AMERICAN UNIVERSITY OF BEIRUT

THE OUTCOME OF COLISTIN USE FOR THE TREATMENT OF MULTIDRUG RESISTANT ACINETOBACTER BAUMANII IN CRITICALLY ILL PATIENTS AT A TERTIARY CARE CENTER

By

Ali H. Zayter

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By

ALI H. ZAYTER

Approved by:

Dr Abla Sibaii, Professor — Advisor
Dept. of Epidemiology and Population Health

Dr Ali Hallal, MD Assistant Professor — Member of Commitee
Dept. of Surgery, Intensive Care Unit

Dr Zeina Kanafani,MD Associate Professor — Member of Commitee
Dept.of internal Medicine, Infectious Diseases

Date of thesis defense:January 28,2015

Date of thesis defense: January 28, 2015

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AN ABSTRACT OF THE THESIS OF

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Title: The Outcome of Colistin Use for the Treatment of Multidrug Resistant Acinetobacter baumanii in Critically Ill Patients at a Tertiary Care Center

The prevalence of nosocomial infections caused by Multi-drug resistant gram negative bacteria (MDRGNB) has been dramatically increased in the past few decades. It was noted that despite the presence of wide range of commercial antibiotics that are intended to treat these types of infections, the emergent resistance and virulence of some gram negative bacterial species and strains had limited the clinicians choices and focused their attention towards the reintroduction of an old antibiotic (polymyxin E) discovered 50 years ago and was abandoned since then because of its questioned safety and efficacy.

This study was conducted to display and examine with an eye to the differences between two types of treatments for gram negative bacteria. Currently, there is no available study that rendered a formal statement about the meaningful impinging of colistin re use on patient's outcomes especially those who acquired intensive care unit infection with pulmonary or blood stream multi drug resistant (MDR) acinetobacter species infection.

The aims of our study are to investigate whether the reintroduction of colistin into clinical practice to treat patients with MDR acinetobacter species have significantly improved the 30-days patient's in-hospital stays compared to those patients who were treated with other used antibiotics and to question the resulting nephrotoxicity status in both groups.

Retrospective cohort with external comparison group; Risk factors for mortality and nephrotoxicity were investigated in both the colistin and the non colistin group. The colistin group consisted of patients who were acinetobacter MDR and treated with intravenous or inhaled or both intravenous/inhaled colistin with or without other combined antimicrobials. The non-colistin group included patients who were treated with combinations of antibiotics used to treat MDR acinetobacter baumanii. Both treatment groups were adjusted to the severity of illness by calculating the SOFA score within 24 hours of admission, Pittsburgh bacteremia score at the onset of bacteremia and Charlson's co-morbidity score, length of intensive unit stay pre and post infection, mechanical ventilation, invasive central lines, arterial line, Nasogastric tube, urinary indwelling catheter, previous antibiotic use, recent surgery, reason for admission, age, gender, underlying disease, immunosuppression including neutropenia, Hypoalbumenia and concomitant use of other possible nephrotoxic agents and antimicrobials were also calculated and recorded. Clinical and demographic characteristics were firstly compared be-

tween the colistin and the non colistin group by which significant differences between groups were noted as indicated by the p-values obtained for Sofa score, 30 days in hospital mortality, admission reason (sepsis), use of carbapenems and Tigacycline.

Bivariate analysis was conducted to examine the association between clinical and demographic variables and 30 days in hospital mortality. All variables with p-value ≤ 0.2 at the bivariate level were included in the multivariate analysis. All the independent variables were tested for both multicollinearity and interaction between each other. Unadjusted and adjusted odd ratios were reported with their respective 95% OR confidence intervals.

55 patients with multidrug resistant acinetobacter baumanii were included in the colistin group compared to 27 patients in the non colistin group. Presence of coronary artery disease (OR 5.55, 95%CI 1.37--22.45, P-value 0.016), Immunosuppressed patients (OR 6.18, 95%CI 1.34--28.47, P- value 0.019) and post infection length of hospital stay (OR 0.88, 95%CI 0.824--0.9527) were independently and significantly associated with 30 days in-hospital mortality. Colistin group, the treatment under investigation was not a significant predictor of 30 days in-hospital mortality with (OR 5.04, 95%CI 0.98---25.94, P-value 0.053).Nephrotoxicity status was not significantly associated with almost all the proposed variables to cause renal impairment in both groups except for the use of chemotherapeutic agents (OR 21.52, 95% CI 1.71--270.30, P-value 0.0.17).

