Synergistic effect of Moxifloxacin and Imipenem against *Acinetobacter baumannii* after co-culture with a resistant *Shigella flexneri*

A Dissertation submitted to the Department of Pharmacy, East West University, as the Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy.

Submitted by

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Declaration by the Research Candidate

I, Atkiya Nazifa ,hereby declare that this dissertation entitled "Synergistic effect of Moxifloxacin and Imipenem against *Acinetobacter baumanni* after co-culture with a resistant strain *Shigella flexneri*" submitted to the Department of Pharmacy, East West University, in partial fulfillment for the requirement of the Degree of Master of Pharmacy ,is an authentic research work done by me under the guidance of Professor Dr. Sufia Islam , Department of Pharmacy , East West University, Dhaka Bangladesh. The content of this dissertation in full or in parts, have not been submitted to any other Institution or University for the award of any Degree or any Diploma of Fellowship.

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Certificate by the Supervisor

This is to certify that the dissertation, entitled "Synergistic effect of Moxifloxacin and Imipenem against *Acinetobacter baumanni* after co-culture with a resistant strain *Shigella flexneri*" is a bona fide research work done under our guidance and supervision by Atkiya Nazifa (ID # 2016-1-79-011), in partial fulfillment for the requirement of the Degree of the Master of Pharmacy.

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Certificate by the Chairperson

This is to certify that the dissertation, entitled "Synergistic effect of Moxifloxacin and Imipenem against *Acinetobacter baumanni* after co-culture with a resistant strain *Shigella flexneri*" is a bona fide research work done by Atkiya Nazifa (ID # 2016-1-79-011), in partial fulfillment for the requirement of the Degree of the Master of Pharmacy.

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Dedication

This research paper is dedicated to my

respected Thesis Supervisor

&

my beloved family

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Abstract

Some microorganisms are capable of causing serious disease has become resistant to most commonly available antibiotics. Treating patients with drug-resistant pathogens have created a need for new therapies. In our study, we investigated the antimicrobial effect of combination antibiotics (clarithromycin, moxifloxacin, Imipenem) against *Acinetobacter baumannii* after co-culture with resistant *Shigella flexneri*. In our study, Kirby–Bauer disc diffusion antibiotic sensitivity testing was used which is essentially a qualitative or semi-quantitative test indicating the sensitivity or resistance of microorganisms to the test materials. Initially *Acinetobacter baumannii* was sensitive against the clarithromycin, imipenem and moxifloxacin in different concentration of 20, 40, 60 and 80 μg/ml. However, same concentrations of clarithromycin, imipenem and moxifloxacin showed no zone of inhibition against *Shigella flexneri*. After co-culture with two bacteria, both of them did not show optimum zone of inhibition against clarithromycin, imipenem and moxifloxacin at concentration of 20, 40, 60, and 80 μg/ml respectively. When high concentrations (120, 160, 180 μg/ml) of clarithromycin, Imipenem and moxifloxacin were used against the co-cultured pathogen, still no sensitivity was observed against the treated antibiotic. Our study shows that sensitive *Acinetobacter baumannii* become resistant after co-culture with resistant *Shigella flexneri*. Treatment with combination of Imipenem and moxifloxacin in different concentration of 20, 40, 60, and 80 μg/ml against newly resistant *Acinetobacter baumannii* showed sensitivity. Combination of Clarithromycin and imipenem with concentration of 20, 40, 60, and 80 μg/ml exhibited satisfactory zone of inhibition. Antibiotic combinations can be used as an alternative treatment approach in multi-drug resistant *Shigella flexneri* and *Acinetobacter baumannii* infections. The result of the combination antibiotic treatment also shows 10% greater effectiveness compared with single antibiotic against resistant pathogen which indicate synergistic effect of antibiotics.

Key words: *Shigella flexneri*; antimicrobial effect; drug resistance; sensitivity; *Acinetobacter baumannii*; synergistic effect.

1.1 Introduction:

Life-threatening bacterial infections are a major concern in health care system. Because of increase in the emergence of antibiotic resistant bacteria, researcher's must look to rarely used antibiotics for alternative treatment options.

1.2 *Acinetobacter baumannii:*

Acinetobacter baumannii is a non-motile, gram-negative, non-fermentative, oxidase-negative and aerobic bacillus. *A. baumannii*is one of the most opportunistic pathogenic agents in humans (Mahgoub S. et al. 2002).AB is considerably resistant against most antibiotics. Various nosocomial infections have been observed by this species with high morbidity and mortality. They include pneumonia, bacteremia, urinary tract, skin and soft tissue infections, especially in patients with severe illness.

The prevalence of multidrug resistance with *A. baumannii* strains have been increasing during recent years. This organism has been shown to cause highly mortal hospital infections (Perez, F. et al. 2007). The bacteria show a wide range of antimicrobial resistance mechanisms and has the ability of acquiring plasmids, transposons and integrons and mostly, the production of carbapenemases (carbapenemases are β-lactamases with versatile hydrolytic capacities, they have the ability to hydrolyze antibiotics , and carbapenems. Bacteria producing these β-lactamases may cause serious infections in which the carbapenemase activity renders many β-lactams ineffective)

1.2.1 Pathophysiology:

Though *Acinetobacter* causes actual infection, the pathological changes that occurred based on the organ system. The pathological changes, as observed in pneumonia patients are indistinguishable from those caused by other aerobic gram-negative bacilli that cause nosocomial pneumonias. Similarly, *Acinetobacter* urinary tract infections are clinically indistinguishable from catheter-associated bacteremia caused by other aerobic gram-negative bacilli.

1.2.2 Epidemiology:

1.2.3 Frequency:

Acinetobacter commonly colonizes patients in the intensive care setting in a hospital. *Acinetobacter* colonization is particularly common in patients are incubated and in those who have multiple intravenous lines or monitoring devices, surgical drains, or indwelling urinary catheters. *Acinetobacter* infections are common and occurred almost in some hospitalized patients.

1.2.4 Mortality/Morbidity:

Although *Acinetobacter* is primarily a colonizer in the hospital environment, it occasionally causes infection. Mortality and morbidity resulting from *A. baumannii* infection relate to the underlying cardiopulmonary immune status of the host rather than the inherent virulence of the organism.

Mortality and morbidity rates in patients who are very ill with multisystem disease are increased because of their underlying illness rather than the superimposed infection with *Acinetobacter*.

1.2.5 Race:

Acinetobacter infection has no known racial predilection.

1.2.6 Sex:

Acinetobacter infection has no known sexual predilection.

1.2.7 Age:

Acinetobacter infection has no known predilection for age.

1.2.8 Antimicrobial Resistance:

Antimicrobial resistance among *Acinetobacter* species has increased in the past decade (Lockhart, SR et al. 2007). The capacity of *Acinetobacter* species for extensive antimicrobial resistance may be due in part to the organism's relatively impermeable outer membrane and its

environmental exposure to a large reservoir of resistance genes. Definitions of multidrug-resistant *Acinetobacter* species vary, referring to a wide array of genotypes and phenotypes (Falagas ME, et al.2006). Two of the most common definitions of multidrug resistance are carbapenem resistance or resistance to ⩾3 classes of antimicrobials (Falagas ME, et al. 2006).

1.2.9 Treatment:

Carbapenem resistance is now observed in *A. baumannii* isolates, leading to limited therapeutic options. Several carbapenem-hydrolyzing lactamases have been documented in *A. baumannii*. Lactamases from class B illustrate highly hydrolyzing activity of carbapenems. Carbapenems, which were the drug of choice, today they are no longer using for treatment of *A. baumannii* infections. Several studies carried out about drug resistance of *Acinetobacter baumannii* and found high resistant rate to most of the antibiotics (Diganta Dey et al. 2014).

Increasing antimicrobial resistance leaves few therapeutic options, and there are no well-designed clinical trials to compare treatment regimens for multidrug-resistant *Acinetobacter* infection like as;

Lactamase inhibitors, particularly sulbactam, have intrinsic activity against many Acinetobacter strains (Wood et al.1993)

Tigecycline, a relatively new glycylcycline agent, has bacteriostatic activity against multidrug-resistant *Acinetobacter* species (Seifert H et al. 2006).

Polymyxin therapy given limited therapeutic options, clinicians have returned to the use of polymyxin B or polymyxin E (colistin) for the most drug-resistant *Acinetobacter* infections. Colistin acts by disturbing the bacterial cell membrane, thus increasing permeability, leading to cell death.

1.3 Synergy and Combination therapy:

Synergistic effect is an effect when two or more agents, entities, factors, or substances that produces an effect greater than the sum of their individual effects. It is opposite of antagonism.

A lack of controlled clinical trials makes it difficult to evaluate the role of synergy or combination therapy for multidrug-resistant *Acinetobacter* infection. Most available data are from uncontrolled case series, animal models, or in vitro studies. A study has shown combinations of rifampin, sulbactam, aminoglycoside agents, colistin, carbapenems, and other agents against multidrug-resistant *Acinetobacter* infection (Rodriguez-Hernandez, MJ, et al. 2001)

Studies have found conflicting results for the same antimicrobial combinations(Garnacho-Montero, J et al. 2015)studied a mouse model of multidrug-resistant *Acinetobacter, pneumonia* and found that the combinations of rifampin with imipenem, tobramycin, or colistin were the most effective regimens. A follow-up clinical pilot study, however, cautioned against the use of rifampin plus imipenem for treatment of carbapenem-resistant *Acinetobacter* infection, because investigators observed a high failure rate and documented the emergence of rifampin resistance in 70% of the patients who were treated with this regimen.

Most results for combination therapy are comparable to cure rates reported for parenteral colistin alone, and the wide variety of other agents used limits the ability to draw conclusions regarding combination therapy. Controlled clinical studies are needed to determine whether any antimicrobial combinations translate into useful therapeutic strategies.

(Li, J, Rayner et al. 2006) found heteroresistance (i.e., subpopulations with varying levels of resistance to colistin) in colistin-susceptible *Acinetobacter* isolates studied in vitro. Serial passage of the isolates in the presence of colistin increased the proportion of colistin-resistant subpopulations.

