Determination of the antibacterial activity against microorganism after co-culture with resistant pathogenic bacteria

A thesis paper submitted to the Department of Pharmacy in partial fulfillment for the requirement of the degree of Master of Pharmacy (M. Phrm.)

Submitted by

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ID: 2015-1-79-003

Submitted to

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Dedication

This thesis paper

is dedicated

to my beloved family&

RESEARCH SUPERVISOR

Declaration by the Research Candidate

I, Tahsin Tabassum, hereby declare that this dissertation, entitled **"**Determination of the antibacterial activity against microorganism after co-culture with resistant pathogenic bacteria" submitted to the Department of Pharmacy, East West University, in partial fulfillment for the requirement of the degree of Master of Pharmacy , is a genuine & authentic research work carried out by me under the guidance of Dr. Sufia Islam, Professor, Department of Pharmacy, East West University, Dhaka, Bangladesh. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any Degree or Diploma of Fellowship.

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Certificate by the Supervisor

This is to certify that the dissertation, entitled **"**Determination of the antibacterial activity against microorganism after co-culture with resistant pathogenic bacteria" is a bona fide research work done, under our guidance and supervision by Tahsin Tabassum (ID: 2015-1-79-003), in partial fulfillment for the requirement of the degree of Master of Pharmacy.

Dr. Sufia Islam,

Professor

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Certificate by the Chairperson

This is to certify that the dissertation, entitled **"**Determination of the antibacterial activity against microorganism after co-culture with resistant pathogenic bacteria" is a bona fide research work done by Tahsin Tabassum (ID: 2014-3-79-012), in partial fulfillment for the requirement of the degree of Master of Pharmacy.

Dr. Shamsun Nahar Khan

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Abstract:

In this study the effect of the antibacterial activity was determined after co-culture of sensitive strain with resistant pathogenic bacteria. Disc diffusion method was used to find out the antibacterial activity after co-culture of sensitive strain with resistant pathogenic bacteria. Here, co-culture was performed with *Acinetobacter baumannii* and *Salmonella typhi* and also with *Acinetobacter baumannii* and *Shigella flexneri*. In our study, before co-culture *Acinetobacter baumannii* and *Salmonella typhi* were sensitive strains, whereas *Shigella flexneri* was resistant. After co-culture of two sensitive strains (*Acinetobacter baumannii* and *Salmonella typhi*), *Acinetobacter baumannii* showed sensitivity when treated with different concentrations (20 μg/ml, 40 μg/ml, 80 μg/ml and 160 μg/ml) of cefixime. However, no zone of inhibition was observed when *Salmonella typhi* was treated with different concentrations (20 μg/ml, 40 μg/ml, 80 μg/ml and 160 μg/ml) of cefixime. Co-culture of sensitive *Acinetobacter baumannii*was done with resistant *Shigella flexneri*. *Acinetobacter baumannii*s till showed sensitivity when treated with different concentrations (20 μg/ml, 40 μg/ml, 80 μg/ml and 160 μg/ml) of azithromycin. However, zone diameter was reduced to 25% when treated with azithromycin after co-culture with resistant strain of *Shigella flexneri*. No zone of inhibition was observed when *Shigella flexneri* was treated with different concentrations (20μg/ml, 40μg/ml, 80μg/ml and 160μg/ml) of azithromycin. Sensitive strain of *Acinetobacter baumannii* has shown 25% reduction of zone of inhibition when co-culture was done with *Shigella flexneri*.

Keywords: Co-culture, resistant pathogenic bacteria, *Acinetobacter baumannii*, *Salmonella typhi*, *Shigella flexneri*, cefixime, azithromycin.

Chapter 1

Introduction

1.1 Introduction:

Septicaemia is associated with morbidity and mortality in ICU patients due to nosocomial infections. It is very costly to treat such infections. It has been estimated that 575000–677000 episodes and 79000–94000 deaths occurred in each year due to the nosocomial infections in North America. These infections are the sixth- and seventh-leading causes of death in Canada and the United States respectively (Rachel D. *et al*, 2016). Sensitive strains become resistant when come in contact with the resistant strain and this problem is usually occurred in ICU patients due to nosocomial infections. The antibiotic resistance in those patients is most prevalent. It creates difficulties to treat critically ill patients. There is an immediate need to develop newer classes of antibiotic because antibiotic resistant has become an urgent threat to individual patient. It is difficult to treat drug-resistant pathogens. It has created the need for new therapies. Public health risk caused by multidrug-resistant (MDR) bacteria has become a serious problem on a global scale. Efforts for treating MDR bacteria continue to be a problem for the clinician for victim of nosocomial infection (Shin B. *et al*, 2015).

It has been shown that *Acinetobacter baumannii* is an important pathogen in healthcare settings as it is associated with nosocomial infections. Infections caused by *Acinetobacter baumanni* include bacteremia, pneumonia, meningitis, wound infections and urinary tract infections. Antibiotic resistance against *A. baumanni* is a major concern because of increasing resistance (Shin Bora et al, 2015). Diarrhoeal disease is a major cause of morbidity and mortality in children under the age of 5. *Shigella* is the most prevalent diarrhoel pathogen which accounts for around 125 million cases of diarrhoea annually. Most of the diarrhoeal cases are from low-income countries. Antibacterial resistance is a major problem in *Shigella* infection, particularly *Shigella* sonnei has become dominant worldwide. This resistant strain is increasingly isolated in Asia and oher continents (Hao C. *et al*, 2016).

Antimicrobial therapy is the mainstay for treating typhoid fever caused by *Salmonella typhi*. It has still remained as unresolved public health problem and fatal in 30% cases because of the complications associated with appropriate antibiotic therapy. The choice of antibiotic is limited to treat typhoid due to the development of resistance by this organism. Treatment failure cases have increased as the susceptibility to the antibiotics have decreased (Dutta shanta, 2014).

Antibiotic treatment regimen should start timely with appropriate therapy to prevent complications and death. Therefore, there is an urgent need to guide appropriate empirical treatment with antimicrobials to improve patient outcomes. (Rachel D. *et al*, 2016)

In addition there is an immediate need to develop newer classes of antibiotic because antibiotic resistant has become an urgent threat to individual patient.

To our knowledge, no study has been carried out to find out the antibiotic sensitivity pattern either alone or combination antibiotic therapy against the clinical resistant pathogen. Therefore, we conducted this study to find out the antibiotic sensitivity against the resistant organsims after performing co-culture with sensitive-sensitive strains and sensitive-resistance strains.

1.2 *Acinetobacter baumannii*

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteo bacteria. *Acinetobacter* species are oxidase-negative and nonmotile, and occur in pairs under magnification. They are important soil organisms, where they contribute to the mineralization of, for example, aromatic compounds. *Acinetobacter* species are a key source of infection in debilitated patients in the hospital, in particular the species *Acinetobacter baumannii* (Abbo, 2007).

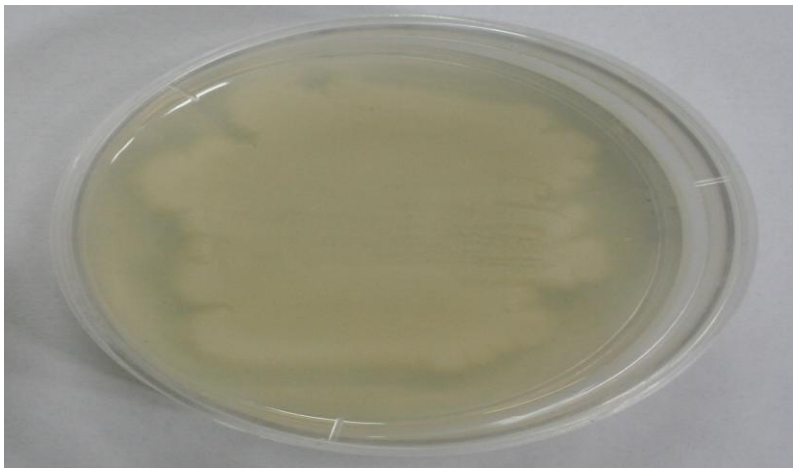

Figure 1.1: *Acinetobacter baumannii*

1.2.1 Etymology:

Acinetobacter is a compound word from scientific Greek [α + κίνητο + βακτηρ(ία)], meaning nonmotile rod. The first element acineto- appears as a somewhat baroque rendering of the Greek morpheme ακίνητο-, commonly transliterated in English is akineto-, but actually stems from the French cinetique and was adopted directly into English.

1.2.2 Description:

Species of the genus *Acinetobacter* are strictly aerobic, nonfermentative, Gram-negative bacilli. They show mostly a coccobacillary morphology on nonselective agar. Rods predominate in fluid media, especially during early growth. The morphology of *Acinetobacter* species can be quite variable in Gram-stained human clinical specimens, and cannot be used to differentiate *Acinetobacter* from other common causes of infection. Most strains of *Acinetobacter*, except some of the *A. lwoffii* strain, grow well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting, they are often partially lactose-fermenting when grown on MacConkey agar. They are oxidase-negative, nonmotile, and usually nitrate-negative. Bacteria of the genus *Acinetobacter* are known to form intracellular inclusions of polyhydroxyalkanoates under certain environmental conditions (e.g. lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources) (Abbo, 2007).

1.2.3 Identification:

Identification of *Acinetobacter* species is complicated by lack of standard identification techniques. Initially, identification was based on phenotypic characteristics such as growth temperature, colony morphology, growth medium, carbon sources, gelatin hydrolysis, glucose fermentation, among others. This method allowed identification of *A. calcoaceticus–A. baumannii* complex by the formation of smooth, rounded, mucoid colonies at 37°C. Closely related species could not be differentiated and individual species such as *A. baumannii* and *Acinetobacter* genomic species could not be positively identified phenotypically. Because routine identification in the clinical microbiology laboratory is not yet possible, *Acinetobacter* isolates are divided and grouped into three main complexes.

1.2.4 Clinical significance:

Acinetobacter is frequently isolated in nosocomial infections, and is especially prevalent in intensive care units, where both sporadic cases and epidemic and endemic occurrences are common. *A. baumannii* is a frequent cause of hospital-acquired pneumonia, especially of late-onset, ventilator-associated pneumonia. It can cause various other infections, including skin and wound infections, bacteremia, and meningitis, but *A. lwoffi* is mostly responsible for the latter. Of the *Acinetobacter*, *A. baumannii* is the greatest cause of human disease, having been implicated in a number of hospital-acquired infections such as bacteremia, urinary tract infections (UTIs), secondary meningitis, infective endocarditis, and wound and burn infections. In particular, *A. baumannii* is frequently isolated as the cause of hospital-acquired pneumonia among patients admitted to the intensive care unit. Risk factors include long-term intubation and tracheal or lung aspiration. In most cases of ventilator-associated pneumonia, the equipment used for artificial ventilation such as endotracheal tubes or bronchoscopes serve as the source of infection and result in the colonization of the lower respiratory tract by *A. baumannii*. In some cases, the bacteria can go on to enter the bloodstream, resulting in bacteremia with mortality rates ranging from 32% to 52%. UTIs caused by *A. baumannii* appear to be associated with continuous catheterization, as well as antibiotic therapy. *A. baumannii* has also been reported to infect skin and soft tissue in traumatic injuries and postsurgical wounds. *A. baumannii* commonly infects burns and may result in complications owing to difficulty in treatment and eradication. Though less common, some evidence also links this bacterium to meningitis, most often following invasive surgery, and in very rare cases, to community-acquired primary meningitis wherein the majority of the victims were children. Case reports also link *A. baumannii* to endocarditis, keratitis, peritonitis, and very rarely fatal neonatal sepsis. The clinical significance of *A. baumannii* is partially due to its capacity to develop resistance against many available antibiotics. Reports indicate that it possesses resistance against broad-spectrum cephalosporins, β-lactam antibiotics, aminoglycosides, and quinolones. Resistance to carbapenems is also being increasingly reported. *A. baumannii* can survive on the human skin or dry surfaces for weeks, and is resistant to a variety of disinfectants, making it particularly easy to spread in a hospital setting. Antibiotic resistance genes are often plasmid-borne, and plasmids present in *Acinetobacter* strains can be transferred to other pathogenic bacteria by horizontal

gene transfer. In healthy individuals, *Acinetobacter* colonies on the skin correlate with low incidence of allergies; *Acinetobacter* is thought to be allergy-protective (Abbo, 2007).

