Developing an Antimicrobial Strategy for Sepsis in Malawi

_

Thesis submitted in accordance with the requirements of the Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy by Joseph Michael Lewis

August 2019

Contents

Pı	refac	e	9		
1	Introduction				
	1.1	Chapter Overview	13		
	1.2	Sepsis in sub-Saharan Africa	13		
	1.3	ESBL-E in sub-Saharan Africa	13		
	1.4	Conclusions	13		
	1.5	Thesis overview	13		
	1.6	Appendix	13		
	1.7	References	13		
2	Methods				
	2.1	Chapter Overview	17		
	2.2	Study site	17		
	2.3	Clinical Study	17		
	2.4	Diagnostic Laboratory Procedures	17		
	2.5	Molecular methods	17		
	2.6	Bioinformatics	17		
	2.7	Statistical Analysis	17		
	2.8	Study Team	17		
	2.9	Data Collection and Storage	17		
	2.10 Ethical Approval, Consent and Participant Remuneration				
3	My	cobacterium tuberculosis BSI: an IPD meta analysis	19		
4	A clinical and microbiological description of sepsis in Blantyre, Malawi				
	4.1	Chapter overview	22		
	4.2	Introduction and chapter aims	22		
	43	Aims and Methods	22		

4 CONTENTS

	4.4	Results	22		
	4.5	Discussion	22		
	4.6	Conclusions and further work	22		
	4.7	Appendix	22		
5	Lon	gitudinal ESBL-E carriage in Malawian adults in health and disease	23		
	5.1	Chapter Overview	23		
	5.2	Introduction and chapter aims	23		
	5.3	Methods	23		
	5.4	Results	23		
	5.5	Discussion	23		
	5.6	Conclusions and further work	23		
6	Wh	ole genome sequencing of ESBL <i>E. coli</i> carriage isolates	25		
	6.1	Chapter overview	27		
	6.2	Methods	27		
	6.3	Results	27		
	6.4	Discussion	27		
	6.5	Appendix	27		
7	Gut	mucosal carriage of ESBL-E in Blantyre, Malawi	29		
\mathbf{R}	References				

List of Tables

6 LIST OF TABLES

List of Figures

8 LIST OF FIGURES

Preface

10 LIST OF FIGURES

Introduction

1.1 Chapter Overview

- 1.2 Sepsis in sub-Saharan Africa
- 1.2.1 Search strategy
- 1.2.2 Defining sepsis
- 1.2.3 Applicability of sepsis-3 definitions in sub-Saharan Africa
- 1.2.4 Sepsis epidemiology in sub-Sahara Africa
- 1.2.4.1 Incidence
- 1.2.4.2 Risk factors: the sepsis population in sub-Saharan Africa
- 1.2.4.3 **Outcomes**
- 1.2.5 Sepsis aetiology in sub-Saharan Africa
- 1.2.5.1 Bacterial zoonoses, Rickettsioses and arboviruses
- 1.2.5.2 HIV opportunistic infections: PCP, histoplasmosis and cryptococcal disease
- 1.2.6 Sepsis management
- 1.2.6.1 Early goal directed therapy
- 1.2.6.2 Evidence to guide antimicrobial therapy in sSA
- 1.2.6.3 Evidence to guide intravenous fluid therapy in sub-Saharan Africa
- 1.3 ESBL-E in sub-Saharan Africa
- 1.3.1 Search strategy
- 1.3.2 Introduction: definition and classification of ESBL-E
- 1.3.3 Global molecular epidemiology of ESBL-E: an overview
- 1.3.3.1 1980s-1990s: First identification of ESBL in nosocomial pathogens
- 1.3.3.2 1990s-2010s: Emergence and globalisation of CTX-M

