luft and Dettenkofer, 2010)***

Traditional antibiotics used in the treatment are not effective against MRSA as it has become resistant to multiple other antimicrobial agents, including aminoglycosides, fluoroquino-

lones, tetracyclines, and macrolides–lincosamides–streptogramins. MRSA has acquired reduced susceptibility to vancomycin, which has been reported in Hong Kong and Taiwan but not in Mainland China *(**Wang et al., 2008**)*.

Because of the multidrug resistance of MRSA and vancomycin-intermediate or resistant S. aureus, there is increasing need to develop new agents for the treatment of S. aureus infections. Tigecycline and linezolid have been used in clinical practice for resistant Gram-positive infections and ceftobiprole is in phase 3 of clinical development ***(Davies et al., 2006).***

New drugs are available in France (i.e. daptomycin, ertapenem, and doripenem) as well as those of antibacterial drugs currently in development (i.e. ceftaroline, dalbavancin, telavancin, oritavancin, iclaprim, and ramoplanin) or available in other countries (i.e. garenoxacin, sitafloxacin, and temocillin) ***(Wang et al., 2008)***.

Aim of the Work

This work is aiming to assess incidence of linezolid resistance in MRSA and in vitro combination of linezolid and ertapenem as highly synergestic effect against MRSA.

Chapter I
Staphylococci

Historical Aspect:

Staphylococci were first recovered from pus by Koch (1878) and Pasteur (1880) who cultivated them in liquid media. In (1881), Rosenbach obtained a pure culture of Staphylococci on solid media and he classified them according to their colony appearance into two species: Staphylococcus (Staph.) aureus (golden yellow colony), and Staph. albus (greyish white colony). Later on, the lemon colored colony, Staph. citrus, was added by Passett in 1885 ***(Thorberg, 2008)*.**

Natural habitat:

Staphylococci are widely distributed in various environments. Natural populations are associated with skin, skin glands and mucous membranes of humans, as well as many animals. They are sometimes found in the intestinal, genitourinary, and upper respiratory tracts of these hosts. They have also been isolated from animal products and other sources, such as soil, sand, seawater, fresh water, dust, and air (***Tomasz et al., 2010).***

Staphylococcus capitis is found primarily in the skin and sebaceous gland of the scalp, forehead and neck whereas Staph. auricularis is found primarily in the external auditory canal (***Winn et al., 2006***).

Morphology:

Bacteria of the genus Staphylococcus are gram-positive cocci that are microscopically observed as individual organisms, in pairs, and in irregular, grapelike clusters. The term Staphylococcus is derived from the Greek term staphyle, meaning "a bunch of grapes." Staphylococci are nonmotile, non–spore-forming, and catalase-positive bacteria. The cell wall contains peptidoglycan and teichoic acid. The organisms are resistant to temperature as high as 50°C, to high salt concentrations, and to drying. Colonies are usually large (6-8 mm in diameter), smooth, and translucent. The colonies of most strains are pigmented, ranging from cream-yellow to orange (***Belkum et al ., 2009).***

Epidemiology

Mode of transmission

Staphylococci are colonizers of various skin and mucosal surfaces. Infections are frequently acquired when:

- S. aureus spread from patient's endogenous strain to normally sterile site by traumatic introduction and from person to person by fomites, unwashed hands of health care coworkers which lead to patient colonization and potentially infected with antibiotic resistant strains ***(Otter et al., 2010)***.