

TABLE OF CONTENTS

1. Introduction	1-5
2. Review of Literature	6-57
2.1. Antibiotics	7
2.2. Antibiotic resistance	8
2.2.1. Innate resistance	9
2.2.2. Adaptive resistance	9
2.3. Carbapenem: Background	9-10
2.3.1. Imipenem	10-11
2.3.2. Ertapenem	11-12
2.3.3. Meropenem	12
2.3.4. Doripenem	13
2.3.5. Tebipenem	13
2.4. Mode of action	13-14
2.5. Mode of resistance	14
2.6. Carbapenem hydrolyzing enzymes	15
2.6.1. Functional or Bush-Jacoby functional classification	16
2.6.2. Molecular or Ambler classification	16
2.6.3. Class A-Serine carbapenemases	16-17
2.6.3.1. Chromosomally encoded class A enzymes	17-18
2.6.3.2. Plasmid encoded class A carbapenemases	18-21
2.6.3.3. Class B carbapenemases (metallo-β-lactamases)	21-22
• Chromosome borne metallo- β -lactamases	23-24
• Plasmid borne metallo- β -lactamases	24-31
2.6.3.4. Class D carbapenemases	31-34
2.7. Non-enzymatic (Efflux pump) mechanism	35-38
2.7.1. Efflux pumps in <i>P. aeruginosa</i>	38
2.7.2. Efflux pump in <i>Acinetobacter</i> species	39

2.8. Non-enzymatic (Reduced membrane permeability) mechanism	39-40
2.9. Carbapenem Resistance-Detection	40-42
2.9.1.1. Modified Hodge test	42-43
2.9.1.2. Inhibitor based method	44-49
2.9.1.3. KPC+MBL detection commercial kit	49-50
2.9.1.4. Culture media for detection of carbapenemase	50
2.9.1.5. Carbapenemase detection by rapid colorimetric Methods	50-52
2.9.1.6. Carbapenem Inactivation Method (CIM)	52-53
2.9.2. Additional tests	53-54
2.9.3. Analysis of carbapenemase genes by genotypic means	59-61
2.9.4. Efflux pump: Detection Methods	56-57
3. Materials and Methods	58-76
3.1. General Methods	58
3.2. Specimen processing	58-60
3.3.1. Determination of (MIC's) of carbapenem drugs	60-61
3.3.2. Modified Hodge test	61-62
3.3.3. Carbapenemase detection based on inhibitor profile	62-63
3.3.4. Rapid CARB Blue Kit	63-64
3.3.5. Neo-Rapid CARB Kit	64
3.3.6 Carba NP test	65
3.3.7. CarbAcineto NP Test	66-67
3.3.8. Blue Carba Test	67-68
3.3.9. Carbapenem Inactivation Method (CIM)	69
3.4 Control Strains	70
3.5. Molecular Methods	71-75
3.5.1. DNA Extraction	71-72
3.5.2. Primer sets	73
3.5.3.. PCR reaction mixture	73
3.5.4. PCR amplification of Carbapenem resistance gene	74

3.5.5. Agarose gel electrophoresis	74
3.5.6. Loading and electrophoresis of the sample	74
3.5.7. Visualization of the gel	75
3.6. Data analysis	75
3.7. Efflux pump mechanism	75-76
4. Results	77-116
4.1. General Resistance pattern	77
4.2. Lack of Agreement between Carbapenem-Resistance Test Results Obtained With Antimicrobial Discs from Different Sources	78
4.2.1. Excluding Intrinsic Resistance	79
4.2.2. Final tally of carbapenem resistant GNB	79-80
4.3. Carbapenem Resistant Enterobacteriaceae (n=49)	81-86
4.3.1 Specimen types	82
4.3.2. Ward-wise distribution	83
4.3.3. Resistance to other antimicrobials	84
4.3.4 Determination of carbapenem (MIC)	85-86
4.4 <i>Acinetobacter calcoaceticus-baumannii</i> complex (n=104)	87-90
4.4.1 Antimicrobial susceptibility testing and MIC determination	88-90
4.5. <i>Pseudomonas aeruginosa</i> (n=31)	91-94
4.5.1. Antimicrobial susceptibility testing and MIC determination	92-94
4.6. Testing Carbapenem MIC with Etest Strips	95
4.7.1. Modified Hodge Test (MHT)	96-97
4.7.2. KPC+MBL Confirm ID kit (for Enterobacteriaceae)	98-99
4.7.3. Rapid CARB Blue test Kit (for <i>Acinetobacter</i> , <i>P. aeruginosa</i> and Enterobacteriaceae)	100-101
4.7.4. Neo-Rapid CARB kit (For Enterobacteriaceae and <i>P. aeruginosa</i>)	102-103
4.7.5. Carba NP, CarbAcineto NP, and Blue Carba tests, along with their modifications	104-105

4.7.6. Carbapenem Inactivation Method for <i>Acinetobacter</i> , <i>P. aeruginosa</i> and Enterobacteriaceae	106-107
4.8.1. Enterobacteriaceae: PCR for carbapenemase gene	110
4.8.2. ACBC: PCR for carbapenemase genes	110
4.8.3. <i>P. aeruginosa</i> : PCR for carbapenemase gene	110
4.9. Efflux pump detection	115-116
5. Discussion	117-135
6. Summary	136-139
7. Conclusion	140-141
8. References	142-177

List of publication and presentation

Appendices

- Master chart: Enterobacteriaceae
- Master Chart: *Acinetobacter calcoaceticus baumannii* complex
- Master Chart: *Pseudomonas aeruginosa*
- Patient Proforma