Colistin treatment is an effective and safe for severely ill patients admitted to the intensive care units in terms of 30 days in-hospital mortality and nephrotoxicity when there is no other alternative therapy. But, it is still the need to conduct clinical trials that represent the most potent studies to pursue and draw rationalized definitive conclusions about the safety and efficacy of colistin use in clinical practice.

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CHAPTER I

INTRODUCTION

The emergence of antibiotic resistance has been acknowledged as an ominous indicator for both clinical and public health outcomes (Landman et al, 2002). The augmented bacterial resistance is assumed to result in higher mortality rates, longer hospitalization periods and consequently increased healthcare cost (Acar, 1997, Holmberg et al, 1987).

In health care settings, the increased prevalence of gram negative bacteria and its outgrowth of multi drug resistant antibiotic especially in intensive care units are of such extreme interest (Gaynes et al, 2005,Fridkin et al, 2001).The emergence of resistance to carbapenems and further antibiotics has been lately renowned world-wide (Grundmann et al, 2010).

Acinetobacter baumanii has been perceived as one of the most virulent opportunistic pathogen that is able to cause both community and hospital acquired infections (Fournier &Richet, 2006).The organism's ability to attain resistance to almost all existing antibiotics and stay alive in different environmental circumstances have passively contributed to its transmission during outbreaks (Lolans et al, 2006, Jawad et al 1998&Wendt et al 1997).

The genus Acinetobacter consists of strictly aerobic, gram-negative coco-Bacilli that are capable of causing both community and health care associated infections and outbreaks including ventilator-associated pneumonia, blood stream infections, urinary tract infections and wound infections (Fournier et al 2006, Hidron et al 2008).

It is evident that the pathogen potentials to cause community and heath care associated infections, continue to exist in different environmental conditions and gain resistance mechanisms had restricted the treatment options for both the clinicians and patients (Fournier &Richet, 2006).In their struggle against bacterial resistance, the infectious disease specialists have been strained to devise into clinical practice an old drug brought to light 50 years ago (Falagas &Kasiakou, 2005).

Colistin is being used as the first line treatment for severe multidrug resistant gram negative bacterial infections. Meanwhile, it is unclear whether Polymyxin E (colistin) has decreased the mortality rates of the treated patients or its use is an independent predictor for nephrotoxicity. A number of questions remain unresolved concerning the clinical outcomes of using colistin to treat MDR A. baumanii mainly hospital mortality rates and potential adverse effects including nephrotoxicity.

The specific objectives of our study are to investigate whether the reintroduction of Colistin (colisimethate sodium) into clinical practice has significantly improved the 30-day in hospital mortality of patients with MDR Acinetobacter species pulmonary and/or blood stream infections treated with colistin compared to those who were not treated with colistin and the resulting nephrotoxicity among both groups.

In Lebanon, Due to the lack of national healthcare facilities network that conduct regular prevalence surveys to assess the burden and extent of multi drug resistant bacteria, the knowledge about opportunistic hospital acquired infections mainly multi drug resistant acinetobacter baumanii was not addressed as other infections till late 2003.

The main limitation was the inability to identify the organism correctly at early times because of non-reactivity in many biochemical tests plus one or more type of non-lactose fermenters gram oxidase negative clones were often coexisted in cultures.

The infection control and prevention program at AUBMC promptly implemented an infection control and prevention standards that effectively helped in interrupting the transmission of infection through special attention to shared items, environmental settings and standard clinical practices.

The thesis is composed of five chapters; the first chapter introduced the topic in its clinical and public outcomes, by brief definition of the studied organism, its ability to acquire resistance, cause outbreaks and the proposed treatments. The second chapter included literature review about the epidemiology and habitat of the organism, clinical characteristics, microbiological definition of acinetobacter baumanii, risk factors, outbreaks and prevalence, patient outcome, treatment challenges ,surveillance and control measures as well as all the studies that pointed this subject by other researcher's. The third chapter consisted of the methodology used to conduct this study, data collection sheet, the primary dependent variable, the secondary dependent variable, the aims and objectives of the study and the analysis plan. The fourth chapter included some descriptive, comparison between the two treatment groups, univariate and multivariate analysis of the primary outcome as well as univariate and multivariate analysis of the secondary outcome. The fifth chapter discussed the final analysis findings, study limitations, strengths and the recommendations.