1.2.5 Treatment:

Acinetobacter species are innately resistant to many classes of antibiotics, including penicillin, chloramphenicol, and often aminoglycosides. Resistance to fluoroquinolones has been reported during therapy, which has also resulted in increased resistance to other drug classes mediated through active drug efflux. A dramatic increase in antibiotic resistance in *Acinetobacter* strains has been reported by the Centers for Disease Control and Prevention (CDC), and the carbapenems are recognised as the gold-standard and treatment of last resort. *Acinetobacter* species are unusual in that they are sensitive to sulbactam, which is commonly used to inhibit bacterial beta-lactamase, but this is an example of the antibacterial property of sulbactam itself. Gene-silencing antisense oligomers in a form called peptide-conjugated phosphorodiamidate morpholino oligomers have also been reported to inhibit growth in tests carried out in animals infected with antibiotic-resistant *A. baumanii* (Abbo,2007).

1.2.6 Species Identification:

*Acinetobacter*s may be identified presumptively to the genus level as gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli. They are short, plump, gram-negative rods that are difficult to destain and may therefore be misidentified as either gram-negative or gram-positive cocci (hence the former designation Mimae). *Acinetobacter* species of human origin grow well on solid media that are routinely used in clinical microbiology laboratories, such as sheep blood agar or tryptic soy agar, at a 37°C incubation temperature. These organisms form smooth, sometimes mucoid, grayish white colonies; colonies of the *A. calcoaceticus-A. baumannii* complex resemble those of Enterobacteriaceae, with a colony diameter of 1.5 to 3 mm after overnight culture, while most of the other *Acinetobacter* species produce smaller and more translucent colonies. Unlike the Enterobacteriaceae,some *Acinetobacter* species outside the *A. calcoaceticus-A. baumannii* complex may not grow on McConkey agar. Isolates of the species A. haemolyticus and several other currently not-well-defined species, such as *Acinetobacter* genomic species 6, 13BJ, 14BJ, 15BJ, 16, and 17, may show hemolysis on sheep blood agar, a property that is never present in *Acinetobacter* isolates

belonging to the *A. calcoaceticus-A. baumannii* complex. Unfortunately, no single metabolic test distinguishes *Acinetobacter*s from other similar nonfermenting gram-negative bacteria. A reliable method for unambiguous identification of *Acinetobacter*s to the genus level is the transformation assay of Juni, which is based on the unique property of mutant *Acinetobacter* strain BD413 trpE27, a naturally transformable tryptophan auxotroph recently identified as A. baylyi to be transformed by crude DNA of any *Acinetobacter* species to a wild-type phenotype. For the recovery of *Acinetobacter*s from environmental and clinical specimens (e.g., skin swabs to detect skin colonization), enrichment culture at low pH in a vigorously aerated liquid mineral medium supplemented with acetate or another suitable carbon source and with nitrate as the nitrogen source has proven useful. To facilitate the isolation of *Acinetobacter*s from mixed bacterial populations, Leeds *Acinetobacter* medium was proposed. Of the few methods that have been validated for identification of *Acinetobacter* species, DNA-DNA hybridization remains the reference standard. The phenotypic identification scheme proposed by Bouvet and Grimont in 1986 is based on 28 phenotypic tests. This identification scheme was refined in 1987 by the same authors and includes growth at 37°C, 41°C, and 44°C; production of acid from glucose; gelatin hydrolysis; and assimilation of 14 different carbon sources. While this simplified identification scheme allows discrimination between 11 of the 12 genomic species initially described and correctly identified to the species level 95.6% of 136 *Acinetobacter* isolates recovered from human skin samples it does not permit identification of the more recently described (genomic) species. In particular, the closely related and clinically most relevant species *A. baumannii* and *Acinetobacter* genomic species 13TU cannot be distinguished, while *A. calcoaceticus* and *Acinetobacter* genomic species 3 can only be separated by their growth properties at different temperatures. Unfortunately, simple phenotypic tests that are commonly used in routine diagnostic laboratories for identification of other bacterial genera to the species level are unsuitable for unambiguous identification of even the most common *Acinetobacter* species.

Both DNA-DNA hybridization and the phenotypic identification system of Bouvet and Grimont are laborious and far from being suitable for routine microbiology laboratories. In fact, these methods are available in only a few reference laboratories worldwide. Molecular methods that have been developed and validated for identification of *Acinetobacter*s include amplified 16S rRNA gene restriction analysis (ARDRA) high-resolution fingerprint analysis by amplified fragment length polymorphism (AFLP) ribotyping tRNA spacer fingerprinting restriction

analysis of the 16S-23S rRNA intergenic spacer sequences sequence analysis of the 16S-23S rRNA gene spacer region and sequencing of the rpoB (RNA polymerase β-subunit) gene and its flanking spacers ARDRA and AFLP analysis are currently the most widely accepted and validated reference methods for species identification of *Acinetobacter*s, with a large library of profiles available for both reference and clinical strains, while tRNA fingerprinting, though generally also suitable for species identification, does not discriminate between *A. baumannii* and *Acinetobacter* genomic species 13TU. Both ribotyping and sequence analysis of the 16S-23S rRNA gene spacer region were found to discriminate between species of the *A. calcoaceticus-A. baumannii* complex but have not been applied to other *Acinetobacter* species, and sequencing of the rpoB gene, although very promising, awaits further validation. All of these methods have contributed to a better understanding of the epidemiology and clinical significance of *Acinetobacter* species during recent years, but they are too laborious to be applied in day-to-day diagnostic microbiology, and their use for the time being is also confined mainly to reference laboratories. More recent developments include the identification of *A. baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species PCR-electrospray ionization mass spectrometry and a simple PCR-based method described by Higgins et al. That exploits differences in their respective gyrB genes to rapidly differentiate between *A. baumannii* and *Acinetobacter* genomic species 13TU. Promising results with matrix-assisted laser desorption ionization-time-of-flight MS have been obtained for species identification of 552 well-characterized *Acinetobacter* strains representing 15 different species Matrix-assisted laser desorption ionization-time-of-flight MS allows for species identification in less than 1 hour, but it requires expensive equipment and needs further evaluation. Species identification with manual and semiautomated commercial identification systems that are currently used in diagnostic microbiology, such as the API 20NE, Vitek 2, Phoenix, and MicroScan WalkAway systems, remains problematic. This can be explained in part by their limited database content but also because the substrates used for bacterial species identification have not been tailored specifically to identify *Acinetobacter*s. Particularly,the three clinically relevant members of the *A. calcoaceticus-A. baumannii* complex cannot be separated by currently available commercial identification systems; in fact, *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU are uniformly identified as *A. baumannii* by the most widely used identification systems. In referring to these species, it therefore seems appropriate to

use the term *A. baumannii* group instead of *A. calcoaceticus-A. baumannii* complex. This reflects the fact that *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU share important clinical and epidemiological characteristics and also eliminates the confusion resulting from inclusion of an environmental species, A. calcoaceticus. However, since the vast majority of studies that have addressed epidemiological and clinical issues related to *Acinetobacter* have not employed identification methods that allow for unambiguous species identification within the *A. baumannii* group, the designation *A. baumannii* in this review, if not stated otherwise, is used in a broader sense to also accommodate *Acinetobacter* genomic species 3 and 13TU. The need for species identification of *Acinetobacter*s in routine clinical laboratories has been questioned by some researchers. From a clinical and infection control point of view, however, it is necessary to distinguish between the *A. baumannii* group and *Acinetobacter*s outside the *A. baumannii* group since the latter organisms rarely have infection control implications. In addition, these organisms are usually susceptible to a range of antimicrobials, and infections caused by these organisms are most often benign. From a research perspective, in contrast, clinical studies using proper methods for species identification of *Acinetobacter*s, including those within the *A. baumannii* group, are mandatory to increase our knowledge of the epidemiology, pathogenicity and clinical impact of the various species of this diverse genus (Abbo, 2007).

1.2.7 ANTIBIOTIC RESISTANCE:

β-Lactams:

Enzymatic mechanisms:

The most prevalent mechanism of β-lactam resistance in *A. baumannii* is enzymatic degradation by β-lactamases. However, in keeping with the complex nature of this organism, multiple mechanisms often work in concert to produce the same phenotype Inherent to all *A. baumannii* strains are chromosomally encoded AmpC cephalosporinases also known as *Acinetobacter*-derived cephalosporinases (ADCs). Unlike that of AmpC enzymes found in other gram-negative organisms, inducible AmpC expression does not occur in *A. baumannii*. The key determinant regulating overexpression of this enzyme in *A. baumannii* is the presence of an upstream IS element known as ISAba1 (described below). The presence of this element highly correlates with

increased AmpC gene expression and resistance to extended-spectrum cephalosporins. Cefepime and carbapenems appear to be stable in response to these enzymes.

Aminoglycosides:

The presence of genes coding for aminoglycoside-modifying enzymes within class 1 integrons is highly prevalent in multidrug-resistant *A. baumannii* strains). All of the major enzyme classes have been described, including acetyltransferases, nucleotidyltransferases, and phosphotransferases More recently, 16S rRNA methylation has been described for *A. baumannii* (armA) strains from Japan, Korea, and the United States.This emerging resistance mechanism impairs aminoglycoside binding to its target site and confers high-level resistance to all clinically useful aminoglycosides, including gentamicin, tobramycin, and amikacin. Interestingly, the genetic surroundings of armA appear very similar across gram-negative organisms, as it is plasmid borne and within a transposon.

Apart from the AdeABC efflux pump, which less effectively transports amikacin and kanamycin due to their more hydrophilic nature aminoglycosides (gentamicin and kanamycin) are also substrates of the recently described AbeM pump, a member of the multidrug and toxic compound extrusion (MATE) family (Afzal-Shah,2001).

Quinolones:

Modifications to DNA gyrase or topoisomerase IV through mutations in the gyrA and parC genes have been well described for *A. baumannii*. These mutations interfere with target site binding. Similar to aminoglycosides, many quinolones are also substrates for multidrug efflux pumps including the RND-type pump AdeABC and the MATE pump AdeM. Thus far, plasmid-mediated quinolone resistance, mediated by qnr genes, has not been reported for *A. baumannii*.