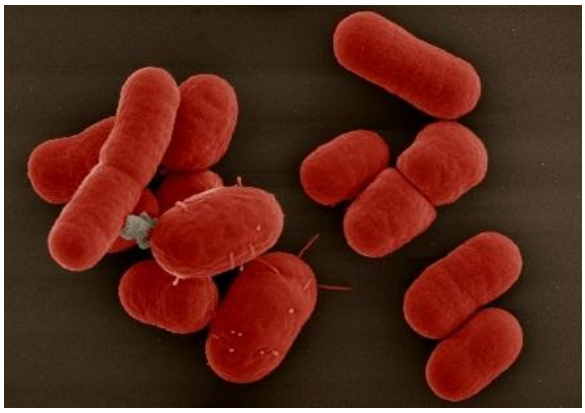


Fig1.1:*Acinetobacter baumannii*

1.4 Shigella flexneri:

Shigella flexneri is a species of Gram-negative bacteria in the genus *Shigella* that can cause diarrhea in humans. Several different serogroups of *Shigella* are described; *S. flexneri* belongs to group *B. S. flexneri* infections can usually be treated with antibiotics, although some strains have become resistant. Less severe cases are not usually treated because they become more resistant in the future.

1.4.1 Classification:

Gram-negative entero-invasive bacterium

1.4.2Genus and Species:

Strains: *Shigella flexneri* 1a. *Shigella flexneri* 1b. *Shigella flexneri* 2a. *Shigella flexneri* 2a strain 2457T. *Shigella flexneri* 2a strain 301. *Shigella flexneri* 3a. *Shigella flexneri* 3b. *Shigella flexneri*5. *Shigella flexneri* 5 strain 8401. *Shigella flexneri* 5a. *Shigella flexneri* 6. *Shigella flexneri* Y.

1.4.3 Pathophysiology:

Shigella infection is a major public health problem in developing countries where sanitation is poor. Humans are the natural reservoir, although other primates may be infected. No natural food products harbor endogenous *Shigella* species, but a wide variety of foods may be contaminated.

Shigellosis is spread by fecal-oral transmission. Other modes of transmission include ingestion of contaminated food or water (untreated wading pools, interactive water fountain), contact with a contaminated inanimate object, and certain mode of sexual contact. Vectors like the housefly can spread the disease by physically transporting infected feces.

The infectivity dose (ID) is extremely low. As few as 10 *S. dysenteriae bacilli* can cause clinical disease, whereas 100-200 *bacilli* are needed for *S. sonnei* or *S. flexneri* infection. The reasons for this low-dose response are not completely clear. One possible explanation is that virulent *Shigellae* can withstand the low pH of gastric juice. Most isolates of *Shigella* survive acidic treatment at pH 2.5 for at least 2 h.

The incubation period varies from 12 hours to 7 days but is typically 2-4 days; the incubation period is inversely proportional to the load of ingested bacteria. The disease is excreted by infected person's stool, which can extend as long as 4 weeks from the onset of illness. Bacterial shedding usually ceases within 4 weeks of the onset of illness; rarely, it can persist for months. Appropriate antimicrobial treatment can reduce the duration of carriage to a few days.

1.4.4 Virulence:

Virulence in *Shigella* species involves both chromosomal-coded and plasmid-coded genes. Virulent *Shigella* strains produce disease after invading the intestinal mucosa; the organism only rarely penetrates beyond the mucosa (Edwards BH. 1999)

The characteristic virulence trait is encoded on a large (220 kb) plasmid responsible for synthesis of polypeptides that cause cytotoxicity. *Shigellae* that lose the virulence plasmid are no longer

pathogenic. *Escherichia coli* that harbor this plasmid clinically behave as *Shigella* bacteria (Friedrich AW,Bielaszewska et al. 2002)

Siderophores, a group of plasmid-coded genes, control the acquisition of iron from host cells from its protein-bound state. In the extra intestinal phase of infection by gram-negative bacteria, iron becomes one of the major factors that limit further growth. This limitation occurs because most of the iron in human body is sequestered in hemoproteins (i.e., hemoglobin, myoglobin) or iron-chelating proteins involved in iron transport (transferrin and lactoferrin). Many bacteria can secrete iron chelating compounds, or siderophores, which chelate iron from the intestinal fluids and which bacteria then take up to obtain iron for its metabolic needs. These siderophores are under the control of plasmids and are tightly regulated by genes under low iron conditions, expression of the siderophore system is high.

Regarding chromosomally encoded enterotoxin, many pathogenic features of *Shigella* infection are due to the production of potent cytotoxins known as Stx, a potent protein synthesis–inhibiting exotoxin. *Shigella* strains produce distinct enterotoxins. These are a family of cytotoxins that contain 2 major immunologically non–cross-reactive groups called Stx1 and Stx2. The homology sequences between Stx1 and Stx2 are 55% and 57% in subunits A and B, respectively.

These toxins are lethal to animals; enterotoxic to ligated rabbit intestinal segments; and cytotoxic for vero, HeLa, and some selected human renal vascular endothelial cells manifesting as diarrhea, dysentery, and hemolytic-uremic syndrome (HUS). Stx1 is synthesized in significant amount by *S dysenteriae* serotype 1 and S flexneri 2a and *E coli* (*Shigella* toxin–producing *E coli*) (Ingersoll MA & Zychlinsky A 2006)

Stx1 and Stx2 are both encoded by a bacteriophage inserted into the chromosome. Stx1 increases inflammatory cytokine production by human macrophages, which, in turn, leads to a burst of interleukin (IL)-8. This could be relevant in recruiting neutrophils to the lamina propria of the intestine in hemorrhagic colitis and accounts for elevated levels of IL-8 in serum of patients with diarrhea-associated HUS.

Stxs have 2 subunits. Stx is transported into nucleoli. Stx nucleolar movement is carrier-dependent and energy-dependent. Subunit A is a 32-kD polypeptide that, when digested by

trypsin, generates A1 with a 28-kD fragment and another small fragment, A2, which is 4 kD. A1 fraction acts like *N* -glycosidase; it removes single adenine residue from 28S rRNA of ribosome and inhibits protein synthesis. The A2 fraction is a pentamer polypeptide of 7.7-kD protein and is required to bind the A1 fraction to the B subunit. The main function of the B subunit is the binding of toxins to the cell surface receptor, typically globotriosyl ceramide (Gb3), on the brush border of intestinal epithelial cells.

In summary, events that occur on exposure to *Shigella* toxin are as follows:

- The B subunit of holotoxin binds to the Gb3 receptor on the cell surface of brush-border cells of the intestines.
- The receptor-holotoxin complex is endocytosed.
- The complex moves to Golgi apparatus and then to the endoplasmic reticulum.

The A1 subunit is released and it targets 28S RNA of the ribosome, inhibiting protein synthesis. Stxs may play a role in the progression of mucosal lesions after colonic cells are invaded, or they may induce vascular damage in the colonic mucosa. Stx adheres to small-intestine receptors and blocks the absorption of electrolytes, glucose, and amino acids from intestinal lumen. The B subunit of Stx binds the host's cell glycolipid in the large intestine and in other cells, such as renal glomerular and tubular epithelia. The A1 domain internalized by means of receptor-mediated endocytosis and causes irreversible inactivation of the 60S ribosomal subunit, inhibiting protein synthesis and causing cell death, microvascular damage to the intestine, apoptosis in renal tubular epithelial cells, and hemorrhage (as blood and mucus in the stool).

Chromosomal genes control lipopolysaccharide (LPS) antigens in cell walls. LPS plays an important role in resistance to nonspecific host defense encountered during tissue invasion. These genes help in invasion, multiplication, and resistance to phagocytosis by tissue macrophages. LPS enhances the cytotoxicity of Stx on human vascular endothelial cells. *Shigella* chromosomes share most of their genes with *E coli* K12 strain MG1655, and the diversity of putative virulence genes acquired by means of bacteriophage-mediated lateral gene transfer is extensive. O convergent evolution involving the gain and loss of

functions, *Shigella* species have become highly specific human pathogens with variable epidemiologic and pathologic features.

A 3-kb plasmid that harbors information for production of bacteriocin by *S flexneri* strains has been described. The production of this bacteriocin may be related to dysenteric diarrhea these bacterial strains produce.

1.4.5 Intestinal adherence factor:

Intestinal adherence factor favors colonization in vivo and in animal models. This is 97-kD outer-membrane protein (OMP) encoded by each gene on chromosomes. This codes for intimin protein, and an anti-intimin response is observed in children with HUS (Schuller S. 2011).

1.4.6 Pathology:

The host or person response to primary infection is characterized by the induction of an acute inflammation, which is accompanied by polymorphonuclear cell (PMN) infiltration, resulting in massive destruction of the colonic mucosa. Apoptotic destruction of macrophages in sub epithelial tissue allows survival of the invading *shigella,* and inflammation facilitates further bacterial entry.

Gross pathology consists of mucosal edema, erythema, friability, superficial ulceration, and focal mucosal hemorrhage involving the recto sigmoid junction primarily.

Microscopic pathology consists of epithelial cell necrosis, goblet cell depletion, PMN infiltrates and mononuclear infiltrates in lamina propria, and crypt abscess formation.

Shigella bacteria invade the intestinal epithelium through M cells and proceed to spread from cell to cell, causing death and sloughing of contiguously invaded epithelial cells and inducing a potent inflammatory response resulting in the characteristic dysentery syndrome. In addition to this series of pathogenic events, only *S dysenteriae* type 1 has the ability to elaborate the potent Shiga toxin that inhibits protein synthesis in eukaryotic cells and that may lead to extra intestinal

complications, including hemolytic-uremic syndrome and death. Invasion of M cells, the specialized cells that cover the lymphoid follicles of the mucosa, overlying Peyer patches, may be the earliest event (Phalipon, A & Sansonetti, PJ 2007).

1.4.7 Mortality/Morbidity:

Although shigellosis-related mortality is rare in developed countries, *S dysenteriae* infection is associated with substantial morbidity and mortality rates in the developing world.