CHAPTER II

LITERATURE REVIEW

A- Epidemiology and Habitat

Acinetobacter is strictly aerobic, non-motile, non-lactose fermenting, oxidase-negative, catalase positive gram negative cocco bacilli. It consists initially of 30 genomic species. Extending to the present time only 17 species have been given names, 3 of these species have been established to cause human disease (Falagas &karageorgopoulos, 2008).

Acinetobacter species are ubiquitous pathogens that can be retrieved and isolated from soil, water, animals, humans, food and arthropods (Ash et al, 2002, La Scola et al, 2001, Houng et al, 2001).

A study conducted by Berlau and his colleagues on 192 healthy volunteers had showed the existence of different isolates of acinetobacter species on human skin (Berlau, Aucken, Malnik& Pitt, 1999)and on fresh fruits and vegetables bought by people (Berlau, Aucken, Houang &Pitt, 1999).Distinctive features of acinetobacter infections have been reported during wars and natural disasters by which blood stream, wound and respiratory acinetobacter species were isolated from military personnel injured in Iraq and Afghanistan and following tsunami stroked southeast Asia in2004(Maegele et al,2005,CDC,2004).Among various acinetobacter species, acinetobacter baumanii has recently brought into focus the clinical attention (Chuang et al, 2011).

Recent study has established the ability of acinetobacter baumanii to cause severe community infections of both pneumonia and bacteremia especially among heavy smokers and alcohol users (Falagas et al, 2007).

B- Clinical characteristics

In hospital settings, acinetobacter baumanii is frequently isolated from the patient's throat, respiratory tract, blood and environmental settings. The organism's outbreaks distribution have been determined due to its seasonality (Leung et al, 2006, MC Donald et al, 1999) where more cases of blood stream infections and pneumonia reported in wet periods from July till October (MC Donald et al, 1999).Several investigators have recognized a significant association between environmental reservoirs and the transmission of organism during outbreaks (Jawad et al 1998, Wendt et al 1997).

Critically ill patients with nosocomial infections are the most affected subjects in terms of increased cost, more days of mechanical ventilation dependency and extended length of intensive care and hospital stays (Blot et al, 2005).Ventilator associated pneumonia and blood stream infections are the most prominent manifestations of acinetobacter baumanii infections(Price &Weinstein, 2008).These infections are thought to increase length of ICU stay by an average of 2.03 and risk of death by 14% (INICC,2011).In Lebanon ,INICC reported an excess length of ICU stay due to nosocomial infections mainly ventilator associated pneumonia by -0.17 but this decrease was not statistically significant with 95%CI(-3.31---2.96)and relative risk of death 0.74, 95%CI(0.21---2.59)(INICC,2011).Despite the organism's negative impacts on patient's outcomes, it is added also its ability to acquire rapid and extensive antimicrobial resistance to almost all commercial antibiotics(Lolans et al, 2006, Garnacho-Montero et al ,2010).

The wide spread of acinetobacter baumanii multidrug resistant strains represent a serious threat to the local and national health (Coelho et al, 2004).It is believed that inadequate antimicrobial therapy for intensive critical patients is associated with increased risk for mortality among these groups (Kollef et al, 2002).

C- Multi drug resistant acinetobacter baumanii

Definition
Risk factors
Outbreaks and Prevalence
Patient Outcome
Treatment challenges
Surveillance and Control measures

1. Definition

Multi drug resistant acinetobacter baumanii is defined as carbapenems resistance or resistance to at least three or more classes of antimicrobials (Maragakis & Perl, 2008).However, there is no standardized definition that renders a formal statement because of the genus different genotypes and phenotypes orders.

2. Risk factors

Colonization and infection with multi drug resistant acinetobacter baumanii is associated with certain risk factors such as use of broad-spectrum antibiotics, colonization pressure, length of ICU stay, immunosuppression, mechanical ventilation, previous sepsis, invasive central lines, recent surgery, multiple traumas, and severity of illness that requires frequent intensive care unit admission(Playford et al,2006,Cisneros et al 2002).

