

CONTENTS	Page No.
Title page	i
Declaration	ii
Certificate by Supervisor	iii
Dedication	iv
Acknowledgement	v
Abstract	---
Contents	--
List of symbols/ abbreviations	xi–xii
Chapter 1 Introduction	1–5
Chapter 2 Literature review	7–39
2.1. The Gram negative bacterial outer membrane (OM)	9–14
2.1.1. Organization of the OM	9–10
2.1.2. Lipopolysaccharides (LPSs)	10–14
2.1.2.1. O-specific chain (O-antigen)	10–12
2.1.2.2. Core Oligosaccharide (core OS)	12–13
2.1.2.3. Lipid A	13–14
2.2. Peptidoglycan layer and Penicillin-binding proteins	14–19
2.2.1. PBP 5, 6 and DacD of <i>E. coli</i>	17–18
2.2.2. PBP 1a and 1b of <i>E. coli</i>	18–19
2.3. Beta-lactam antibiotics	19–23
2.3.1. The mode of action of beta-lactam antibiotics	21–22
2.3.1.1. DD-Carboxypeptidases and beta-lactams	22–23
2.4. Mechanisms involved in the development of beta-lactam resistance	23–38
2.4.1. Alteration or prevention the antibiotic permeability into the cell	25–27
2.4.2. Production of beta-lactam hydrolyzing or inactivating enzymes	27–31
2.4.2.1. Major Beta-lactamases present in Gram negative bacteria	28–31
2.4.3. Presence of efflux pump systems	31–33
2.4.3.1 Efflux pumps in <i>E. coli</i>	32–33
2.4.4. Modifications or alterations of PBPs	33–38
2.4.4.1. MecA in MRSA	34–35

2.4.4.2. Mosaic PBPs in <i>Streptococcus pneumoniae</i>	35–35
2.4.4.3. Altered PBPs in <i>Neisseria gonorrhoeae</i>	35–36
2.4.4.4. Mosaic PBPs in <i>Neisseria sp</i>	36–36
2.4.4.5. Beta-lactam resistance associated with PBP changes in other organisms	36–37
2.4.4.6. Deletion or overexpression of PBPs alter the beta-lactam resistance	38–38
Objectives of the present thesis	39–39
Chapter 3 Identifying the role of O8-antigen in altering beta-lactam antibiotic sensitivity in <i>Escherichia coli</i>	41–60
3.1. Introduction	43–43
3.2. Materials	44–48
3.3. Methods	48–51
3.4. Results and discussion	51–60
3.4.1. Beta-lactam sensitivities of <i>E. coli</i> 2443 and CS109	41–53
3.4.2. Labeling of O-antigen with ConA-AF 488	53–54
3.4.3. Detection of O-antigen by LPS staining	55–55
3.4.4. Beta lactam sensitivities of AGTO2-1K	55–56
3.4.5. Sensitivities of CS109, 2443 and AGTO2-1K to structurally unrelated antibiotics	56–57
3.4.6. Possible mechanisms for the effects of O8-antigen on beta-lactam sensitivity	57–60
3. 5. Conclusion	60–60
Chapter 4 Evaluation of the relationships between the LMM PBPs (PBP5, 6 and DacD) and sensitivity towards beta-lactam antibiotics	61–96
4.1. Introduction	63–64
4.2. Materials	64–67
4.3. Methods	68–76
4.4. Result	76–92
4.4.1. The role of PBP5 in beta-lactam resistance	77–85

4.4.1.1. Construction of PBP5 deleted mutants	77–77
4.4.1.2. Confirmation of PBP5 deletion by PCR amplification	77–78
4.4.1.3. Beta-lactam sensitivity of PBP5 deleted mutants	78–79
4.4.1.4. Sensitivities against other structurally unrelated antibiotics	79–80
4.4.1.5. Complementation with PBP5 clones	80–80
4.4.1.6. The beta-lactam sensitivity after complementation with PBP5 clone	80–81
4.4.1.7. The effect of active site Serine mutation of PBP5 in beta-lactam resistance	82–82
4.4.1.8. Beta-lactam sensitivities after complementation with <i>dacA</i> homologs	83–85
4.4.2. Role of PBP6 in beta-lactam resistance	85–87
4.4.2.1. PBP6 deletion mutant construction	85–86
4.4.2.2. Confirmation of PBP6 deletion	86–86
4.4.2.3. The effect of PBP6 deletion on beta-lactam sensitivity	86–87
4.4.3. The effect of PBP5, 6 double deletion on beta-lactam susceptibility	87–92
4.4.3.1. Confirmation of PBP5, 6 double deletion through PCR amplification	87–88
4.4.3.2. Beta-lactam susceptibility pattern of double (PBP5 and 6) mutants	88–89
4.4.3.3. Complementation of SK2O56-3 with PBP clones	89–89
4.4.3.4. Beta-lactam sensitivity of SK2O56-3 after complementation with PBP5 and 6 clones	90–91
4.4.3.5. Beta-lactam sensitivity of SK2O56-3 after complementation with DacD	91–92
4.5. Discussion	92–95
4. 6. Conclusion	95–96
Chapter 5 Designing a drug combination with the commonly used beta-lactam agents by evaluating the role of HMM PBPs (PBP1a and 1b) in beta-lactam sensitivity	97–123

5.1. Introduction	99–101
5.2. Materials	101–103
5.3. Methods	103–107
5.4. Result	107–121
5.4.1. Role of PBP1b in beta-lactam resistance	107–113
5.4.1.1. Deletion of PBP1b from CS109	107–108
5.4.1.2. Effect of PBP1b deletion effect on beta-lactam sensitivity	108–110
5.4.1.3. Effect of O-antigen on beta-lactam sensitivity in PBP1b mutant	110–113
5.4.2. Role of PBP1a in beta-lactam sensitivity	113–115
5.4.3. Ectopic complementation and beta-lactam sensitivity	115–117
5.4.4. Effect of sub-inhibitory level of Cefsulodin in combination with other beta-lactam agents	117–118
5.4.5. Application of Cefsulodin in combination with other beta-lactams on different Gram negative bacteria	118–119
5.4.6. Detection of beta-lactamase activity of bacterial isolates	119–121
5.5. Discussion	121–122
5. 6. Conclusion	123–123
Chapter 6 Summary and Conclusions	125–130
6.1. Summary	127–128
6.2. Conclusions	129–129
6.3. Contributions of the thesis	129–130
6.4. Possible Future Perspectives	130–130
References	131–146
Publications	----
Copyright permission from JOHN WILEY AND SONS	----
Copyright permission from ELSEVIER	----
Curriculum vitae	----