Tetracyclines and Glycylcyclines:

Resistance to tetracyclines and their derivatives can be mediated by efflux or ribosomal protection. Tetracycline-specific efflux pumps include those encoded by the tet(A) to tet(E) determinants, most often found within gram-negative organisms, and the tet(K) determinant found in Staphylococcus aureus. Thus far, the tet(A) and tet(B) determinants have been described for *A. baumannii*. tet(A) was found within a transposon similar to Tn1721, in

association with an IS element. tet(A) confers resistance to tetracycline but not minocycline, an agent with greater activity against *A. baumannii*. Ribosomal protection is mediated by the tet(M) and tet(O) determinants, with tet(M) being described rarely for *A. baumannii*. Interestingly, this tet(M) determinant was identical to that described for S. aureus.

Apart from tetracycline-specific efflux pumps, this class of antimicrobials is also susceptible to efflux by the multidrug efflux systems, such as the AdeABC pump. Importantly, tigecycline, which is the first of a new class of modified tetracycline antimicrobials known as glycylcyclines, is also a substrate for this emerging efflux system. By performing real-time PCR with the adeB gene in clinical and laboratory exposed isolates with increased MICs of tigecycline, increased adeB gene expression was identified. It was of concern that the rise in MIC of tigecycline occurred rapidly with in vitro passage, suggesting that the expression of this multidrug efflux pump can be upregulated swiftly in response to selective pressure. The role of the AdeABC efflux pump in reduced susceptibility to tigecycline was confirmed by insertional inactivation of the adeB gene, which led to a significant drop in the MIC of tigecycline (4 μg/ml to 0.5 μg/ml). These data suggest that caution should be used in considering tigecycline treatment for *A. baumannii* infection in sites where drug levels may be suboptimal, such as the bloodstream.

Polymyxins:

Despite recent reports demonstrating increase in vitro resistance and heteroresistance to the polymyxins in A. baumanni. The mechanism of resistance remains unknown. It has previously been shown that reduced binding to the lipopolysaccharide (LPS) target site can lead to resistance in *E.coli, Salmonella spp*., and *P. aeruginosa* .Also, changes in OMPs causing reduced susceptibility to polymyxins have been described for *P. aeruginosa* (Afzal Shah, 2001).

Other Antibiotics:

The prevalence of trimethoprim-sulfamethoxazole resistance in *A. baumannii* is high in many geographic regions. As discussed above, integrons are very common among strains of *A. baumannii* that have a multidrug resistance phenotype. The 3′-conserved region of an integron most commonly contains a qac gene fused to a sul gene, conferring resistance to antiseptics and sulfonamides, respectively. Consequently, sulfonamide resistance has been shown to be highly predictive of integron-carrying strains of *A. baumannii*. Similarly, genes coding for trimethoprim

(dhfr) and chloramphenicol (cat) resistance have also been reported within integron structures in *A. baumannii*. Efflux may also contribute to resistance against these agents.

1.2.8 CLINICAL MANIFESTATIONS OF *ACINETOBACTER BAUMANNII* INFECTIONS:

In the vast majority of publications on the clinical manifestations of *Acinetobacter* infections, the methods used for species identification were not appropriate according to current standards. However, with an acceptable level of uncertainty, we can assume that what has been published on nosocomial *Acinetobacter* infection in general, or on *A. baumannii* infection in particular, is indeed applicable to *A. baumannii*. Case reports or small series on clinical manifestations of infections caused by *Acinetobacter* infections outside the *A. baumannii* group should be interpreted with caution if (semi)-automated methods for species identification were employed.

Hospital-Acquired Pneumonia:

In most institutions, the majority of *A. baumannii* isolates are from the respiratory tracts of hospitalized patients. In many circumstances, it is very difficult to distinguish upper airway colonization from true pneumonia. There is no doubt, however, that true ventilator-associated pneumonia (VAP) due to *A. baumannii* occurs. In large surveillance studies from the United States, between 5 and 10% of cases of ICU-acquired pneumonia were due to *A. baumannii*. However, it is highly likely that in certain institutions, the proportion of ICU-acquired pneumonia due to *A. baumannii* is much higher. Typically, patients with *A. baumannii* infections have had prolonged ICU stays although in outbreak situations, earlier acquisition of infection may occur.

Community-Acquired Pneumonia

Community-acquired pneumonia due to *A. baumannii* has been described for tropical regions of Australia and Asia. The disease most typically occurs during the rainy season among people with a history of alcohol abuse and may sometimes require admission to an ICU. It is characterized by a fulminant clinical course, secondary bloodstream infection, and mortality rate of 40 to 60%. The source of infection may be throat carriage, which occurs in up to 10% of community residents with excessive alcohol consumption.

Bloodstream Infection:

In a large study of nosocomial bloodstream infection in the United States *A. baumannii* was the 10th most common etiologic agent, being responsible for 1.3% of all monomicrobial nosocomial bloodstream infections (0.6 bloodstream infection per 10,000 admissions). *A. baumannii* was a more common cause of ICU-acquired bloodstream infection than of non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections, respectively, in those locations). Crude mortality overall from *A. baumannii* bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU. *A. baumannii* bloodstream infection had the third highest crude mortality rate in the ICU, exceeded only by *P. aeruginosa* and Candida sp. infections. *A. baumannii* infections were the latest of all bloodstream infections to occur during hospitalization, occuring a mean of 26 days from the time of hospital admission. It is therefore not certain if the high crude mortality rate represents its occurrence in patients with ongoing underlying critical illness or whether the organism does have significant attributable mortality Sources of bloodstream infection were not described in the study mentioned above but are typically line related or attributed to underlying pneumonia, UTI, or wound infection. It is notable that 102 patients had bloodstream infections at sites treating U.S. military members injured in Iraq or Afghanistan from 1 January 2002 and 31 August 2004.

Urinary Tract Infection:

Acinetobacter baumannii is an occasional cause of UTI, being responsible for just 1.6% of ICU-acquired UTIs in one study (187). Typically, the organism is associated with catheter-associated infection or colonization. It is not usual for this organism to cause uncomplicated UTI in healthy outpatients.

Meningitis:

Nosocomial, postneurosurgical *A. baumannii* meningitis is an increasingly important entity. The microbial epidemiology of nosocomial meningitis is evolving to include more gram-negative pathogens so it is not surprising that multidrug-resistant *A. baumannii* is among the pathogens implicated. Typical patients have undergone neurosurgery and have an external ventricular drain. Mortality may be as high as 70%, although the cause of mortality is often difficult to discern (Afzal-Shah, 2001).

1.3 *Salmonella typhi*:

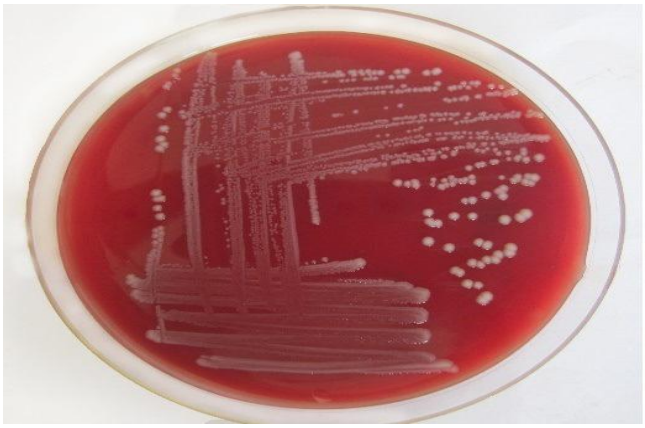

Figure 1.2: *Salmonella typhi*

1.3.1 Introduction:

Worldwide, typhoid fever affects roughly 17 million people annually, causing nearly 600,000 deaths. The causative agent, *Salmonella typhi* , is an obligate parasite that has no known natural reservoir outside of humans. Little is known about the historical emergence of human *S. typhi* infections, however it is thought to have caused the deaths of many famous figures such as British author and poet Rudyard Kipling, the inventor of the airplane, Wilbur Wright, and the Greek Empire's Alexander the Great. The earliest recorded epidemic occurred in Jamestown, VA where it is thought that 6,000 people died of typhoid fever in the early 17th Century. This disease is rare in the United States and developed nations, but always poses the risk of emergence

(Wain J., 2015).

1.3.2 History:

Originally isolated in 1880 by Karl J. Erberth, *S. Typhi* is a multi-organ pathogen that inhabits the lympathic tissues of the small intestine, liver, spleen, and bloodstream of infected humans. It is not known to infect animals and is most common in developing countries with poor sanitary systems and lack of antibiotics, putting travelers to Asia, Latin America, and Africa in a high risk group. Of the 266 people infected in the United States in 2002, approximately 70% had traveled internationally within 6 weeks of the onset of disease (Wain J., 2015).

1.3.3 Microbiological Characteristics:

This gram-negative enteric bacillus belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobe that is susceptible to various antibiotics. Currently, 107 strains of this organism have been isolated, many containing varying metabolic characteristics, levels of virulence, and multi-drug resistance genes that complicate treatment in areas that resistance is prevalent. Diagnostic identification can be attained by growth on MacConkey and EMB agars, and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in TSI media, which is used to differentiate it from other Enterobacteriaceae.

1.3.4 Typhoid/ Enteric Fever:

Infection of *S. typhi* leads to the development of typhoid, or enteric fever. This disease is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea, and loss of appetite. Other symptoms include constipation or diarrhea, enlargement of the spleen, possible development of meningitis, and/or general malaise. Untreated typhoid fever cases result in mortality rates ranging from 12-30% while treated cases allow for 99% survival.

1.3.5 Signs and symptoms:

Classically, the course of untreated typhoid fever is divided into four distinct stages, each lasting about a week. Over the course of these stages, the patient becomes exhausted and emaciated.

- In the first week, the body temperature rises slowly, and fever fluctuations are seen with relative bradycardia (Faget sign), malaise, headache, and cough. A bloody nose (epistaxis) is seen in a quarter of cases, and abdominal pain is also possible. A decrease in the number of circulating white blood cells (leukopenia) occurs with eosinopenia and relative lymphocytosis; blood cultures are positive for *Salmonella typhi* or S. para*typhi*. The widal test is negative in the first week.
- In the second week, the person is often too tired to get up, with high fever in plateau around 40 °C (104 °F) and bradycardia (sphygmothermic dissociation or Faget sign), classically with a dicrotic pulse wave. Delirium is frequent, often calm, but sometimes agitated. This delirium gives to typhoid the nickname of "nervous fever". Rose spots

appear on the lower chest and abdomen in around a third of patients. Rhonchi are heard in lung bases.

- The abdomen is distended and painful in the right lower quadrant, where borborygmi can be heard. Diarrhea can occur in this stage: six to eight stools in a day, green, comparable to pea soup, with a characteristic smell. However, constipation is also frequent. The spleen and liver are enlarged (hepatosplenomegaly) and tender and liver transaminases are elevated. The widal test is strongly positive, with antiO and antiH antibodies. Blood cultures are sometimes still positive at this stage.
- In the third week of typhoid fever, a number of complications can occur:
  - Intestinal haemorrhage due to bleeding in congested Peyer's patches; this can be very serious, but is usually not fatal.
  - Intestinal perforation in the distal ileum: this is a very serious complication and is frequently fatal. It may occur without alarming symptoms until septicaemia or diffuse peritonitis sets in.
  - Encephalitis
  - Respiratory diseases such as pneumonia and acute bronchitis
  - Neuropsychiatric symptoms (described as "muttering delirium" or "coma vigil"), with picking at bedclothes or imaginary objects.
  - Metastatic abscesses, cholecystitis, endocarditis, and osteitis
  - The fever is still very high and oscillates very little over 24 hours. Dehydration ensues, and the patient is delirious (typhoid state). One-third of affected individuals develop a macular rash on the trunk.
  - Platelet count goes down slowly and risk of bleeding rises.
  - By the end of third week, the fever starts subsiding (Wain J.,2015).