3. Outbreaks and Prevalence

Healthcare-associated outbreaks of multi drug resistant acinetobacter infections have been reported in Asia, Europe, America, and Middle East (Lolans et al, 2006,Coelho et al, 2006, CDC,2004,Landman et al,2002).Landman and his colleagues were able to collect 419A.baumanii isolate from 15 Brooklyn hospitals within 3-months period(Landman et al, 2002).Another Surveillance conducted in France in 2001 included 305,656 patients and 1533 health care facility had showed a prevalence rate of 0.075% of acinetobacter baumanii isolates(Fournier &Richet, 2006).In a recent study that investigated the risk factors associated with the increased incidence of acinetobacter baumanii infections among intensive care patients; age, acute renal failure, thrombocytopenia and subsequent other bacteremia were statistically significant causes for higher mortality rates(Katsaragakis et al ,2010).Other individual risk factors for acquisition of multidrug resistant acinetobacter baumanii include male sex, coronary vascular disease, mechanical ventilation and metronidazole(Abbo et al, 2005).

The prevalence of acinetobacter baumanii outbreaks have been noted in many European countries since 1980s mainly in France, Germany, Italy, Spain, England and Netherland(Kempf et al,2011).In a recent surveillance study that included 14 European country, the overall rate of Imipenem resistance was 48.9% with much higher rates in Italy, Greece and England (Kempf et al,2011).

Recent reports and reviews of surveillance data have confirmed a dramatic massive increase in the prevalence of acinetobacter isolates resistance to almost available antibiotics susceptibility(Kanafani&Kanj,2013).These reviews have made a certain of the active contribution of heavy antibiotic use in the development of carbapenems resistance(Kanafani and Kanj,2014).

Other studies have stressed the risk factors associated with the increased prevalence of MDR acinetobacter baumanii across urban US hospitals (Nachiket et al, 2013).

4. Patient Outcome

It was difficult to correlate between multi drug resistant Acinetobacter infections dominantly occurred in severely ill patients and extremely high crude mortality rates (Sunshine et al, 2007).But it is believed that acquiring resistant strains prolongs hospital length of stay, increases medical costs and excesses mortality rates (Giske al ,2008).Systemic reviews showed that the attributable mortality among patients with multidrug resistant acinetobacter baumanii ranged between 7.8% and 23% in hospital patients and 10% to 43% among ICU patients (Kempf et al, 2011).

5) Treatment challenges:

The limited therapeutic options rooms and remarkable negative patient's outcomes due to increased antimicrobial resistance and dearth of clinical trials that impose novel treatments have driven clinical specialists to reintroduce polymyxin E into clinical practice.

Colistin (Polymyxin E) was isolated from Bacillus polymyxa colistinus in 1949, it started in clinical use on 1959(Kumatzawa et al, 2002).But, the drug lost its vividness rapidly after several reports of neurotoxicity and nephrotoxicity (Spapen et al, 2011).

The active ingredients and formulation of colistin varies widely by the region, In Lebanon, colistimethate sodium is the active formula being used to treat multi drug resistant acinetobacter baumanii. It acts primarily by disturbing the bacterial cell membrane, increasing permeability and causing cell death (Falagas et al, 2005).

Colistimethate is eliminated by the kidneys through renal tubular secretion (Li J et al, 2006).Several observational studies have reported improvement in outcome among severely ill patients who received parenteral colistin to treat MDR A. baumanii (Kallel et al, 2006, Garnacho et al, 2003, Levin et al, 1996).

Colistin could be used as monotherapy or combined therapy with other anti-microbials. Better infection outcome have been demonstrated with intravenous colistin as monotherapy or combined therapy with Meropenem (Falagas et al, 2010). It is believed that after adjusting for renal function, increasing average daily dose of colistin will enhances survival chances(Falagas et al,2010).In a clinical trial conducted to test the synergistic effect of colistin combined with rifampicin in treating multi drug resistant acinetobacter baumanii particularly among critically ill patients (Basseti et al, 2008).No definitive effect results neither optimal dosing regimen were able to be specified, but the overall end point showed effective and safe outcomes(Bassetti et al, 2008).Petrosiilo and his colleagues were able to prove better patient outcome from the synergistic effect of combining Colistin with glycol peptides in the treatment of both acinetobacter baumanii and gram positive bacterial infections(Petrosillo et al, 2013).