Methods

2.1 Chapter Overview

- 2.2 Study site
- 2.2.1 Malawi
- 2.2.2 Queen Elizabeth Central Hospital
- 2.2.3 Participating Laboratories
- 2.2.3.1 Malawi-Liverpool-Wellcome Clinical Research Programme
- 2.2.3.2 Malawi College of Medicine Tuberculosis Laboratory
- 2.2.3.3 Wellcome Trust Sanger Institute
- 2.3 Clinical Study
- 2.3.1 Entry Criteria
- 2.3.2 Study Visits and Patient Sampling
- 2.3.2.1 Enrollment assessment and first six hours
- 2.3.2.2 Subsequent visits
- 2.3.2.3 Blood, urine, and stool, sputum and CSF collection
- 2.3.2.4 Imaging: chest x-ray and ultrasound scanning
- 2.3.3 Outcomes and sample size calculations
- 2.4 Diagnostic Laboratory Procedures
- 2.4.1 Point of care diagnostics
- 2.4.2 Laboratory diagnostics
- 2.4.2.1 Haematology and biochemistry
- 2.4.2.2 Aerobic blood and CSF culture
- 2.4.2.3 Mycobacterial blood culture

Mycobacterium tuberculosis BSI: an IPD meta analysis

A clinical and microbiological description of sepsis in Blantyre, Malawi

- 4.1 Chapter overview
- 4.2 Introduction and chapter aims
- 4.3 Aims and Methods
- 4.4 Results
- 4.4.1 Study population
- 4.4.2 Symptoms and health-seeking behaviour
- 4.4.3 Admission physiology and laboratory investigations
- 4.4.4 Aetiology
- 4.4.5 Treatment
- 4.4.6 Outcome
- 4.4.7 Determinants of mortality
- 4.5 Discussion
- 4.5.1 Demographics and outcome: significant longer-term mortality
- 4.5.2 Aetiology: TB dominates as a cause of sepsis
- 4.5.3 Determinants of 28-day mortality: an expanded role for TB therapy?
- 4.5.4 Limitations
- 4.6 Conclusions and further work
- 4.7 Appendix

Longitudinal ESBL-E carriage in Malawian adults in health and disease

D		1 1	1 1	
М	lace	no	l (1	er.

- 5.1 Chapter Overview
- 5.2 Introduction and chapter aims
- 5.3 Methods
- 5.4 Results
- 5.4.1 Study population
- 5.5 Discussion
- 5.5.1 Limitations
- 5.6 Conclusions and further work

24CHAPTER 5.	LONGITUDINAL	ESBL-E CARRIA	GE IN MALAWIA	N ADULTS IN HE	ALTH AND DISEA

Whole genome sequencing of ESBL $E.\ coli$ carriage isolates

 $26 CHAPTER\ 6.\ \ WHOLE\ GENOME\ SEQUENCING\ OF\ ESBL\ E.\ COLI\ CARRIAGE\ ISOLATES$

6.1 Chapter overview

- 6.2 Methods
- 6.2.1 Bioinformatic pipeline
- 6.2.2 Global *E. coli* collection
- 6.2.3 Statistical analysis
- 6.3 Results
- 6.3.1 Samples and quality control
- 6.3.2 Phylogroup, MLST and core genome phylogeny of study isolates
- 6.3.3 Study isolates in a global context
- 6.3.4 Antimicrobial resistance determinants
- 6.3.4.1 β -lactam resistance
- 6.3.4.2 Quinolone resistance
- 6.3.4.3 Aminoglycoside resistance
- 6.3.4.4 Chloramphenicol, co-trimoxazole, tetracycline and other resistance determinants
- 6.3.4.5 Clustering and lineage association of AMR determinants
- 6.3.5 Plasmid replicons
- 6.3.6 Testing metadata associations: SNP distance, hierBAPS sequence clusters and ESBL-clusters
- 6.3.6.1 Hierarchical BAPS clustering of core gene pseudosequences
- 6.3.6.2 ESBL-clusters
- 6.3.6.3 Assessing for healthcare-associated lineages
- 6.3.6.4 Assessing for within-patient conservation of lineage or MGE
- 6 1 Diagnasian

 $28 CHAPTER\ 6.\ \ WHOLE\ GENOME\ SEQUENCING\ OF\ ESBL\ E.\ COLI\ CARRIAGE\ ISOLATES$

Gut mucosal carriage of ESBL-E in Blantyre, Malawi

References