1.3.6 Cause:

Transmission:

A 1939 conceptual illustration showing various ways that typhoid bacteria can contaminate a water well (center)

The bacterium that causes typhoid fever may be spread through poor hygiene habits and public sanitation conditions, and sometimes also by flying insects feeding on feces. Public education campaigns encouraging people to wash their hands after defecating and before handling food are an important component in controlling spread of the disease. According to statistics from the United States Centers for Disease Control and Prevention (CDC), the chlorination of drinking water has led to dramatic decreases in the transmission of typhoid fever in the United States

(Anna E., 2014).

Bacteria:

The cause is the bacterium *Salmonella typhi*, also known as *Salmonella* enterica serotype *typhi*. There are two main types of *typhi* namely the ST1 and ST2 based on MLST subtyping scheme, which are currently widespread globally.

1.3.7 Diagnosis:

Diagnosis is made by any blood, bone marrow or stool cultures and with the Widal test (demonstration of antibodies against *Salmonella* antigens O-somatic and H-flagellar). In epidemics and less wealthy countries, after excluding malaria, dysentery, or pneumonia, a therapeutic trial time with chloramphenicol is generally undertaken while awaiting the results of the Widal test and cultures of the blood and stool. The widal test is time-consuming, and often, when a diagnosis is reached, it is too late to start an antibiotic regimen. The term 'enteric fever' is a collective term that refers to severe typhoid and paratyphoid.

1.3.8 Treatment:

The rediscovery of oral rehydration therapy in the 1960s provided a simple way to prevent many of the deaths of diarrheal diseases in general. Where resistance is uncommon, the treatment of

choice is a fluoroquinolone such as ciprofloxacin. Otherwise, a third-generation cephalosporin such as ceftriaxone or cefotaxime is the first choice. Cefixime is a suitable oral alternative. Typhoid fever, when properly treated, is not fatal in most cases. Antibiotics, such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, amoxicillin, and ciprofloxacin, have been commonly used to treat typhoid fever in microbiology. Treatment of the disease with antibiotics reduces the case-fatality rate to about 1%.Death occurs in 10% to 30% of untreated cases. In some communities, however, case-fatality rates may reach as high as 47%.

1.3.9 Resistance:

As resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and streptomycin is now common, these agents have not been used as first–line treatment of typhoid fever for almost 20 years. Typhoid resistant to these agents is known as multidrug-resistant typhoid (MDR typhoid). Ciprofloxacin resistance is an increasing problem, especially in the Indian subcontinent and Southeast Asia. Many centres are shifting from using ciprofloxacin as the first line for treating suspected typhoid originating in South America, India, Pakistan, Bangladesh, Thailand, or Vietnam. For these people, the recommended first-line treatment is ceftriaxone. Also, azithromycin has been suggested to be better at treating typhoid in resistant populations than both fluoroquinolone drugs and ceftriaxone. Azithromycin significantly reduces relapse rates compared with ceftriaxone. A separate problem exists with laboratory testing for reduced susceptibility to ciprofloxacin: current recommendations are that isolates should be tested simultaneously against ciprofloxacin (CIP) and against nalidixic acid (NAL), and that isolates that are sensitive to both CIP and NAL should be reported as "sensitive to ciprofloxacin", but that isolates testing sensitive to CIP but not to NAL should be reported as "reduced sensitivity to ciprofloxacin". However, an analysis of 271 isolates showed that around 18% of isolates with a reduced susceptibility to ciprofloxacin (MIC 0.125–1.0 mg/l) would not be picked up by this method. How this problem can be solved is not certain, because most laboratories around the world (including the West) are dependent on disk testing and cannot test for MICs(Anna E., 2014).

1.3.10 Virulence Factors:

S. typhi has a combination of characteristics that make it an effective pathogen. This species contains an endotoxin typical of Gram negative organisms, as well as the VI antigen which is thought to increase virulence. It also produces and excretes a protein known as "invasin" that allows non-phagocytic cells to take up the bacterium, where it is able to live intracellularly. It is also able to inhibit the oxidative burst of leukocytes, making innate immune response ineffective.

1.3.11 Epidemiology:

The encounter of humans to *S. typhi* is made via fecal-oral route from infected individuals to healthy ones. Poor hygiene of patients shedding the organism can lead to secondary infection, as well as consumption of shellfish from polluted bodies of water. The most common source of infection, however, is drinking water tainted by urine and feces of infected individuals. The estimated inoculum size necessary for infection is 100,000 bacteria. Typhoid fever also represents the second most commonly reported laboratory infection. The entry of this bacterial species into the human body is most commonly achieved by ingestion, with the importance of aerosol transmission unknown. Once ingested, the organisms multiply in the small intestine over the period of 1-3 weeks, breech the intestinal wall, and spread to other organ systems and tissues. The innate host defenses do little to prevent infection due to the inhibition of oxidative lysis and the ability to grow intracellularly after uptake. Transmission of *S. Typhi* has only been shown to occur by fecal-oral route, often from asymptomatic individuals. 2-5% of previously infected individuals become chronic carriers who show no signs of disease, but actively shed viable organisms capable of infecting others. A famous example is "Typhoid" Mary Mallon, who was a food handler responsible for infecting at least 78 people, killing 5. These highly infectious carriers pose a great risk to public health due to their lack of disease-related symptoms. The damage caused by typhoid fever is reversible and limited if treatment is started early in the infection. This leads to a mortality rate of less than 1% among treated individuals who have an antibiotic-susceptible strain of S. *Typhi*, making the outcome and prognosis for patients a positive one (Anna E., 2014).

1.3.12 Prevention:

The key to avoid infection by *S. Typhi* is prevention of fecal contamination in drinking water and food supplies. These measures are attained in developed societies, attributing to the low incidence. The United States has an average of around 400 infections annually, almost exclusively among people who have recently traveled to developing countries. Prevention can also be aided by vaccination to the bacteria, however the effectiveness of this has been questionable. In addition, it is shown that large inoculums sizes can overwhelm the developed immunity and result in disease. Typhoid fever has played a significant role in history. This pathogen thrives in developing societies or areas where disasters have compromised sanitation. Although the incidence in the United States is very low, outbreaks and substantial epidemics still remain possible due to worldwide travel and unknowing carriers of the disease. The development of antibiotic treatments and several vaccines have presented the possibility of worldwide eradication. Until this is achieved, however, *S. typhi* and its characteristic typhoid fever will remain a threat for future epidemics (Wain J., 2015).

1.4 *Shigella flexneri*

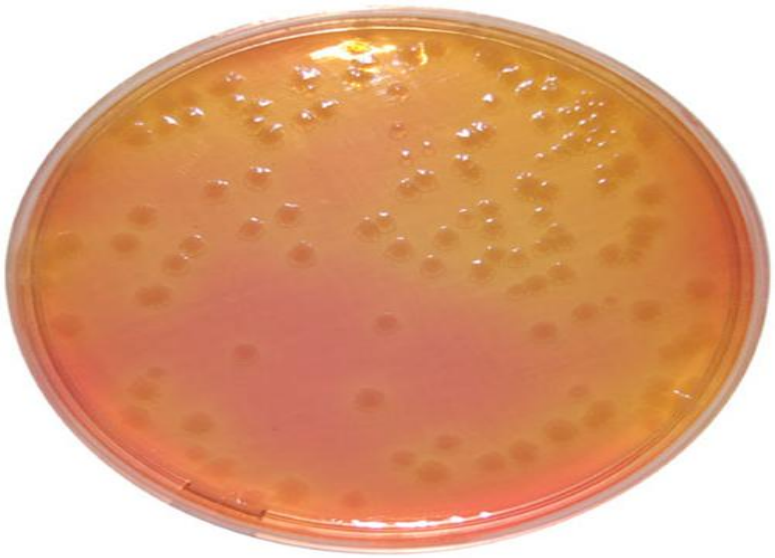

Figure 1.3: *Shigella flexneri*

Shigella (/ʃɪˈgɛlə/) is a genus of Gram-negative, facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacteria closely related to *Salmonella*. The genus is named after Kiyoshi Shiga, who first discovered it in 1897. The causative agent of human shigellosis, *Shigella* causes disease in primates, but not in other mammals. It is only naturally found in humans and gorillas. During infection, it typically causes dysentery. *Shigella* is one of the leading bacterial causes of diarrhea worldwide, causing an estimated 80-165 million cases. The number of deaths it causes each year is estimated at between 74,000 and 600,000 deaths. It is in the top four pathogens that cause moderate-to-severe diarrhea in African and South Asian children. (Yabuuchi, 2002)

1.4.1Classification:

Shigella species are classified by four serogroups:

- Serogroup A: *S. dysenteriae* (15 serotypes)
- Serogroup B: *S. flexneri* (six serotypes)
- Serogroup C: *S. boydii* (19 serotypes)
- Serogroup D: *S. sonnei* (one serotype)

Groups A–C are physiologically similar; *S. sonnei* (group D) can be differentiated on the basis of biochemical metabolism assays. Three *Shigella* groups are the major disease-causing species: *S. flexneri* is the most frequently isolated species worldwide, and accounts for 60% of cases in the developing world; *S. sonnei* causes 77% of cases in the developed world, compared to only 15% of cases in the developing world; and *S. dysenteriae* is usually the cause of epidemics of dysentery, particularly in confined populations such as refugee camps. Each of the *Shigella* genomes includes a virulence plasmid that encodes conserved primary virulence determinants. The *Shigella* chromosomes share most of their genes with those of E. coli K12 strain MG1655. Phylogenetic studies indicate *Shigella* is more appropriately treated as subgenus of Escherichia, and that certain strains generally considered E. coli – such as E. coli O157:H7 – are better placed in *Shigella* (Yabuuchi, 2002).