Case fatality is as high as 15% among patients with *S dysenteriae* type 1 who require hospitalization; this rate is increased by delayed arrival and treatment with ineffective antibiotics. Infants, non-breastfed children, children recovering from measles, malnourished children, and adults older than 50 years have a more severe illness and a greater risk of death

The overall mortality rate in developed countries is less than 1%.

1.4.8 Race:

No racial predilection is known.

1.4.9 Sex:

No sexual predilection is known.

1.4.10 Application to Biotechnology:

Shigella flexneri is a bacterial pathogen that is not used for biotechnology.

1.5 Current Research:

The suppressed immune systems of AIDS patients make them more vulnerable to diarrhea caused by *Shigella flexneri* and other pathogens. The lack of helper T cells makes AIDS patients susceptible to illness in general, and diarrhea happens to be one of the most common illnesses. However, an intensive handwashing regimen can be used to lower the incidence of diarrhea.

There is an inverse relationship among AIDS patients between handwashing frequency and the occurrence of diarrhea (Huang, DB and Zhou, J 2007).

Shigella flexneri 2a can be detected quickly from stool samples at bedside. The detection test involves a dipstick coated with monoclonal antibodies specific for *Shigella flexneri* 2a LPS, which includes a repeating, branched Penta saccharide as part of its O-antigen. Strain 2a was selected because it is the strain most associated with endemics. The test can detect low levels of *Shigella flexneri* within 15 minutes and was shown to have both high specificity and sensitivity (Nato, F et al. 2007).

Shigella flexneri appears to be able to inhibit apoptosis in epithelial cells. HeLa cells infected with *Shigella flexneri* resisted apoptosis after exposure to staurosporine, whereas uninfected cells appeared apoptotic. Infected cells had cytochrome c release and activated caspase 9 but no activated caspase 3, suggesting that *Shigella flexneri* inhibits caspase 3 activation. The bacteria must invade, have a functional type III secretion system, and have a functioning mxiE gene to block apoptosis in epithelial cells. The mxiE gene encodes a transcriptional activator for intracellular genes, some of which are presumably involved in apoptosis inhibition (Clark,CS, & Maurelli, AT 2007)

Fig 1.2: Shigella flexneri

1.6 Antibiotic and resistance:

Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines.

Bacteria, not humans or animals, become antibiotic-resistant. These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria.

Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.

So, there is urgent need to change the way it prescribes and uses antibiotics. Even if new medicines are developed, without behavior change, antibiotic resistance will remain a major threat. Behavior changes must also include actions to reduce the spread of infections through vaccination, hand washing, practicing safer sex, and good food hygiene.

1.7 Prevention and control:

Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. Steps can be taken at all levels of society to reduce the impact and limit the spread of resistance

1.8 Impact:

When infections can no longer be treated by first-line antibiotics, more expensive medicines must be used. A longer duration of illness and treatment, often in hospitals, increases health care costs as well as the economic burden on families and societies.

Antibiotic resistance is putting the achievements of modern medicine at risk. Organ transplantations, chemotherapy and surgeries such as caesarean sections become much more dangerous without effective antibiotics for the prevention and treatment of infections.

1.9 Combination therapy:

In important infectious diseases, including tuberculosis, combination therapy (i.e., the concurrent application of two or more antibiotics) has been used to delay or prevent the emergence of resistance. In acute bacterial infections, antibiotics as part of combination therapy are prescribed for their synergistic effects to improve treatment outcome as the combined effect of both antibiotics is better than their individual effect. *Methicillin* infections may be treated with a combination therapy of Fusidic acid and Rifampicin. Antibiotics used in combination may also be antagonistic and the combined effects of the two antibiotics may be less than if the individual antibiotic was given as part of a monotherapy. For example, chloramphenicol and tetracyclines are antagonists to penicillin and aminoglycosides. However, this can vary depending on the species of bacteria. In general, combinations of a bacteriostatic antibiotic and bactericidal antibiotic are antagonistic.

1.10 Research on antibiotic therapies against resistant pathogens:

1.10.1 Alternatives:

The increase in bacterial strains that are resistant to conventional antibacterial therapies together with decreasing number of new antibiotics currently being developed in the drug pipeline has prompted the development of bacterial disease treatment strategies that are alternatives to conventional antibacterial. Non-compound approaches that target bacteria or approaches that target the host including phage therapy and vaccines are also being investigated to combat the problem.

1.11 Development of new antibiotics:

In April 2013, the Infectious Disease Society of America (IDSA) reported that the weak antibiotic pipeline does not match bacteria's increasing ability to develop resistance. Since 2009, only 2 new antibiotics were approved in the United States. The number of new antibiotics approved for marketing per year declines continuously. The report identified seven antibiotics against the Gram-negative *bacilli* (GNB) currently in phase 2 or phase 3 clinical trials. However,

these drugs do not address the entire spectrum of resistance of GNB. Some of these antibiotics are combination of existent treatments:

- Ceftolozane/tazobactam (CXA-201; CXA 101/tazobactam):
- Antipseudomonal cephalosporin/β-lactamase inhibitor combination (cell wall synthesis inhibitor). FDA approved on 12/19/2014.
- Ceftazidime/avibactam (ceftazidime/NXL104): Antipseudomonal cephalosporin/β-lactamase inhibitor combination (cell wall synthesis inhibitor). In phase 3.
- Ceftaroline/avibactam (CPT-avibactam; ceftaroline/NXL104): Anti-MRSA cephalosporin/ β-lactamase inhibitor combination (cell wall synthesis inhibitor)
- Imipenem/MK-7655: Carbapenem/ β-lactamase inhibitor combination (cell wall synthesis inhibitor). In phase 2.
- Plazomicin (ACHN-490): Aminoglycoside (protein synthesis inhibitor). In phase 2.
- Eravacycline (TP-434): Synthetic tetracycline derivative / protein synthesis inhibitor targeting the ribosome. Development by Tetraphase, Phase 2 trials complete.
- Brilacidin (PMX-30063): Peptide defense protein mimetic (cell membrane disruption). In phase 2.

Streptomyces research is expected to provide new antibiotics, including treatment against MRSA and infections resistant to commonly used medication. Efforts of John Innes Centre and universities in the UK, supported by BBSRC, resulted in the creation of spin-out companies, for example Novacta Biosystems, which has designed the type-b lantibiotic-based compound NVB302 (in phase 1) to treat *Clostridium difficile* infections. Possible improvements include clarification of clinical trial regulations by FDA. Furthermore, appropriate economic incentives could persuade pharmaceutical companies to invest in this endeavor. In the US, the Antibiotic Development to Advance Patient Treatment (ADAPT) Act was introduced with the aim of fast tracking the drug development of antibiotics to combat the growing threat of 'superbugs'. Under this Act, FDA can approve antibiotics and antifungals treating life-threatening infections based on smaller clinical trials. The CDC will monitor the use of antibiotics and the emerging resistance, and publish the data. The FDA antibiotics labeling process, 'Susceptibility Test Interpretive Criteria for Microbial Organisms' or 'breakpoints', will provide accurate data to healthcare professionals.

1.12 Literature review:

It has been shown that the combinations of imipenem with rifampicin, tigecycline and colistin are recommended as the best therapeutic approach for treatment of nosocomial infections of *A. baumannii* because they are efficacious and show low toxicity (Al-Agamy, MH, et al. 2013).

A study conducted on90 patients infected with AB with COPD exacerbation. Co-infection with other bacterial species was shown in 41 patients. The *A.* baumannii strains showed a high resistance (90%) to fluoroquinolones, ceftazidime, piperacillin/tazobactam. The most common combination drug amoxicillin with clavulanic acid most often combined with fluoroquinolone was effective among 10% of patients (Grochowalska, A, et al. 2017).

It has been shown from another study that Gram-negative strains reported from 15 studies (12 exclusively for P. aeruginosa, 2 exclusively for Enterobacteria, 1 for both, and 1 for *Acinetobacter baumannii*) suggested that fosfomycin showed synergistic effect with gentamicin, amikacin, ceftazidime, cefepime, ciprofloxacin and levofloxacin (kastoris, AC, et al. 2010).

Sulbactam was prospectively evaluated in patients with non-life-threatening multiresistant *Acinetobacter baumannii* infections. During this period, 47 patients were treated with sulbactam; of them, five were excluded. Of the 42 patients, 39 improved or were cured and showed *A. baumannii* eradication and one patient had persistence of wound infection after 8 days of sulbactam/ampicillin requiring surgical debridement. Two patients died after 3 days of therapy. It has been shown that sulbactam was bacteriostatic; no synergy was observed between ampicillin and sulbactam and indicate that sulbactam may prove effective for non-life-threatening *A. baumannii* infections. However, the current formulation of sulbactam alone may allow its use at higher doses and provide new potential synergic combinations, particularly for those infections by *A. baumannii* resistant to imipenem (Corbella, X, et al. 1998).

A study has been conducted to compare monotherapy with combination therapy (ampicillin/sulbactam, doripenem and tigecycline) against multidrug-resistant *A.baumannii* where *in-vitro* pharmacodynamics model was used. The specific combination therapy showed additive effect against these multidrug-resistant (MDR) *A.baumannii* (Housman, ST, et al. 2013).

A study hasbeen conducted to treat carbapenem-resistant Gram-negative infections. Organ transplantation appears to be a risk factor for infections with *Klebsiella pneumoniae* and carbapenemase-producing *Enterobacteriaceae*. Aminoglycosides, polymyxins, and tigecycline have been exhibited to treat these infections. Isolation of these MDR bacteria is increasing and may be associated with mortality rate (Patel, GI, Perez, F & Bonomo, RA 2010).

Combination of two carbapenems was successfully evaluated in three patients in carbapenem-resistant *Klebsiella pneumoniae* (CR-KP)strains and administered of 1 g ertapenem 24 hourly followed 1 hour later by meropenem (2 g) every 8 hours in an infusion and carried out over 3 hours duration. The same regimen tested subsequently in 21 patients with carbapenem-resistant *K. pneumoniae* isolation showed 80.7% clinical success and 96% microbiological cure (Giamarellou H. et al. 2013).