1.4.2 Pathogenesis:

Shigella infection is typically by ingestion. Depending on the health of the host, fewer than 100 bacterial cells can be enough to cause an infection. *Shigella* species generally invade the epithelial lining of the colon, causing severe inflammation and death of the cells lining the colon. This inflammation results in the diarrhea and even dysentery that are the hallmarks of *Shigella* infection. Some strains of *Shigella* produce toxins which contribute to disease during infection. *S. flexneri* strains produce ShET1 and ShET2 which may contribute to diarrhea. *S. dysenteriae* strains produce the enterotoxin Shiga toxin, which is similar to the verotoxin produced by Enterohemorrhagic E. coli. Both shiga toxin and verotoxin are associated with causing potentially-fatal hemolytic uremic syndrome. *Shigella* species invade the host through the M-cells interspersed in the gut epithelia of the small intestine, as they do not interact with the apical surface of epithelial cells, preferring the basolateral side. *Shigella* uses a type-III secretion system, which acts as a biological syringe to translocate toxic effector proteins to the target human cell. The effector proteins can alter the metabolism of the target cell, for instance leading to the lysis of vacuolar membranes or reorganization of actin polymerization to facilitate intracellular motility of *Shigella* bacteria inside the host cell. For instance, the IcsA effector protein triggers actin reorganization by N-WASP recruitment of Arp2/3 complexes, helping cell-to-cell spread. After invasion, *Shigella* cells multiply intracellularly and spread to neighboring epithelial cells, resulting in tissue destruction and characteristic pathology of shigellosis. The most common symptoms are diarrhea, fever, nausea, vomiting, stomach cramps, and flatulence. It is also commonly known to cause large and painful bowel movements. The stool may contain blood, mucus, or pus. Hence, *Shigella* cells may cause dysentery. In rare cases, young children may have seizures. Symptoms can take as long as a week to appear, but most often begin two to four days after ingestion. Symptoms usually last for several days, but can last for weeks. *Shigella* is implicated as one of the pathogenic causes of reactive arthritis worldwide (Ryan, 2004).

1.4.3 Diagnosis:

The diagnosis of shigellosis is made by isolating the organism from diarrheal fecal sample cultures. *Shigella* species are negative for motility and are generally not lactose fermenters, but *S. sonnei* can ferment lactose. They typically do not produce gas from carbohydrates (with the exception of certain strains of S. flexneri) and tend to be overall biochemically inert. *Shigella*

should also be urea hydrolysis negative. When inoculated to a triple sugar iron (TSI) slant, they react as follows: K/A, gas -, and H2S -. Indole reactions are mixed, positive and negative, with the exception of *S. sonnei*, which is always indole negative. Growth on Hektoen enteric agar produces bluish-green colonies for *Shigella* and bluish-green colonies with black centers for *Salmonella* (Ryan, 2004).

1.4.4 Prevention and treatment:

Hand washing before handling food and thoroughly cooking all food before eating decreases the risk of getting shigellosis. Currently, no licensed vaccine targeting *Shigella* exists. *Shigella* has been a longstanding World Health Organization target for vaccine development. Sharp declines in age-specific diarrhea/dysentery attack rates for this pathogen indicate natural immunity does develop following exposure; thus, vaccination to prevent the disease should be feasible. Several vaccine candidates for *Shigella* are in various stages of development. Treatment mainly consists of replacing fluids and salts lost due to diarrhea. Severe dysentery can be treated with ampicillin, TMP-SMX, or fluoroquinolones, such as ciprofloxacin. Medical treatment should only be used in severe cases or for certain populations with mild symptoms (elderly, immunocompromised, food service industry workers, child care workers). For *Shigella*-associated diarrhea, antibiotics shorten the length of infection, but they are usually avoided in mild cases because many *Shigella* strains are becoming resistant to common antibiotics. Furthermore, effective medications are often in short supply in developing countries, which carry the majority of the disease burden from *Shigella*. Antidiarrheal agents may worsen the sickness, and should be avoided. Also extensive research has been conducted into therapies, involving treatment with bacteriophages

(Pond, 2005).

1.4.5 Symptoms of *Shigella flexneri*:

Symptoms of shigellosis typically start 1–2 days after exposure and include:

- Diarrhea (sometimes bloody)
- Fever
- Abdominal pain
- Tenesmus, a painful sensation of needing to pass stools even when bowels are empty

(Scallan E., 2011).

1.4.6 Diagnosis & Testing:

Many different kinds of germs can cause diarrhea, so establishing the cause will help guide treatment. Healthcare providers can order laboratory tests to identify *Shigella* in the stools of an infected person. The laboratory can also do special tests to determine which antibiotics, if any, would be best to treat the infection. (Scallan E., 2011)

1.4.7 Antibiotic Resistance:

In 2013, CDC declared antibiotic-resistant *Shigella* an urgent threat in the United States . Resistance to traditional first-line antibiotics like ampicillin and trimethoprim - sulfamethoxazole is common among *Shigella* globally, and resistance to some other important antibiotics is increasing. While travelers to the developing world are at particular risk of acquiring antibiotic-resistant shigellosis, outbreaks of shigellosis resistant to ciprofloxacin or azithromycin—the two antibiotics most commonly used to treat shigellosis—have been reported recently within the United States and other industrialized countries . About 27, 000 *Shigella* infections in the United States every year are resistant to one or both of these antibiotics.When pathogens are resistant to common antibiotic medications, patients may need to be treated with medications that may be less effective, but more toxic and expensive (Christopher PR, 2010).

1.4.8 Transmission:

Shigella germs are present in the stools of infected persons while they have diarrhea and for up to a week or two after the diarrhea has gone away. *Shigella* is very contagious; exposure to even a tiny amount of contaminated fecal matter—too small to see-- can cause infection. Transmission of *Shigella* occurs when people put something in their mouths or swallow something that has come into contact with stool of a person infected with *Shigella*. This can happen when:

- Contaminated hands touch your food or mouth. Hands can become contaminated through a variety of activities, such as touching surfaces (e.g., toys, bathroom fixtures, changing tables, diaper pails) that have been contaminated by stool from an infected person. Hands can also become contaminated with *Shigella* while changing the diaper of an infected child or caring for an infected person.
- Eating food contaminated with *Shigella*. Food may become contaminated if food handlers have shigellosis. Produce can become contaminated if growing fields contain human sewage. Flies can breed in infected feces and then contaminate food when they land on it.
- Swallowing recreational (for example lake or river water while swimming) or drinking water that was contaminated by infected fecal matter.
- Exposure to feces through sexual contact (Christopher PR, 2010).

1.4.9 People at Risk:

- Young children are the most likely to get shigellosis, but people from all age groups are affected 19. Many outbreaks are related to childcare settings and schools, and illness commonly spreads from young children to their family members and others in their communities because it is so contagious.
- Gay, bisexual, and other men who have sex with men (MSM) are more likely to acquire shigellosis than the general adult population. *Shigella* passes from stools or soiled fingers of one person to the mouth of another person, which can happen during sexual activity. Many shigellosis outbreaks among MSM have been reported in the United States, Canada, Tokyo, and Europe since 1999. HIV-infected persons can have more severe and prolonged shigellosis, including having the infection spread into the blood, which can be life-threatening.

- Large outbreaks of *Shigella* have occurred in traditionally observant Jewish communities documented outbreaks in traditionally observant Jewish communities often begin in childcare settings and spread within and between households during social gatherings.
- Travelers to developing countries may be more likely to get shigellosis, and to become infected with strains of *Shigella* that are resistant to important antibiotics Travelers may be exposed through contaminated food, water (both drinking and recreational water), or surfaces. Travelers can protect themselves by strictly following food and water precautions, and washing hands with soap frequently. For more information, see Travelers' Health - Food and Water Safety.

The term men who have sex with men is used in CDC surveillance systems because it indicates the behaviors that transmit *Shigella* infection, rather than how individuals self-identify in terms of their sexuality.

1.5 Antibiotic:

Antibiotics, also called antibacterials, are a type of antimicrobial drug used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity. Antibiotics are not effective against viruses such as the common cold or influenza, and their inappropriate use allows the emergence of resistant organisms. In 1928, Alexander Fleming identified penicillin, the first chemical compound with antibiotic properties. Fleming was working on a culture of disease-causing bacteria when he noticed the spores of a little green mold (*Penicillium chrysogenum*), in one of his culture plates. He observed that the presence of the mold killed or prevented the growth of the bacteria. Antibiotics revolutionized medicine in the 20th century, and have together with vaccination led to the near eradication of diseases such as tuberculosis in the developed world. Their effectiveness and easy access led to overuse, especially in livestock raising, prompting bacteria to develop resistance. This has led to widespread problems with antimicrobial and antibiotic resistance, so much as to prompt the World Health Organization to classify antimicrobial resistance as a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". The era of antibacterial treatment began with the discovery of arsphenamine, first synthesized by Alfred Bertheim and Paul Ehrlich in 1907, and used to treat

syphilis. The first systemically active antibacterial drug, prontosil was discovered in 1933 by Gerhard Domagk, for which he was awarded the 1939 Nobel Prize. All classes of antibiotics in use today were first discovered prior to the mid 1980s. Sometimes the term antibiotic is used to refer to any substance used against microbes, synonymous with antimicrobial, leading to the widespread but incorrect belief that antibiotics can be used against viruses. Some sources distinguish between antibacterial and antibiotic; antibacterials are used in soaps and cleaners generally and antibiotics are used as medicine (Tan, 2015).

1.5.1 Medical uses:

Antibiotics are used to treat or prevent bacterial infections, and sometimes protozoan infections. (Metronidazole is effective against a number of parasitic diseases). When an infection is suspected of being responsible for an illness but the responsible pathogen has not been identified, an empiric therapy is adopted. This involves the administration of a broad-spectrum antibiotic based on the signs and symptoms presented and initiated pending laboratory results that can take several days. When the responsible pathogenic microorganism is already known or has been identified, definitive therapy can be started. This will usually involve the use of a narrow-spectrum antibiotic. The choice of antibiotic given will also be based on its cost. Identification is critically important as it can reduce the cost and toxicity of the antibiotic therapy and also reduce the possibility of the emergence of antimicrobial resistance. To avoid surgery antibiotics may be given for non-complicated acute appendicitis. Effective treatment has been evidenced. Antibiotics may be given as a preventive measure (prophylactic) and this is usually limited to at-risk populations such as those with a weakened immune system (particularly in HIV cases to prevent pneumonia), those taking immunosuppressive drugs, cancer patients and those having surgery. Their use in surgical procedures is to help prevent infection of incisions made. They have an important role in dental antibiotic prophylaxis where their use may prevent bacteremia and consequent infective endocarditis. Antibiotics are also used to prevent infection in cases of neutropenia particularly cancer-related.

1.5.2 Resistance:

The emergence of resistance of bacteria to antibiotics is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibiotic therapy. The antibiotic treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant bacteria, while growth of susceptible bacteria is inhibited by the drug. For example, antibacterial selection for strains having previously acquired antibacterial-resistance genes was demonstrated in 1943 by the Luria–Delbruck experiment. Antibiotics such as penicillin and erythromycin, which used to have a high efficacy against many bacterial species and strains, have become less effective, due to the increased resistance of many bacterial strains. Resistance may take the form of biodegredation of pharmaceuticals, such as sulfamethazine-degrading soil bacteria introduced to sulfamethazine through medicated pig feces. The survival of bacteria often results from an inheritable resistance, but the growth of resistance to antibacterials also occurs through horizontal gene transfer. Horizontal transfer is more likely to happen in locations of frequent antibiotic use. Antibacterial resistance may impose a biological cost, thereby reducing fitness of resistant strains, which can limit the spread of antibacterial-resistant bacteria, for example, in the absence of antibacterial compounds. Additional mutations, however, may compensate for this fitness cost and can aid the survival of these bacteria. Several molecular mechanisms of antibacterial resistance exist. Intrinsic antibacterial resistance may be part of the genetic makeup of bacterial strains. For example, an antibiotic target may be absent from the bacterial genome. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. Antibacterial-producing bacteria have evolved resistance mechanisms that have been shown to be similar to, and may have been transferred to, antibacterial-resistant strains. The spread of antibacterial resistance often occurs through vertical transmission of mutations during growth and by genetic recombination of DNA by horizontal genetic exchange. For instance, antibacterial resistance genes can be exchanged between different bacterial strains or species via plasmids that carry these resistance genes. Plasmids that carry several different resistance genes can confer resistance to multiple antibacterials. Cross-resistance to several antibacterials may also occur when a resistance mechanism encoded by a single gene conveys resistance to more than one antibacterial compound. Antibacterial-resistant strains and species, sometimes referred to as "superbugs", now

contribute to the emergence of diseases that were for a while well controlled. For example, emergent bacterial strains causing tuberculosis (TB) that is resistant to previously effective antibacterial treatments pose many therapeutic challenges. Every year, nearly half a million new cases of multidrug-resistant tuberculosis (MDR-TB) are estimated to occur worldwide. For example, NDM-1 is a newly identified enzyme conveying bacterial resistance to a broad range of beta-lactam antibacterials. The United Kingdom's Health Protection Agency has stated that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections (Brooks, 2015).

1.5.3 Classification of antibiotic:

They are divided into four major groups:

- Fluoroquinolone : ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, ofloxacin.
- Aminoglycosides :
  - Narrow- spectrum: streptomycin, dihydrostreptomycin.
  - Broad -spectrum: neomycin, kanamycin, framycetin, gentamycin, tobramycin, amikacin.
    - β-lactam antibiotic :
      - narrow-spectrum: pen-G, pen-V.
      - Broad -spectrum: amoxicillin, ampicillin.
      - β-lactam resistant: cloxacillin, flucoxacillin, oxacillin.
    - Cephalosporin :

1. 1st generation: cefazolin, cephalexin.
2. 2nd generation: cefaclor, cefuroxime.
3. 3rd generation: cefixime, ceftazidime, ceftriaxone, cefotaxime.
4. 4th generation: cefepime.
5. 5th generation: ceftaroline.

- Macrolides: azithromycin, clarithromycin, josamycin, fidaxomycin, vancomycin, telithromycin, chloramphenicol (Berger, 2015).

In this study, we took resistant and sensitive strains. These strains were co-cultured with each other. We tried to see whether the sensitive strain become resistant after co-cultured with

resistant bacteria when treated with antibiotic azithromycin and cefixime. The antibacterial activity of *Acinetobacter baumannii*, *Salmonella Typhi* and *Shigella flexneri* was observed. Those three samples were collected from the endocrine department of BIRDEM and ICDDR,B. Disc diffusion method was used to find out the antibacterial activity after co-culture of sensitive strain with resistant pathogenic bacteria. Basically two major classes of antibiotic e.g. macrolides and β-lactams antibiotic were used to determine the zone of inhibition by co-culture of pathogenic bacteria.

CHAPTER 2

OBJECTIVES

2.1 Research Objectives:

The objectives of the study are

1. To develop antibacterial resistant by co-culturing of bacteria with the resistant pathogenic strains and
2. To determine the antibacterial activity against microorganism after co-culture with resistant pathogenic bacteria.

CHAPTER 3

METHODS & MATERIALS

3.1Methods and materials:

- **Media :**
    - Nutrient agar
    - Nutrient broth
    - MacConkey agar

3.2 Nutrient Agar: Composition, Preparation and Uses

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

3.2.1 Composition of Nutrient Agar

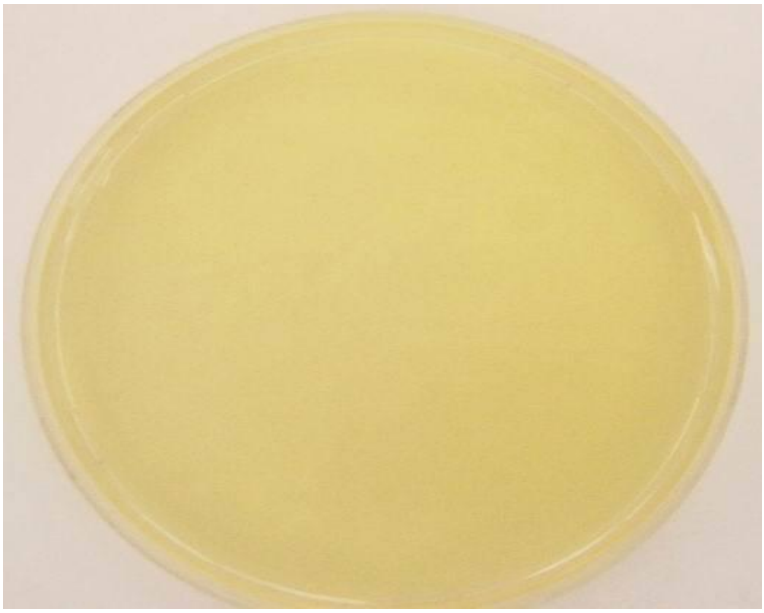

Figure 3.1: Nutrient agar

1. **0.5% Peptone**

It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria.

2. **0.3% beef extract/yeast extract**

It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

3. **1.5 % agar**

It is the solidifying agent.

4. **0.5% NaCl**

The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

5. **Distilled water**

Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

6. **pH is adjusted to neutral (7.4) at 25 °C.**

3.2.2 Preparation of Nutrient Agar:

1. 28 g of nutrient agar powder was suspended in 1 litre of distilled water.

2. This mixture was heated while stirring to fully dissolve all components.

3. The dissolved mixture was autoclaved at 121 degrees Celsius for 15 minutes.

4. Once the nutrient agar has been autoclaved, it was allowed to cool but not solidify.

5. Nutrient agar was poured into each plate and plates were left on the sterile surface until the agar has solidified.

6. The lid of each Petri dish was replaced and stored the plates in a refrigerator.

3.2.3 Uses of Nutrients Agar:

1. It is frequently used for isolation and purification of cultures.

2. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media specially formulated for that purpose.

3.3 Nutrient broth:

3.3.1 Preparation:

1. 13 g of nutrient broth powder was added to 1 litre of distilled water.
2. It was mixed well and sterilized

3.3.2 Composition:

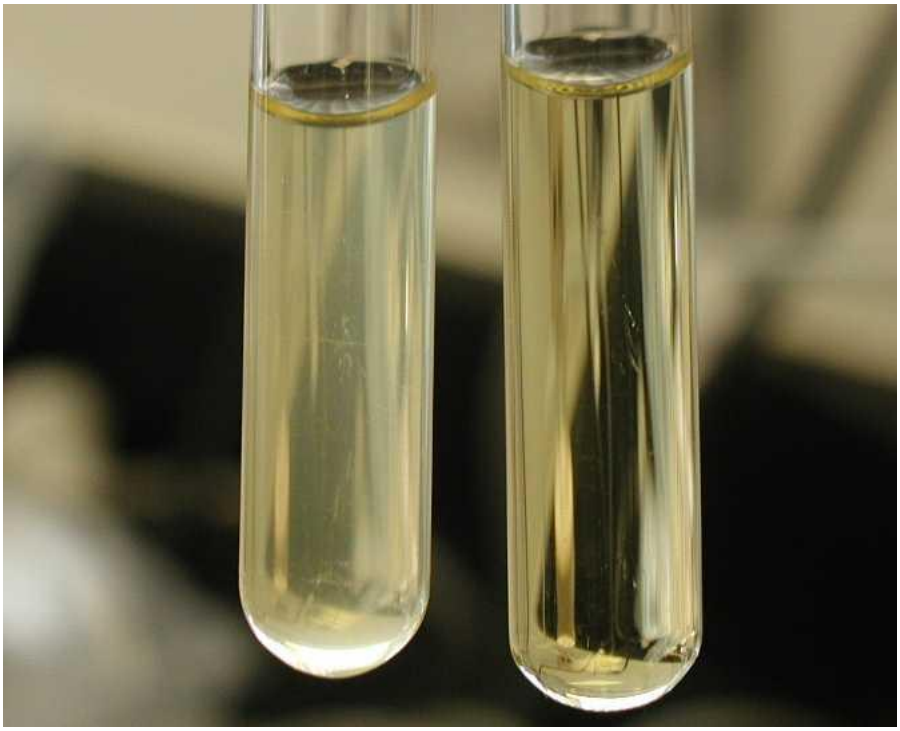

Figure 3.2: Nutrient Broth

1. **0.5% Peptone**

It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria.

2. **0.3% beef extract/yeast extract**

It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

3. **0.5% NaCl**

The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

4. **Distilled water**

Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

3.3.3 Container: Test tube

Color: Light yellow

Consistency: Liquid medium

Uses:

Used for

1. Bacterial growth

2. Coagulase test.

3.4 MacConkey Agar- Composition, Principle, Uses, Preparation:

MacConkey agar (MAC) was the first solid differential media to be formulated which was developed at 20th century by Alfred Theodore MacConkey. MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus Pseudomonas.

3.4.1 Principle of MacConkey Agar

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent.

3.4.2 Composition of MacConkey Agar:

Figure 3.3: MacConkey Agar

Ingredients	Amount
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 g
Agar	13.5 gm
Distilled Water	1 Liter

Table 3.1: composition of MacConkey Agar

3.4.3 Uses of MacConkey Agar:

MacConkey agar is used for the isolation of gram-negative enteric bacteria. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. It is used for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

3.4.4 Preparation of MacConkey Agar:

1. 49.53 grams of dehydrated medium was suspended to 1000 ml purified/distilled water.
2. The medium was heated to dissolve completely.
3. Sterilization was done by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. The mixture was cooled to 45-50°C.
5. It was mixed well before pouring into sterile Petri plates.

3.5 Bacteria sample:

1. *Acinetobacter baumannii*
2. *Salmonella typhi*
3. *Shigella flexneri*

3.6 Materials and glasswares:

1. Petridish
2. Distiled water
3. Cotton bud
4. Filter paper
5. 0.9% Saline
6. Forceps
7. Eppendorf tube
8. Punch machine
9. Reagent bottle

3.7 Reagents:

1. Ethanol
2. Methanol
3. Propylene glycol

3.8 Machines:

1. Autoclave
2. Hot air oven
3. Laminar flow

4. Incubator
5. Refrigerator

3.9 Antibiotics:

1. Azithromycin
2. Cefixime

3.10 Bacterial sample preparation and preservation:

Autoclave of cultured media was done.

⬇

agar media was poured into the petridish.

⬇

loop was heated for 2-3 seconds and then bacteria sample was inoculated in the agar media with help of loop.

⬇

The petridish was kept for overnight observation in the incubator at 37^0 C.

⬇

After 24 hour, glycerin and the nutrient were autoclaved.

⬇

Then, the inoculum was transferred to a culture test tube containing 10 ml nutrient broth.

⬇

The test tube was kept for overnight observation.

⬇

The next day, 3 ml of glycerin was added to the test tube for preservation of bacteria sample to the refrigerator at -80^0 C.