The suppressed immune systems of AIDS patients make them more vulnerable to diarrhea caused by *Shigella flexneri* and other pathogens. The lack of helper T cells makes AIDS patients susceptible to illness and diarrhea happens which is one of the most common illnesses. However, an intensive handwashing regimen can be used to lower the incidence of diarrhea. There is an inverse relationship among AIDS patients between handwashing frequency and the occurrence of diarrhea (Huang, DB & Zhou, J 2007).

Sulbactam and imipenem were compared in an experimental pneumonia model in immunocompetent mice, using of *Acinetobacter baumannii*, and in an experimental endocarditis model in rabbits, using an intermediately susceptible strain. In the former, sulbactam was as efficacious as imipenem in terms of survival, sterility of lungs and in the bacterial clearance from lungs and blood, provided that the $t >$ MIC for sulbactam (1.84 h) was similar to that for imipenem (2.01 h). imipenem ($t >$ MIC, 2.12 h) was more efficacious than sulbactam ($t >$ MIC, 1.17 h) in bacterial clearance from vegetation. These results show the efficacy of sulbactam in infections caused by susceptible strains of *A. baumannii*, with an MIC up to 4 mg/L, provided that doses reach a$t >$ MIC similar to imipenem (Rodriguez-Hernandez, MJ et al. 2001)

The activities of monotherapy of 17 antimicrobial agents or in combination against 70 clinical isolates of *Acinetobacter baumannii* from Singapore were determined by broth microdilution. The MICs of amoxicillin, ampicillin, ceftazidime, ceftriaxone, gentamicin, and piperacillin for 90% of the strains were > or = 128 micrograms/ml. Combination of sulbactam with ampicillin produced improved activity, whereas adding tazobactam to piperacillin did not. The MICs of amikacin, ciprofloxacin, and imipenem for 90% of the strains were 32, 32, and 16 micrograms/ml, respectively (Kuah, BG et al. 1994).

2.1 Research objectives

Our research objectives are

- To develop a resistant organism after co-culture with a resistant pathogenic bacteria.
- To determine the synergistic effect of different combination of antibiotics against resistant *A baumannii.*

3.1 Agar diffusion test:

The agar diffusion test known to Kirby–Bauer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing, is a test of the antibiotic sensitivity of bacteria. The disk diffusion agar method tests the effectiveness of antibiotics on a specific microorganism. An agar plate is first spread with bacteria, then paper disks of antibiotics are added. The bacteria is allowed to grow on the agar media, and then observed. The amount of space around each antibiotic plate indicates the lethality of that antibiotic on the bacteria in question. Highly effective antibiotics (disk C) will produce a wide ring of no bacterial growth, while an ineffective antibiotic (disk A) will show no change in the surrounding bacterial concentration at all. The effectiveness of intermediate antibiotics (disk B) can be measured using their zone of inhibition. This method is used to determine the best antibiotic to use against a new or drug-resistant pathogen.

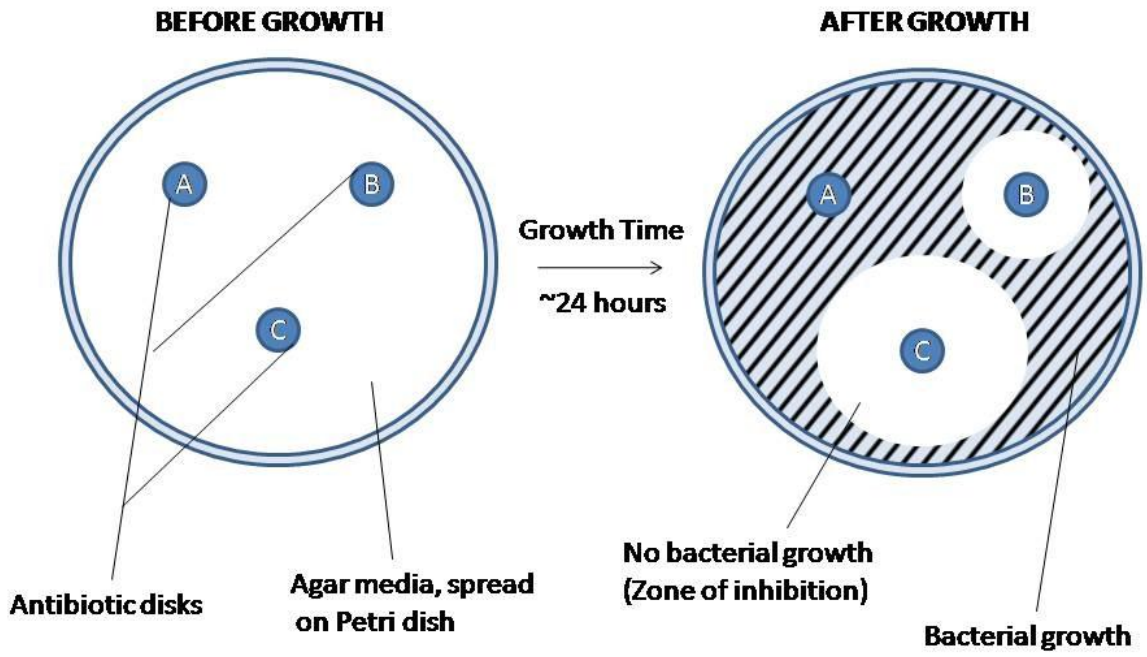


Fig 3.1: Agar diffusion test

3.2 Apparatus and reagents:

- Filter paper discs
- MacConkey agar medium
- Antibiotic:(Clarithromycin, Moxifloxacin Imipenem)
- Petri dishes
- Sterile cotton
- Micropipette
- Inoculating loop
- Sterile forceps
- Screw cap test tubes
- Autoclave
- Laminar air flow hood
- Spirit burner
- Refrigerator
- Incubator
- 0.9%Nacl solution for preparing Imipenem
- Acetone for preparing Clarithromycin
- Nose mask and Hand gloves

Fig 3.2: Apparatus

3.3 Antibiotic:

- Clarithromycin
- Moxifloxacin
- Imipenem

Drug: Clarithromycin 500mg

Drug: Imipenem 500 mg

Drug: moxifloxacin Iv 400mg

Fig 3.3 antibiotics

3.4 Test materials:

In our presence study, the anti-microbial activity

3.5 Test organisms:

These organisms were collected from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB).

3.6 List of bacteria used for screening of antimicrobial activity:

- *Salmonella typhi*
- *Shigella flexneri*

3.7 Composition of culture medium:

The following media was used to demonstrate the antimicrobial activity and for growth of the test organisms.

3.8 MacConkey Agar medium :

3.8.1 Composition:

- Bile salt
- Crystal violet
- Lactose
- Neutral red
- A selective and differential media used to differentiate between gram negative bacteria while inhibiting the growth of gram positive bacteria
- The additional bile salt and crystal violet to the agar inhibits the growth of most Gram-positive bacteria, making MacConkey agar selective
- Lactose and neutral red are added to differentiate the lactose fermenters which form pink colonies, from lactose no fermenters that form clear colonies
- we used MacConkey agar in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

3.9 Nutrient agar medium:

3.9.1 Composition:

- Bacto peptone
- Sodium chloride
- Bacto yeast extract
- Bacto agar
- Distilled water q.s.
- PH
- Nutrient agar medium is the most frequently used

3.10 Preparation of the medium:

MacConkey Agar media was prepared by adding water to a dehydrated product that contains all the ingredients. Normally all media are available commercially in powdered form.

Media of the MacConkey Agar type was prepared by compounding the required individual ingredients or, more conveniently, by adding water to a dehydrated product which contains all the ingredients. Practically all media are available commercially in powdered form. The following steps were involved in the preparation of bacteriological media :

- Definite amounts of MacConkey agar were accurately weighed.
- It was taken in a volumetric flask containing distilled water (half of the required volume).
- A clear medium was obtained by thorough dissolving agar over a water bath with occasional shaking.
- Then the final volume was adjusted.
- The medium was then transferred in 16 ml and 5 ml volume respectively, to prepare plates and slants, in a number of test tubes.
- The test tubes were then plugged with cotton and sterilized in an autoclave at a temperature of 121°C and pressure of 15 lbs./sq. inch for 15 minutes.

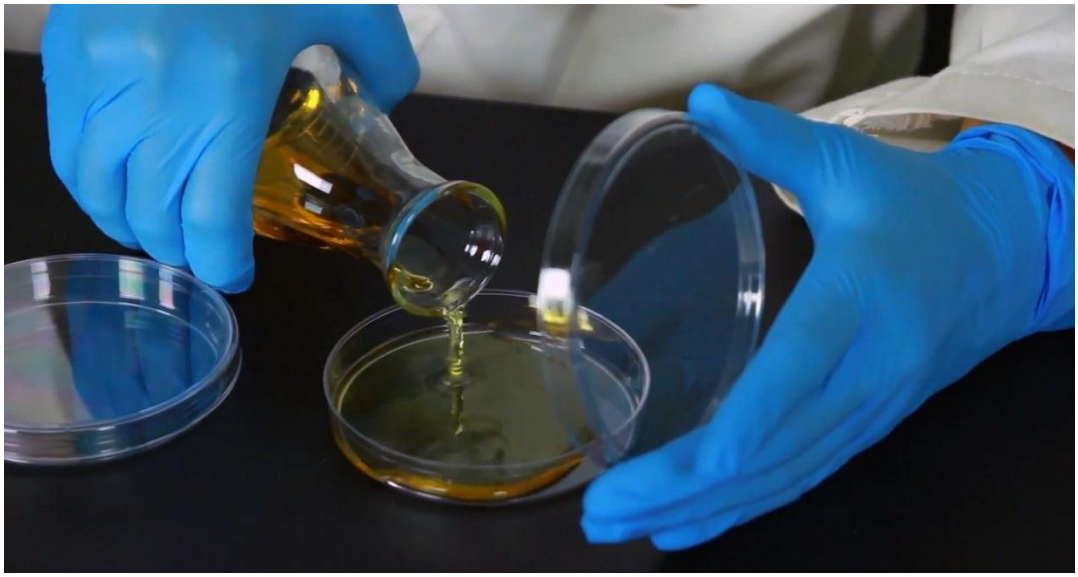


Fig 3.4: medium

3.11 Sterilization procedure:

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

3.12 Preparation of sub-culture:

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

Fig 3.5: sub-culture

3.13 Preparation of test plates:

- Each of the test organisms were transferred from the subculture to the test tube containing 16 ml autoclaved media with the help of the sterilized inoculating loop at 45°c in an aseptic area.
- The test tubes were shaken by rotation to get a uniform suspension of organism. The bacterial suspensions were immediately transferred to the sterile Petri dishes aseptically.
- The Petri-dishes were rotated several times, first clockwise and then anticlockwise, to assure homogeneous distribution of the test organisms.
- The medium was poured into Petri-dishes in such a way as to give a uniform layer, after the medium became cooled and stored in a refrigerator (4°c).