3.11 Disc diffusion method:

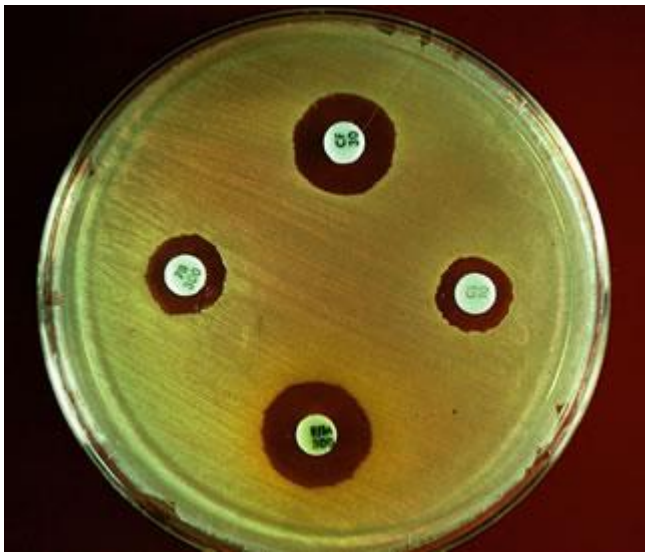

Figure3.4: Disc diffusion

0.9% NaCl solution was taken into 1ml of appendorf tube.

⬇

Using an aseptic technique, a sterile swab was placed into the NaCl containing specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.

⬇

Using the swab, streak the agar plate was streaked to form a bacterial lawn.

⬇

To obtain uniform growth, the plate was streaked with the swab in one direction, the plate was rotated 90° and the plate was streaked again in that direction.

⬇

This rotation was repeated 3 times.

⬇

The plate was allowed to dry for approximately 5 minutes.

⬇

An Antibiotic Disc Dispenser was used to dispense discs containing specific antibiotics onto the plate.

⬇

Using a flame-sterilized forceps, each disc was pressed gently to the agar to ensure that the disc is attached to the agar.

⬇

Plates should be incubated overnight at an incubation temperature of 37 °C (98.6 °F).

CHAPTER 4

RESULTS

Result:

In the experiment, I have investigated the effect of antibacterial activity after co-culture of *Acinetobacter baumannii* with *Salmonella typhi* and *Shigella flexneri*. The result showed that *Acinetobacter baumannii* was sensitive to azithromycin and cefixime whereas, *shigella flexneri* was resistant. In some instances *Salmonella typhi* showed sensitivity while in other cases there was no effect on antibacterial activity of *Salmonella typhi*.

Table 4.1: Zone of inhibition of *Salmonella typhi* treated with different concentration of cefixime.

Bacteria	Antibiotic	Concentration	Zone of inhibition
Salmonella typhi	cefixime	20 µg/ml	3.1 cm
Salmonella typhi	cefixime	40 µg/ml	3.3 cm
Salmonella typhi	cefixime	80 µg/ml	3.5 cm
Salmonella typhi	cefixime	160 µg/ml	3.6 cm

According to table 4.1, *Salmonella typhi* showed sensitivity against the antibiotic cefixime. The zone of inhibition was 3.1cm when the concentration of cefixime was 20µg/ml. Zone of inhibition was increased to 3.3 cm,3.5 cm and 3.6 cm when different concentrations (40 µg/ml,80 µg/ml, 160 µg/ml respectively) of cefixime was applied to the culture plates of *Salmonella typhi.*

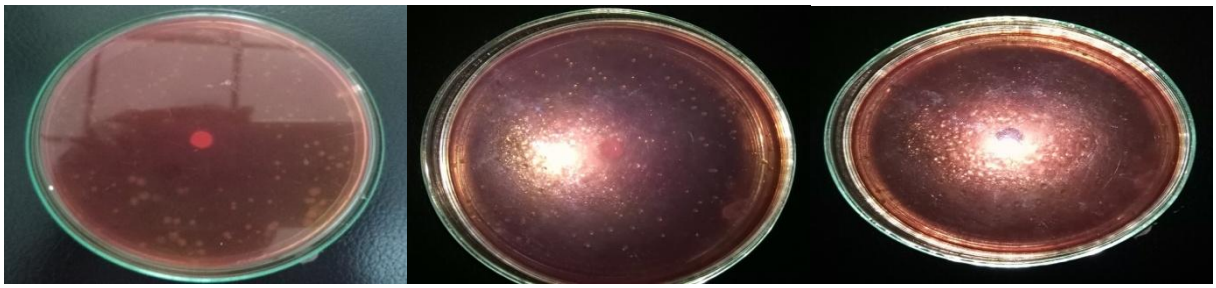

Figure 4.1: Different zone of inhibition of *Salmonella typhi* against the antibiotic cefixime.

Table4.2: Zone of inhibition of *Acinetobacter baumannii* treated with different concentrations of cefixime

Bacteria	Antibiotic	Concentration	Zone of inhibition
Acinetobacter baumannii	cefixime	20 µg/ml	3 cm
Acinetobacter baumannii	cefixime	40 µg/ml	3.5 cm
Acinetobacter baumannii	cefixime	80 µg/ml	4 cm
Acinetobacter baumannii	cefixime	160 µg/ml	4.5 cm

According to table 4.2, *Acinetobacter baumannii* showed sensitivity against the antibiotic cefixime. The zone of inhibition was 3 cm when the concentration of cefixime was 20 µg/ml. Zone of inhibition was increased to 3.5 cm, 4 cm and 4.5 cm when different concentrations (40 µg/ml,80µg/ml, 160µg/ml respectively) of cefixime was applied to the culture plates of *Acinetobacter baumannii.*

Figure 4.2: Different zone of inhibition of *Acinetobacter baumannii* against the antibiotic cefixime.

Co-culture of Bacteria strains (*Acinetobacter baumannii* and *Salmonella typhi*)

Co-culture was performed between the strain of *Acinetobacter baumannii* and *Salmonella typhi*. Both the strain were sensitive.

Table 4.3: Zone of inhibition of *Acinetobacter baumannii* treated with different concentrations of cefixime

Bacteria	Antibiotic	Concentration	Zone of inhibition
Acinetobacter baumannii	cefixime	20 µg/ml	2.8 cm
Acinetobacter baumannii	cefixime	40 µg/ml	3 cm
Acinetobacter baumannii	cefixime	80 µg/ml	3.3 cm
Acinetobacter baumannii	cefixime	160 µg/ml	3.6 cm

According to table 4.3, *Acinetobacter baumannii* showed sensitivity against the antibiotic cefixime.The zone of inhibition was 2.8cm when the concentration of cefixime was 20µg/ml.Zone of inhibition was increased to 3cm,3.3cm and 3.6cm when different concentration(40 µg/ml,80 µg/ml, 160 µg/ml) of cefixime was applied to the culture plates of *Acinetobacter baumannii*.After co-culture the zone of inhibition of *Acinetobacter baumannii* was reduced so that the antibacterial activity was close to resistant.

Table 4.4: Zone of inhibition of *Salmonella typhi* treated with different concentration of cefixime.

Bacteria	Antibiotic	Concentration	Zone of inhibition
Salmonella typhi	Cefixime	20 µg/ml	No zone was observed
Salmonella typhi	Cefixime	40 µg/ml	No zone was observed
Salmonella typhi	Cefixime	80 µg/ml	No zone was observed
Salmonella typhi	Cefixime	160 µg/ml	No zone was observed

Table 4.4 shows that *Salmonella typhi* was resistant when treated with the antibiotic Cefixime. No zone was observed when treated with different concentrations of cefixime (20µg/ml, 40µg/ml, 80µg/ml,160µg/ml). *Salmonella typhi* has become resistant after the co-culture with the sensitive strain of *A baumannii*.

Figure 4.3: Resistant pathogen of *Salmonella typhi* treated with Cefixime

Table 4.5: Zone of inhibition of *Acinetobacter baumannii* treated with different concentration of azithromycin

Bacteria	Antibiotic	Concentration	Zone of inhibition
Acinetobacter baumannii	Azithromycin	20 µg/ml	3.6 cm
Acinetobacter baumannii	Azithromycin	40 µg/ml	3.6 cm
Acinetobacter baumannii	Azithromycin	80 µg/ml	4 cm

Table 4.5 shows that *Acinetobacter baumannii* showed sensitivity when treated with antibiotic azithromycin (20, 40 and 80 µg/ml). The zone of inhibition was 3.6 cm when the concentration of azithromycin was 20µg/ml. The zone of inhibition was increased to 3.6 cm, 4cm when treated with 40 µg/ml,80 µg/ml of azithromycin respectively.

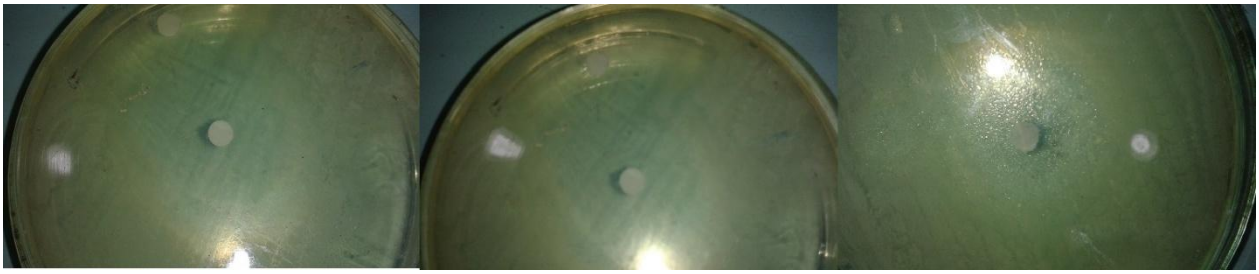

Figure 4.4: Different zone of inhibition of *Acinetobacter baumannii* against the antibiotic azithromycin.

Table 4.6: Zone of inhibition of *Shigella flexneri* treated with different concentration of azithromycin.

Bacteria	Antibiotic	Concentration	Zone of inhibition
Shigella flexneri	Azithromycin	20 µg/ml	No zone was observed
Shigella flexneri	Azithromycin	40 µg/ml	No zone was observed
Shigella flexneri	Azithromycin	80 µg/ml	No zone was observed

According to table 4.6, *Shigella flexneri* showed resistant when treated with the antibiotic azithromycin. No zone of inhibition was observed when treated with different concentrations of azithromycin (20µg/ml, 40µg/ml, 80µg/ml). *Shigella flexneri* was resistant when different concentration of azithromycin was applied to culture plates.

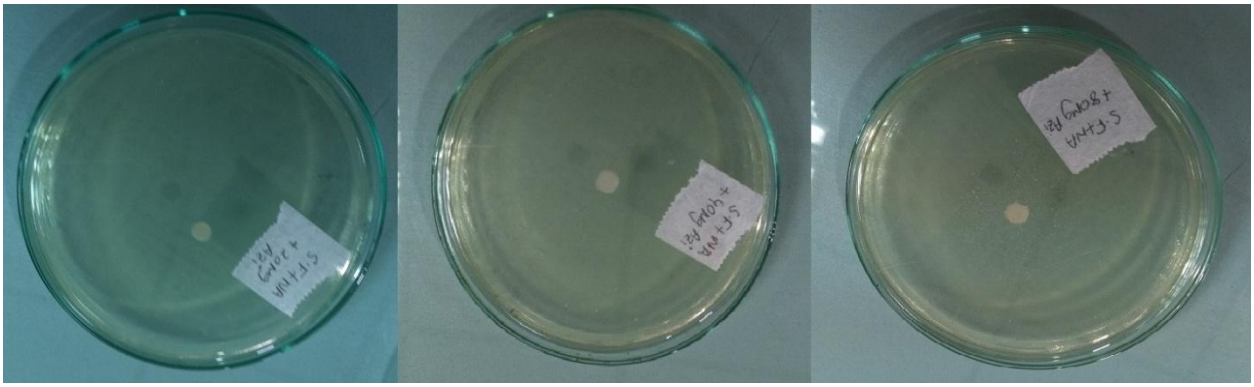

Figure 4.5: Resistant strain of *Shigella flexneri* against azithromycin

Co-culture of Bacteria strains (*Acinetobacter baumannii* and *Shigella flexineri*)

Co-culture has been done with *Acinetobacter baumannii* and *Shigella flexineri. A Baumanni* was a sensitive strain whereas *S flexneri* was a resistant strain.

Table 4.7: Zone of inhibition of *Acinetobacter baumannii* treated with different concentration of azithromycin

Bacteria	Antibiotic	Concentration	Zone of inhibition
Acinetobacter baumannii	Azithromycin	20 µg/ml	2.6 cm
Acinetobacter baumannii	Azithromycin	40 µg/ml	2.8 cm
Acinetobacter baumannii	Azithromycin	80 µg/ml	3 cm

According to table 4.7, *Acinetobacter baumannii* showed sensitivity when treated with the antibiotic azithromycin. The zone of inhibition was 2.6 cm when the concentration of azithromycin was 20µg/ml. The zone of inhibition was increased to 2.8cm, 3cm when treated with 40 µg/ml and 80 µg/ml of azithromycin respectively. After co-culture the zone of inhibition of *Acinetobacter baumannii* was reduced so that the antibacterial activity was close to resistant.

Table 4.8: Zone of inhibition of *Shigella flexneri* treated with different concentration of Azithromycin.

Bacteria	Antibiotic	Concentration	Zone of inhibition
Shigella flexneri	Azithromycin	20 µg/ml	No zone was observed
Shigella flexneri	Azithromycin	40 µg/ml	No zone was observed
Shigella flexneri	Azithromycin	80 µg/ml	No zone was observed

Table 4.8 shows that *Shigella flexneri* was resistant when treated with the antibiotic azithromycin. No zone of inhibition was observed when the different concentration of azithromycin (20 µg/ml 40 µg/ml,80 µg/ml) was applied. *Shigella flexneri* was resistant when different concentration of azithromycin was applied to culture plates.

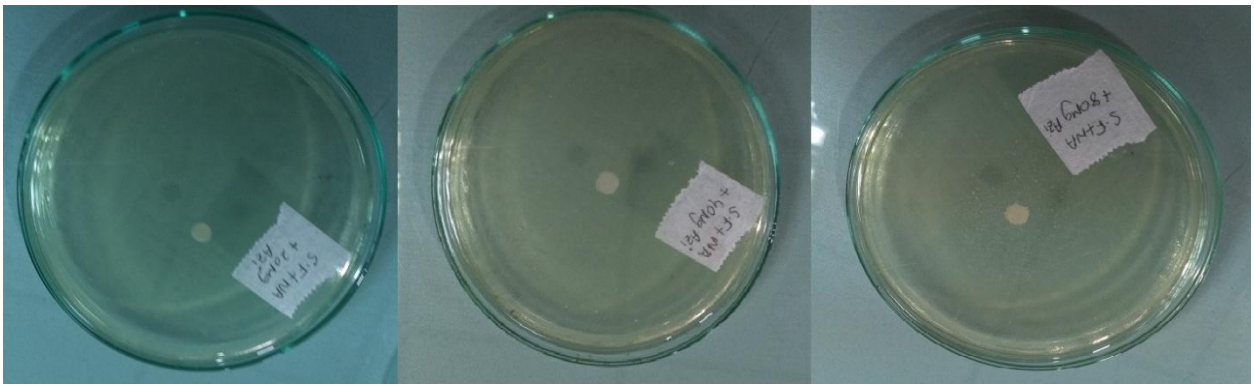

Figure 4.6: Resistant strain of *Shigella flexneri* after treatment with azithromycin

Table4.9: Percent (%) reduction of zone after treatment with azithromycin before and after co-culture of *acinetobacter baumannii.*

Bacteria	Anitbiotic	Concentration	Zone of inhibition (before co-culture)	Zone of inhibition (after co-culture)	% reduction
acinetobacter baumannii	azithromycin	20 µg/ml	3.6 cm	2.6 cm	27.8%
acinetobacter baumannii	azithromycin	40 µg/ml	3.6 cm	2.8 cm	22.2%
acinetobacter baumannii	azithromycin	80 µg/ml	4 cm	3 cm	25%
					Avg=25.2%

Table 4.9 shows that before co-culture the zone of inhibition of *Acinetobacter baumannii* was 3.6, 3.6 and 4 cm respectively when different concentration (20, 40, 80 µg/ml) of azithromycin was applied. After co-culture the zone of inhibition of *acinetobacter baumannii* was 2.6, 2.8 and 3 cm respectively when different concentration (20, 40, 80 µg/ml) of azithromycin was applied. The percent reduction of zone was 27.8%, 22.2% and 25% respectively before and after co-culture. Average percent reduction of zone was 25.2%.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion and Conclusion:

The antibacterial activity of cefexime and azithromycin was investigated after co-culture of *Acinetobacter baumannii* with *Salmonella typhi*. In addition co-culture of *Acinetobacter baumannii* with *Shigella flexneri* was carried out to examine the effect of cefexime and azithromycin. The result showed that *Acinetobacter baumannii* was sensitive to azithromycin and cefixime whereas, *shigella flexneri* was resistant. After co-culture of two sensitive strains (*Acinetobacter baumannii* and *Salmonella typhi*), *Acinetobacter baumannii* showed sensitivity when treated with different concentrations (20 μg/ml, 40 μg/ml, 80 μg/ml and 160 μg/ml) of cefixime. However, no zone of inhibition was observed when *Salmonella typhi* was treated with different concentrations (20μg/ml, 40μg/ml, 80μg/ml and 160μg/ml) of cefixime. Co-culture of sensitive *Acinetobacter baumannii* was done with resistant *Shigella flexneri*. *Acinetobacter baumannii* still showed sensitivity when treated with different concentrations (20μg/ml, 40 μg/ml, 80μg/ml and 160μg/ml) of azithromycin. However, zone diameter was reduced to 25% when treated with azithromycin after co-culture with resistant strain of *Shigella flexneri*.

It is difficult to treat multidrug-resistant pathogens in intensive care unit(ICU) patients.To solve multi-drug resistant (MDR) problem, combination therapy can be used instead of monotherapy. Owing to the lack of adequate studies and to the increasing antimicrobial resistance, therapeutic options for *A. baumannii* infections often represent a challenge. Furthermore, it is also unclear whether combination therapy is more effective than monotherapy, because only observational studies compared different treatment regimens for MDR *Acinetobacter* infections. (Matteo *et al,* 2008). In our study the *Acinetobacter baumannii* was co-cultured with resistant strain of *Shigella flexneri*. The present study showed that zone diameter was reduced to 25% when treated *Acinetobacter baumannii* with azithromycin. After co-culture of sensitive *Acinetobacter baumannii* with resistant *Shigella flexneri,* it showed that *Acinetobacter baumannii* is no longer remain adequately sensitive with monotherapy (azithromycin). May be combination therapy would be helpful to overcome this problem.

There are at least three clinical trials of *A. baumannii* infections in critically ill patients treated with a colistin-rifampicin combination. The first study by Petrosillo *et al.* evaluated the clinical outcome of carbapenem-resistant *A. baumannii*-infected patients treated with a combination of colistin and rifampicin in 14 mechanically ventilated, critically-ill patients with *A. baumannii*-

associated pneumonia. Of the 14 treated patients, seven recovered from *A. baumannii* infections and nine had microbiological clearance (Petrosillo *et al,* 2008).

Motaouakkil *et al.* conducted an observational study to evaluate the efficacy of intravenous and aerosolized colistin combined with rifampicin in the treatment of critically ill patients with nosocomial infections caused by multiresistant *A. baumannii* in a medical ICU patients. The clinical outcome was favorable for all patients. More recently, Bassetti *et al.* conducted the largest study. They treated 29 patients, of whom 19 cases of nosocomial pneumonia and ten cases of bacteraemia, with intravenous colistin sulfomethate sodium (2 million International Units three-times daily) in addition to intravenous rifampicin (10 mg/kg every 12 h). Clinical and microbiological responses were observed in 22 out of 29 cases (76%). Three of the 29 evaluated patients (10%) developed nephrotoxicity during treatment with colistin, all of whom had previous renal failure. No cases of renal failure were observed among patients with normal base line renal function (Matteo *et al*, 2008).

Salmonella typhi remains a serious public health problem in many developing countries for enteric fever. The highest incidence in parts of Africa is 50 per 100.000 person-years and Asia is 274 per 100.000 person-years. Resistance strains have been emerged with *S. typhi* to multiple clinically relevant antibiotics from 1990. In India *S. typhi* is the commonest etiological agent for enteric fever. It is a major public health problem accounting for more than 300,000 cases per year.Outbreaks of multidrug-resistant (MDR) of *S. typhi*. has been experienced by most developing countries as well. In a recent study combination of trimethoprim-sulfamethoxazole are more effective against clinically resistant *S. typhi* rather than azithromycin or cefixime alone (Smith et al,2016). Our *in vitro* study has shown that *Salmonella typhi* becomes resistant when treated with cefixime. May be combination antibiotic regimen will be appropriate to get the sensitivity against *Salmonella typhi*.

Three hundred and thirty-three *Shigella* isolates obtained in 1986 to 1995 were tested for their susceptibilities to 19 antimicrobial agents. Nalidixic acid resistance had emerged in 59.6% of *Shigella flexneri* isolates during 1994 to 1995. Multiresistance (resistance to four or more agents) was more common in *S. flexneri* than in *Shigella sonnei* (Yiu-wai C., 1995).

Bacillary dysentery caused by *Shigella* species is an important cause of diarrhea. Appropriate antibiotic therapy reduces the duration of symptoms and excretion of organisms but increases the risk of developing antibiotic resistance. High frequencies of resistance have been observed in *Shigella flexneri* and *Shigella sonnei*. A study was carried out to see the antimicrobial susceptibility patterns of *Shigella* spp. isolated in Hong Kong from 1986 to 1995, with particular reference to resistance to the 4-quinolones. These isolates were tested for their susceptibility to different antimicrobials. The 19 antimicrobial agents used in this study were ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole, trimethoprim, and tetracycline , amoxicillin-clavulanic acid , amikacin, and cefepime, cefotaxime and gentamicin , cefuroxime**,** and ceftazidime , piperacillin , ciprofloxacin, imipenem , netilmicin , ofloxacin and sparfloxacin.
(Yiu-wai C., 1995). It has been reported that Nalidixic acid resistance had emerged in 59.6% of *Shigella flexneri* isolates. It has also been shown that resistance to four or more agents was more common in *S. flexneri* than in *Shigella sonnei*. Our strains of *S flexneri* did not show any sensitivity when treated with azithromycin.
Our present study shows that sensitive strains become resistant after performing co-culture with either resistance or sensitive strains. Monotherapy did not show adequate sensitivity with *A. baumannii* when co-culture was done with resistant *Shigella flexneri.*

Furthermore studies are needed to develop newer alternative Combination therapy to treat resistant pathogenic bacteria.

CHAPTER 6

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