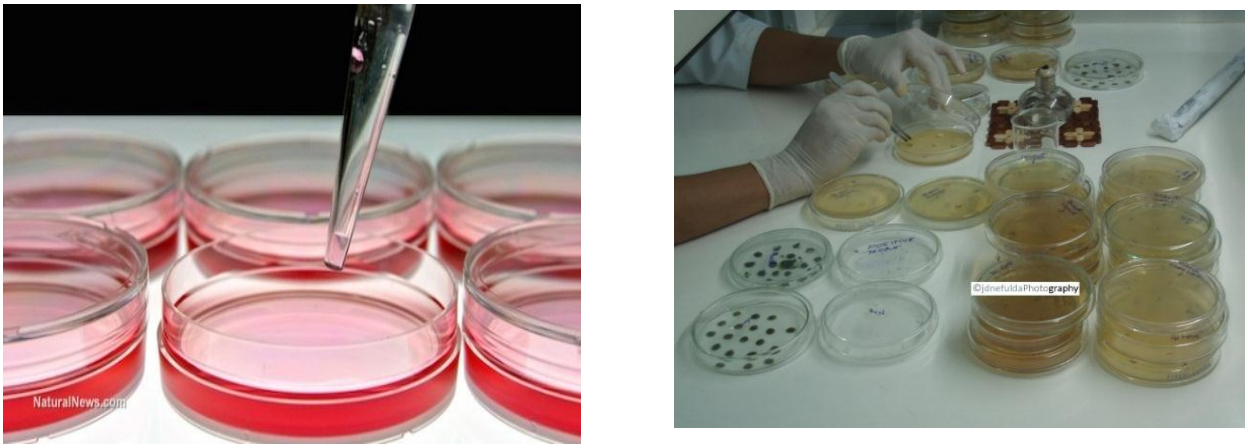


Fig 3.6: Preparation of test plates

3.14 Preparation of Clarithromycin antibiotic stock solution:

- Antibiotic powders were obtained commercially.
- Known weight of antibiotic powder was dissolved in sterile distilled water to obtain the stock solution.
- The stock solution was diluted at the time of disc preparation to obtain the working solution.
- A paper disc of 6mm diameter can absorb 0.02ml or 20µl of solutions. The concentrations of antibiotic solutions were expressed in µg/ml.

3.15 Preparation of Moxifloxacin antibiotic stock solution:

- Antibiotic powders were dissolved in sodium chloride solution (0.8% w/v)
- The stock solution was diluted at the time of disc preparation to obtain the working solution.
- A paper disc of 6mm diameter can absorb 0.02ml or 20µl of solutions. The concentrations of antibiotic solutions were expressed in µg/ml.

3.16 Preparation of Imipenem antibiotic stock solution:

- Antibiotic powders were dissolved in saline water (0.9% NaCl solution).
- Before using the saline water, this was sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes.
- The stock solution was diluted at the time of disc preparation to obtain the working solution.
- A paper disc of 6mm diameter can absorb 0.02ml or 20µl of solutions. The concentrations of antibiotic solutions were expressed in µg/ml.

3.17 Disk diffusion method using antibiotic drug:

3.18 Preparation of disk:

Three types of disks were used for antibacterial screening:

- Sample disks
- Standard disks and
- Blank disks

3.19 Methods of disk preparation:

- Preparation of the filter paper discs involves the punching of holes of approximately 6mm diameter in Whatman filter paper and was sterilized in an autoclave.
- The next step in the study was the preparation of antibiotic stock solutions, where a known weight of the antibiotic powder is dissolved in the sterile distilled water and was stored in the refrigerator for future use. The stock solution was diluted to obtain the working solution.
- The following step was the impregnation of the discs where the antibiotic solutions are loaded on each disc using a mechanical pipette.
- The discs were dried in an incubator and stored in small ampoules with a desiccant at minus 20 °C.

Fig 3.7: Disk preparation

3.20 Disc diffusion and incubation period:

The sample discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours for bacteria culture.

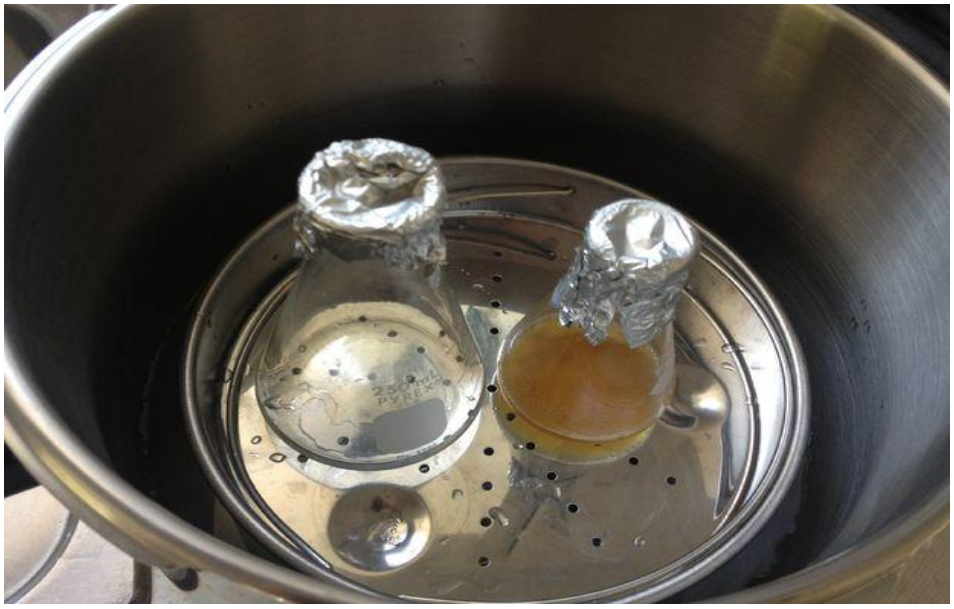


Fig 3.8: incubation

3.21 Apply the disc into agar plate:

Sample impregnated discs, standard antibiotic discs (clarithromycin discs) and negative control discs (blank discs) were placed gently on the 09 separate agar plates, with the test organisms with the help of a sterile forceps to assure complete contact with medium surface. The arrangement of the discs was such that the discs were not closer than 15mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in refrigeration for about 4 hours at 4°c. This was sufficient time for the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°c for 12-18 hours.

Fig 3.9: Disc diffusion

3.22 Determination of the zone of inhibition:

The zone of inhibition is measured by their activity to prevent the growth of the microorganisms surrounding the discs. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent measuring scale.

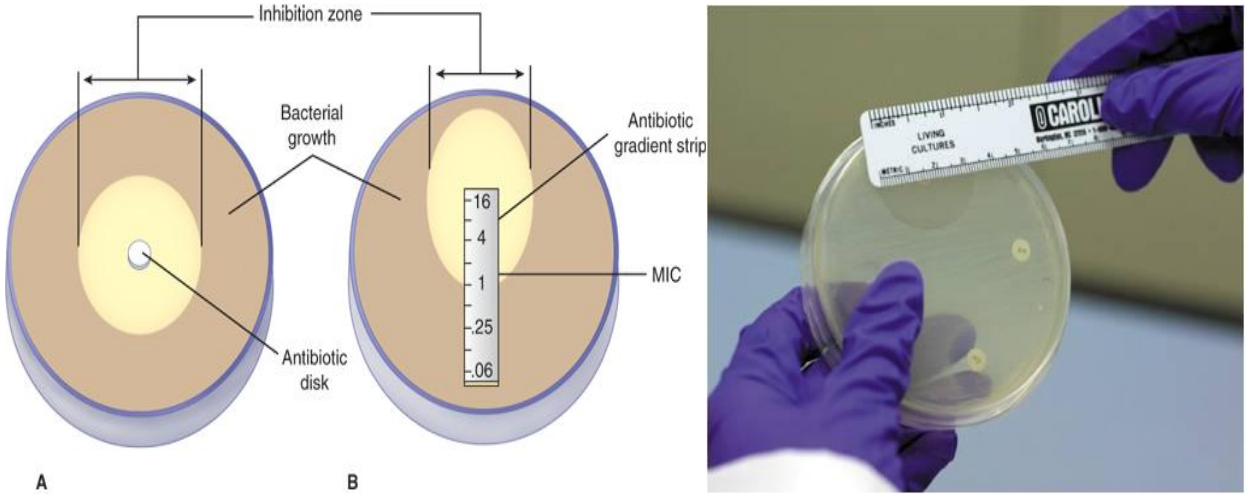


Fig 3.10 : zone of inhibition

Result

In this experiment we investigated the antimicrobial effect of combination antibiotics (clarithromycin, moxifloxacin, Imipenem) against *Acinetobacter baumannii* after co-culture with resistant *Shigella flexneri* .

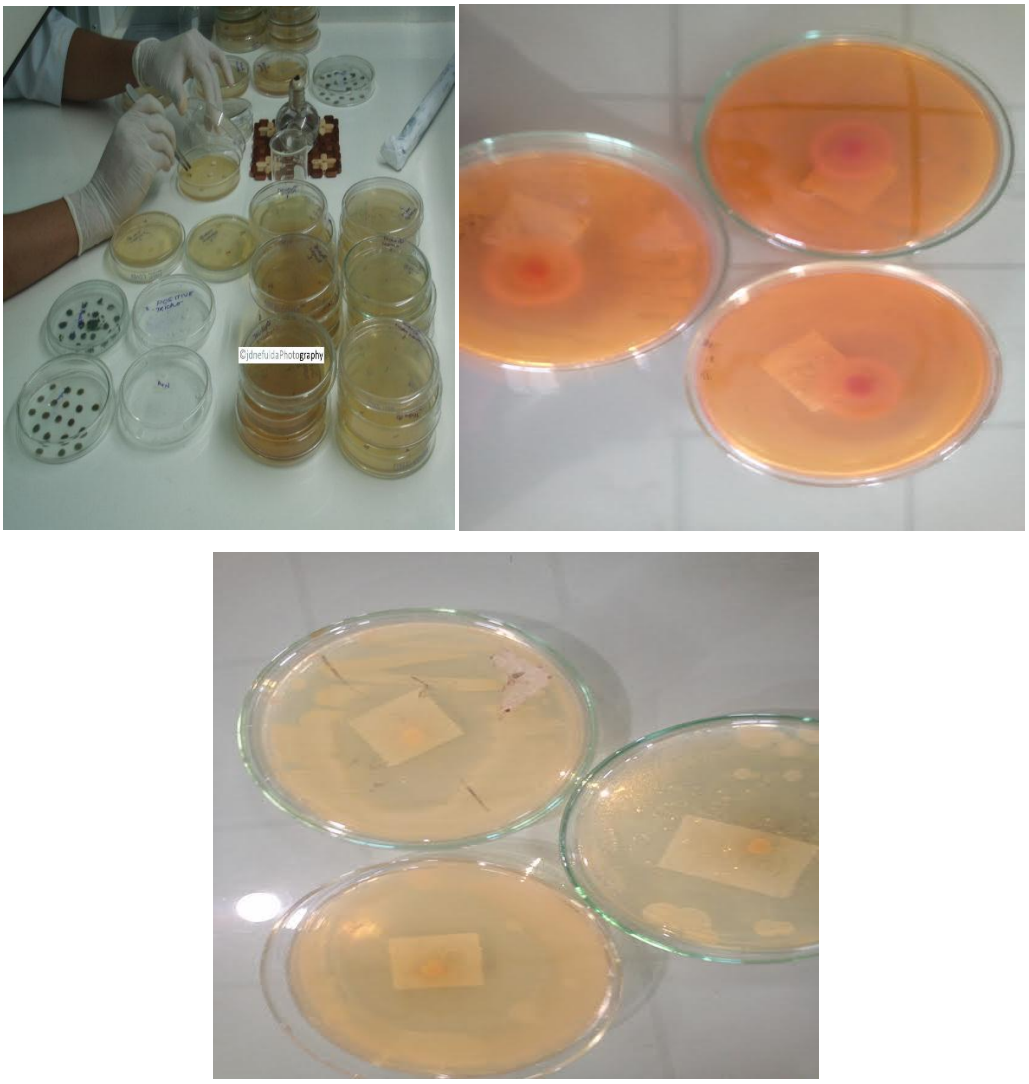


Figure 4.1 Clear zone of inhibition

Table no. 4.1

Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem (with cilastatin) against *Shigella flexneri* and *Acinetobacter baumannii* with different concentration.

Organism	Media	Antibiotic	Strength	Concentration µg/ml	Zone of inhibition (mm)
Acinetobacter baumannii	MacConkey Agar	Clarithromycin	500 mg	20	19
				40	21
				60	22
				80	24
		Moxifloxacin	400 mg	20	26
				40	28
				60	29
				80	30
		Imipenem	500 mg	20	26
				40	27
				60	28
				80	30
Shigella flexneri	MacConkey Agar	Clarithromycin	500 mg	20	0
				40	0
				60	0
				80	0
		Moxifloxacin	400 mg	20	0
				40	0
				60	0
				80	5
		Imipenem	500 mg	20	0
				40	0
				60	0
				80	4

According to table 4.1 Over the course of 24 h, we observed that co-cultured *Acinetobacter baumannii* showed sensitivity against Clarithromycin, Moxifloxacin, imipenem. The zone of inhibition (ZOI) was 19mm21mm, 22 mm and 24 mm when the concentration of Clarithromycin was 20, 40, 60, 80 (µg/ml). While zone of inhibition was 26mm 28 mm 29mm 30 mm of Moxifloxacin with the concentration of 20,40,60,80(µg/ml). Zone of inhibition of Imipenem was 26mm 27 mm28mm 30mm with the concentration of 20, 40, 60, 80 (µg/ml). On the other hand, *Shigella flexneri* did not show any zone when treated with the same antibiotic with same concentration.

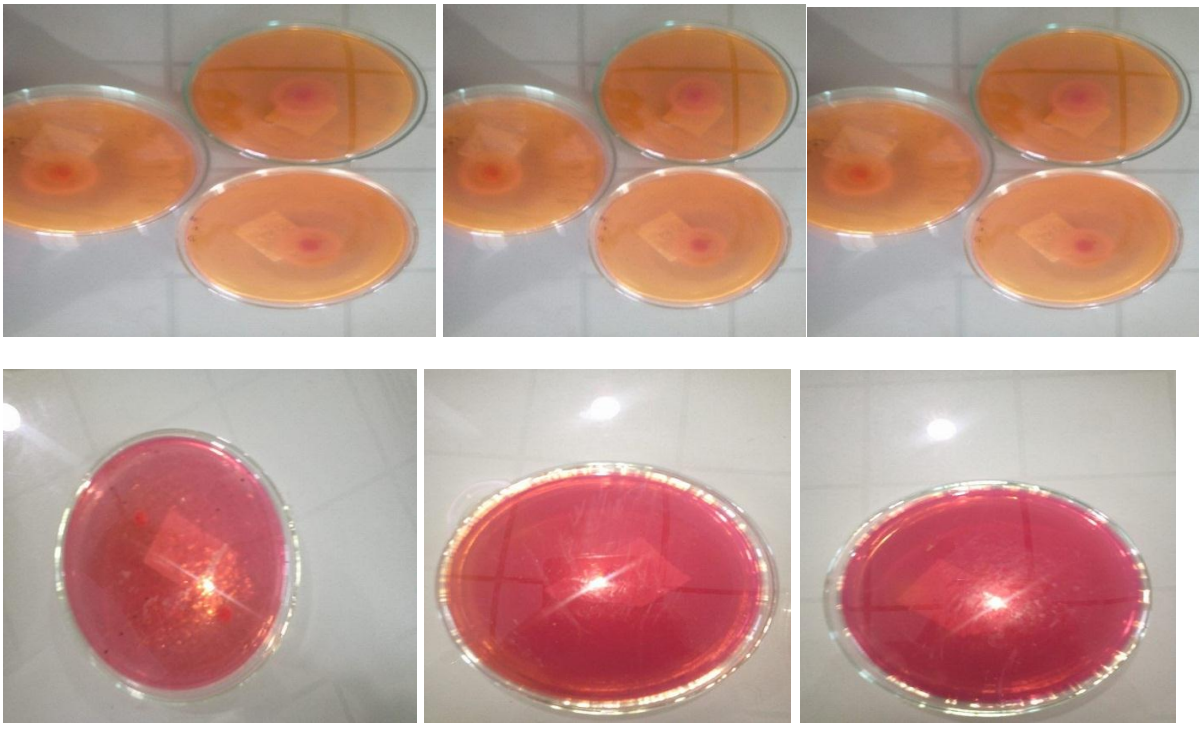


Fig 4.2 : Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem against *Shigella flexneri* and *Acinetobacter baumannii* with different concentration.

Table no. 4.2

Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem (with cilastatin) after Co culture of *Acinetobacter baumannii* with resistant *Shigella flexneri*

Organism	Media	Antibiotic	Strength	Concentration (µg/ml)	Zone of inhibition (mm)
Acinetobacter baumani & *Shigella flexneri*	MacConkey Agar	Clarithromycin	500 mg	20 µg/ml	0
				40 µg/ml	0
				60µg/ml	0
				80 µg/ml	0
		Moxifloxacin	400 mg	20 µg/ml	0
				40 µg/ml	0
				50 µg/ml	0
				80 µg/ml	5
		Imipenem	400 mg	20 µg/ml	0
				40 µg/ml	4
				60 µg/ml	8
				80 µg/ml	14

According to table 4.2 Over the course of 24 hr., we observed that co-cultured *Acinetobacter baumani* and *Shigella flexneri* showed resistant against Clarithromycin. The zone of inhibition (ZOI) was 0mm when the concentration of Clarithromycin was increased 20µg/ml, 60µg/ml, 80µg/ml. Respectively of clarithromycin. Moxifloxacin, Imipenem was applied to the co-culture plates of *Acinetobacter baumani* and *Shigella flexneri* with the concentration of 20µg/ml, 60µg/ml, 80µg/ml and the ZOI was less.

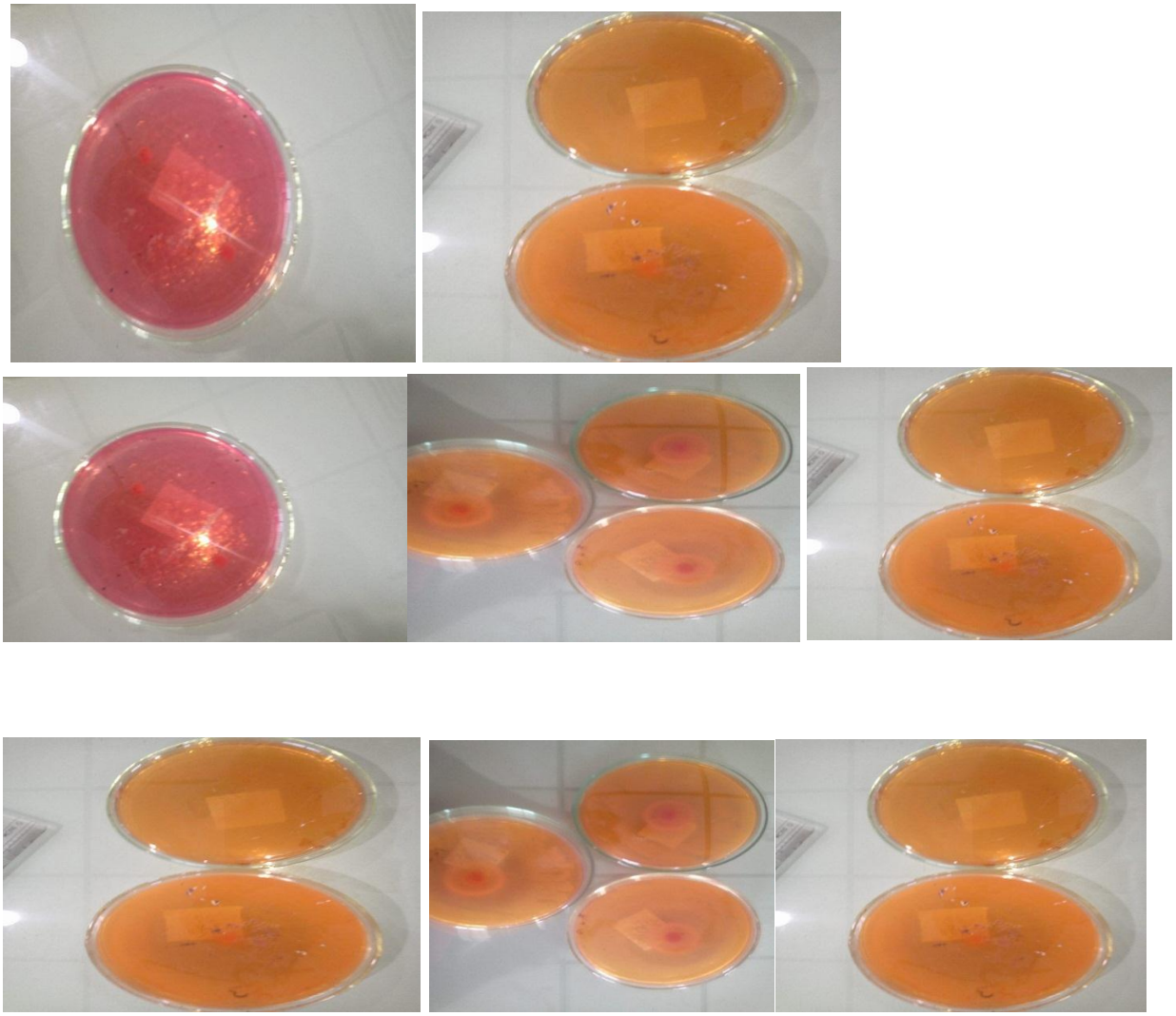


Fig 4.3 : Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem (with cilastatin) after Co culture of *Acinetobacter baumanii* with resistant *Shigella flexneri*

Table no. 4.3

Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem (with cilastatin) with increasing concentration after Co culture of *Acinetobacter baumanii* with resistant *Shigella flexneri*

Organism	Media	Antibiotic	Strength	Concentration µg/ml	Zone of inhibition Mm
Shigella flexneri & *Acinetobacter baumanii*	MacConkey Agar	Clarithromycin	500 mg	120	0
				160	0
				180	3
		Moxifloxacin	400 mg	120	0
				160	0
				180	6
		Imipenem	500 mg	120	4
				160	6
				180	8

According to table 4.3 Over the course of 24 hr., we observed that co-cultured *Acinetobacter baumani* and *Shigella flexneri* showed resistant against Clarithromycin. The zone of inhibition (ZOI) was 0mm when the concentration of Clarithromycin was increased 120µg/ml, 160µg/ml, 180µg/ml. Respectively of Moxifloxacin, Imipenem was applied to the co-culture plates of *Acinetobacter baumannii* and *Shigella flexneri* with concentration of 120µg/ml, 160µg/ml, 180µg/m and the zone of inhibition was less.

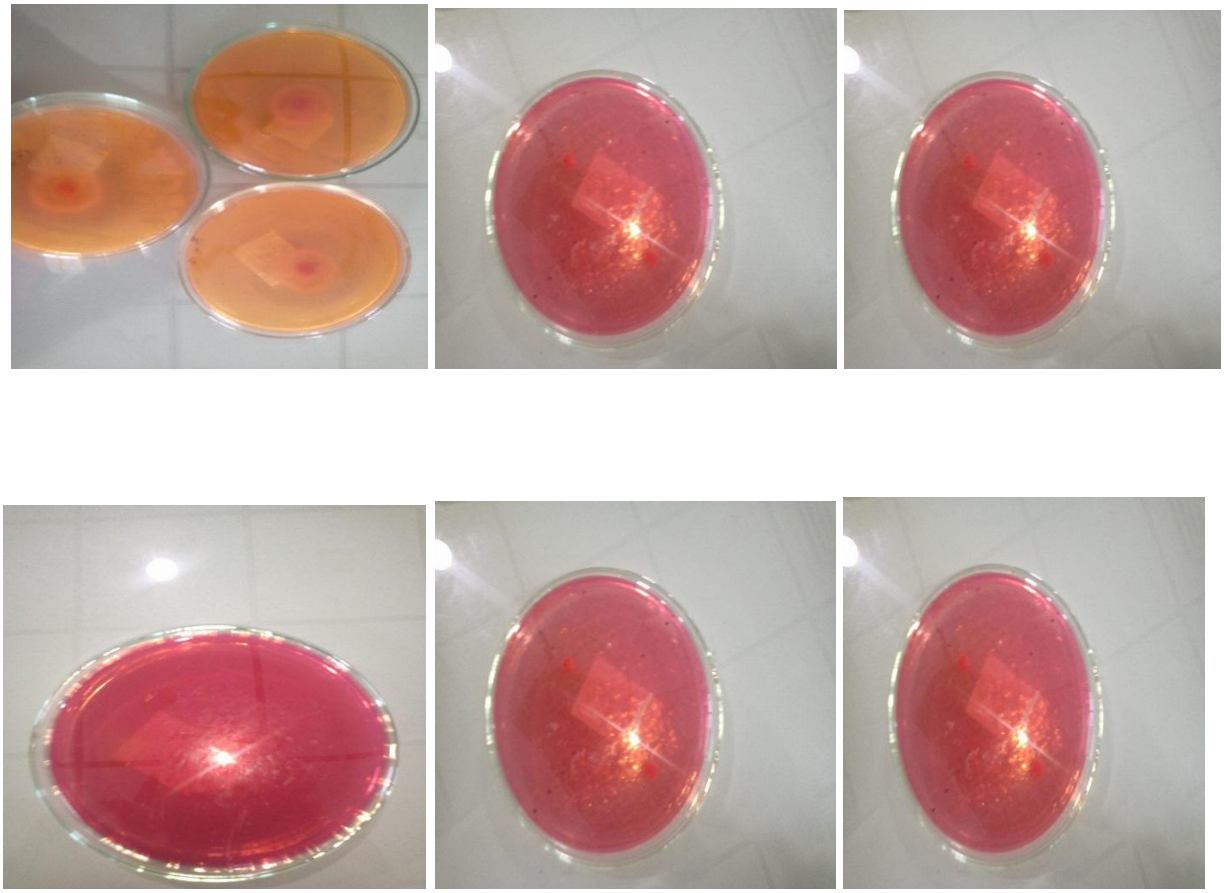
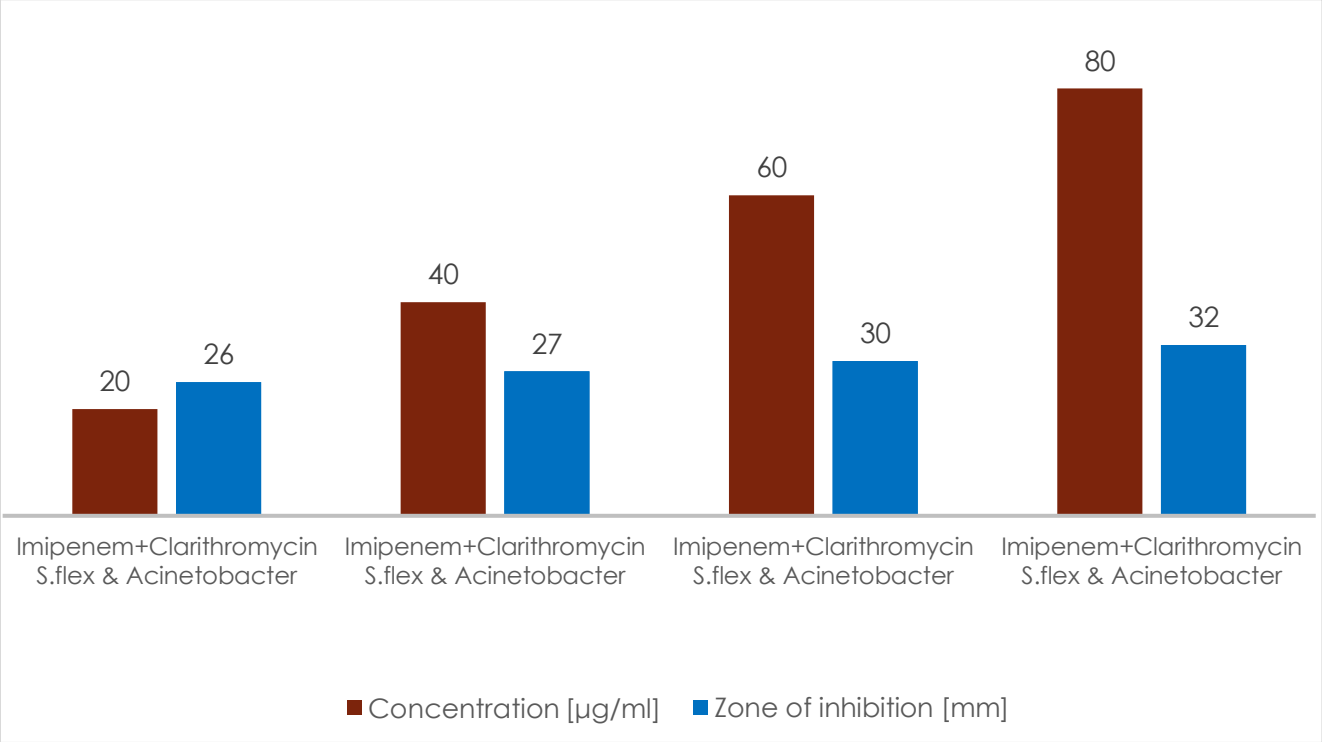


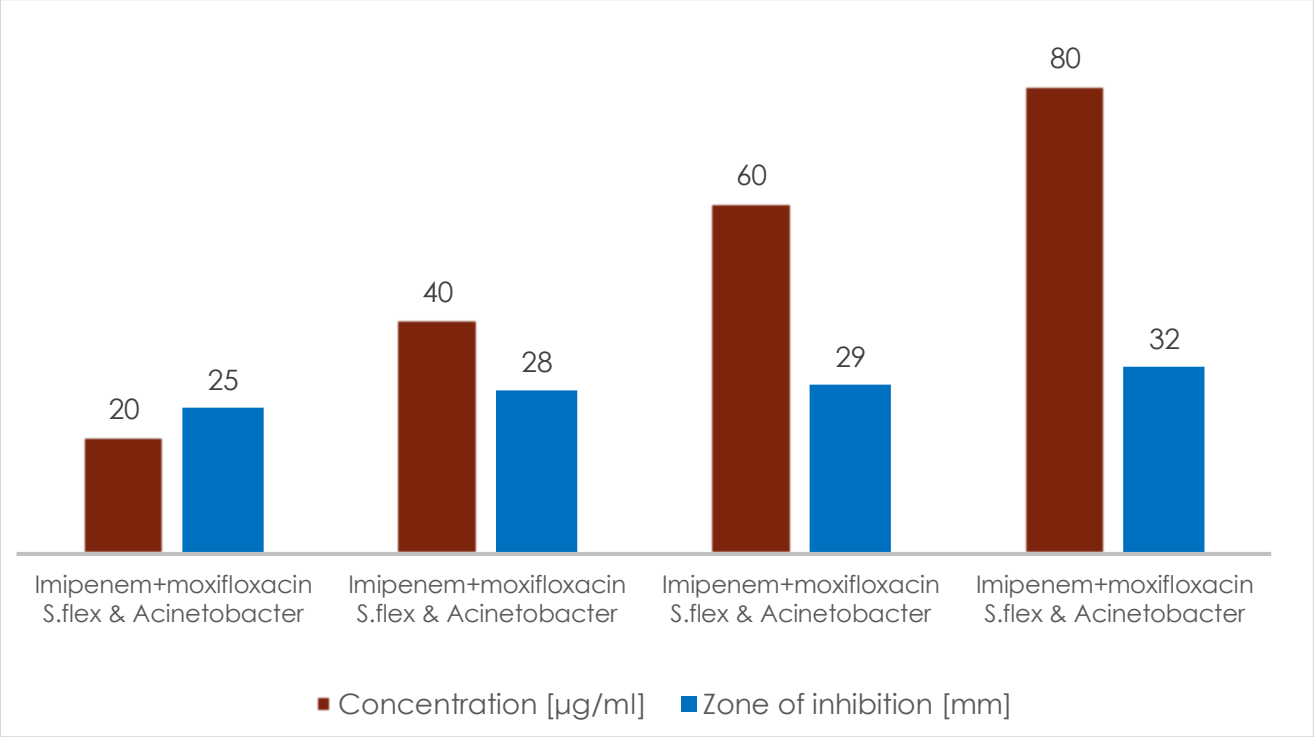
Fig 4.4 : Zone of inhibition after treatment with Clarithromycin, Moxifloxacin, imipenem (with cilastatin) with increasing concentration after Co culture of *Acinetobacter baumanii* with resistant *Shigella flexneri*

Figure 4.5: ZOI of *Acinetobacter baumannii* after treatment with clarithromycin & imipenem combination



According to the figure 4.5 Over the course of 24 hr., we observed that co-culture of Acinetobacter *baumani* and *Shigella flexneri* showed sensitivity against the combination of Clarithromycin and Imipenem . The zone of inhibition (ZOI) was respectively 26 mm,27mm,30mm and 32 mm against the concentration of 20µg/ml, 40 µg/ml, 60µg/ml, 80µg/ml.

Figure 4.6: ZOI of *Acinetobacter baumannii* after treatment with Moxifloxacin & imipenem combination



According to the Figure 4.6 Over the course of 24 hr., we observed that co-culture of *Acinetobacter baumani* and *Shigella flexneri* showed sensitivity against the combination of Moxifloxacin and Imipenem. The zone of inhibition (ZOI) was 25 mm,28mm,29 mm and 32 mm respectively against the concentration of 20µg/ml. 40 µg/ml, 60µg/ml, 80µg/ml. ZOI has increased with the increasing concentration of combination antibiotics.

5.1 Discussion and Conclusion:

Despite a reputation for relatively low virulence, multidrug-resistant *Acinetobacter* infection poses a formidable threat to patients. The cause of many outbreaks, this organism is increasingly endemic in the health care setting. Multidrug-resistant *Acinetobacter and Shigella* infections have extremely high crude mortality rate and occur most frequently in severely ill patients (Fournier, PE, Richet, H & Weinstein, RA 2006). The prevalence of multidrug resistance with *A. baumannii* strains have been increasing during recent years (Mahgoub S,Ahmed, J & Glatt, AE 2002). This organism has been shown to cause highly mortal hospital infections (Patel, G1, Perez, F & Bonomo, RA 2010)

Acinetobacter baumannii constitutes a severe problem in many ICUs worldwide. The very limited therapeutic options available for these organisms are a matter of great concern. No specific guidelines exist addressing the prevention and management of *A. baumannii* infections in the critical care setting (Garnacho-Montero J et al. 2015).

In our study, initially *Acinetobacter baumannii* was sensitive against the clarithromycin, imipenem and moxifloxacin in different concentration of 20, 40, 60 and 80µg/ml. However, same concentrations of clarithromycin, imipenem and moxifloxacin showed no zone of inhibition against *Shigella flexneri*. After co-culture with two bacteria, both of them did not show optimum zone of inhibition against clarithromycin, imipenem and moxifloxacin at concentration of 20, 40, 60, and 80µg/ml respectively. When high concentrations (120, 160, 180µg/ml) of clarithromycin, Imipenem and moxifloxacin were used against the co-cultured pathogen, still no sensitivity was observed against the treated antibiotics.

A baumannii (AB) is a pathogen which may cause nosocomial infections in critically illed patients particularly the patients who are on the support of ventilator. Antibacterial resistance has been observed in patients infected with AB. It has been discussed in literature that thoughtful antibiotic strategies should be developed to inhibit this notorious pathogen. Combination of antimicrobial therapy of existing antibiotics may require to prevent the infections (Lynch, JP 3rd, Zhanel, GG & Clark, NM 2017). In our study, we have found the sensitivity of different

combination antibiotics against AB. The combination antibiotics were Imipenem and moxifloxacin in different concentration of 20, 40, 60, and 80μg/ml.

It has been shown from another study that the combinations of imipenem with rifampicin, tigecycline and colistin are recommended as the best therapeutic approach for treatment of nosocomial infections of *A. baumannii* because they are efficacious and show low toxicity (Al-Agamy, MH, Shibl, AM & Ali, MS, 2013). Although our study was only based on *in vitro* experiment, we found that the combination of Clarithromycin and imipenem exhibited satisfactory zone of inhibition against AB.

A study conducted on 90 patients infected with AB with COPD exacerbation. The *A. baumanni* strains showed a high resistance (90%) to fluoroquinolones, ceftazidime, piperacillin/tazobactam. It has been shown that the most common combination drug amoxicillin with clavulanic acid most often combined with fluoroquinolone was effective among 10% of patients (Grochowalska, A, Koziol-Montewka, M & Sobieszczanska, A 2017). Antibiotic combinations can be used as an alternative treatment approach in multi-drug resistant infections.

It has been shown that gram-negative strains reported from 15 studies showed synergistic effect of fosfomycin with gentamicin, amikacin, ceftazidime, cefepime, ciprofloxacin and levofloxacin Kastoris, AC, 2010). In our study the result of the combination antibiotic treatment also shows 10% greater effectiveness compared with single antibiotic against resistant pathogen which indicates synergistic effect of antibiotics.

Combination of two carbapenems was successfully evaluated and tested in 21 patients with carbapenem-resistant *K. pneumoniae* isolates. About 81% clinical successes and 96% microbiological cure were observed (Giamarellou H. et al. 2013). *K. pneumonia* (KP) is responsible for nosocomial infection in hospitalized patients, sensitivity of the antibiotic combinations should be tested in our lab against the clinical isolates of KP in Bangladeshi patients.

The activities of monotherapy of 17 antimicrobial agents or in combination against 70 clinical isolates of *Acinetobacter baumannii* from Singapore were determined by broth microdilution. The MICs of amoxicillin, ampicillin, ceftazidime, ceftriaxone, gentamicin, and piperacillin for

90% of the strains were > or = 128 micrograms/ml. Combination of sulbactam with ampicillin produced improved activity, whereas adding tazobactam to piperacillin did not. The MICs of amikacin, ciprofloxacin, and imipenem for 90% of the strains were 32, 32, and 16 micrograms/ml, respectively (Kuah, B. G. et al. 1994). In our present study, we did not perform experiment on MICs against our resistant pathogens. Future studies are required to observe the MICs of antibiotics used in the study.

The combination of antibiotics against various resistant pathogens may be effective in clinical setting. As the antibiotics resistance is increasing day by day, combination antimicrobials with the existing antibiotics may have greater values to inhibit MDR pathogens.

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