Gounden and his team had compared the independent use of colistin and tobramycin for the treatment of multi drug resistant acinetobacter baumanii by which no significant difference was noted in terms of mortality and nephrotoxicity in both treatment groups.(Gounden et al, 2009).

Colistin treatment could be administered as intravenous or inhaled, it was noted that aerosolized colistin is associated with better infection cure as adjunct therapy in patients with acinetobacter baumanii pneumonia (Jian li et al, 2006).

Aerosolized colistin treatment combined with other antimicrobials has been considered as an effective and safe adjunct treatment of multi drug resistant acinetobacter baumanii ventilator associated pneumonia among critically ill patients (Michalopoluos et al, 2005).

In a matched case-control study that tested the efficacy and safety of aerosolized colistin in the treatment of ventilator associated pneumonia compared to aerosolized plus intravenous colistin, no significant outcome were observed between the studied groups in terms of pathogen eradication, clinical cure and mortality (Kofteridis et al, 2010).In a systemic review that assessed the efficacy and safety of colistin versus other antibiotics in the treatment of multi drug resistant acinetobacter baumanii, no significant differences was noted between different treatment regimens and colistin regarding hospital mortality and nephrotoxicity(Florescu et al ,2012).

Nephrotoxicity is the most cited and notorious side effect of colistin use. Recent meta-analysis study has illustrated that the use of extensively sensitive and validated criteria in measuring renal status have exaggerated the side effect of colistin use(Spapen et al,2011).It is believed that baseline creatinine levels at the day of starting colistin and the underlying disease process play a vital roles in identifying the occurrence of renal impairment during therapy(Kallel et al,2006).Other studies have stressed the importance of the administered dose of colistin plus the duration of treatment in the development of renal impairment(Falagas et al,2005).

Nephrotoxicity is prominent in patients with preexisting renal deficiencies and therefore the dose of intravenous colistin administered should be adjusted regularly (Levin et al,1999).In one of the largest retrospective studies that measured different colistin outcomes including nephrotoxicity, none of the studied variables was significantly associated with the renal insufficiency(Falagas et al,2009).

6. Surveillance

Surveillance represents the corner stone for any infection control course; it permits the detection of different type of organisms that inhibit our settings, provides us with the epidemiological trends and allow evaluating the current implemented infection control plans.

Routine clinical cultures taken from the patient and or the health care professionals allow the detection of new types and onsets of infections and guide the specialists to prescribe the proper antimicrobial treatments (Orsi et al, 2011).

7. Control measures

The organism's ability to exhibit various resistance and colonization mechanisms and survive for months in different environmental conditions represents the prominent reasons behind the emergence of rapid outbreaks in hospital settings and the implementation of preventive control measures require all levels of the health care professionals (Karageogopoulos et al, 2008).

According to Karageogopoulos, these preventive measures would include three levels:

A- Health care professionals: staff education, enforcement of hand hygiene with ready hand rub antiseptic solutions or soap and water when indicated, adherence to strict contact isolation, regular cultures taken from health care providers, coherence of colonized or actively infected patients to the same staff to avoid cross transmission of infection.

B- Environmental level: proper time and concentration for the applied disinfectants, identification of possible reservoirs (curtains, door handles, bedrails, sinks, pillows, mattresses) and active use of closed suction system for intubated patients.

C- Medical equipment: use of disposable medical equipment whenever possible (oxygen analyzers, resuscitation bags, humidifiers, spirometers and blood pressure cuffs), proper and dedicated sterilization techniques for the reusable items (mechanical ventilators, vital signs monitors, bronchoscopes).

CHAPTER II

METHODOLOGY

A- Data source

1- Study Design

This is a retrospective cohort study with external comparison group conducted at the American university of Beirut-Medical center (AUBMC) which is a 400-bed tertiary care center in Lebanon with nine beds of adult medical and surgical intensive care capacity. This study was approved by the institutional Review Board of the hospital.

2- Patient characteristics

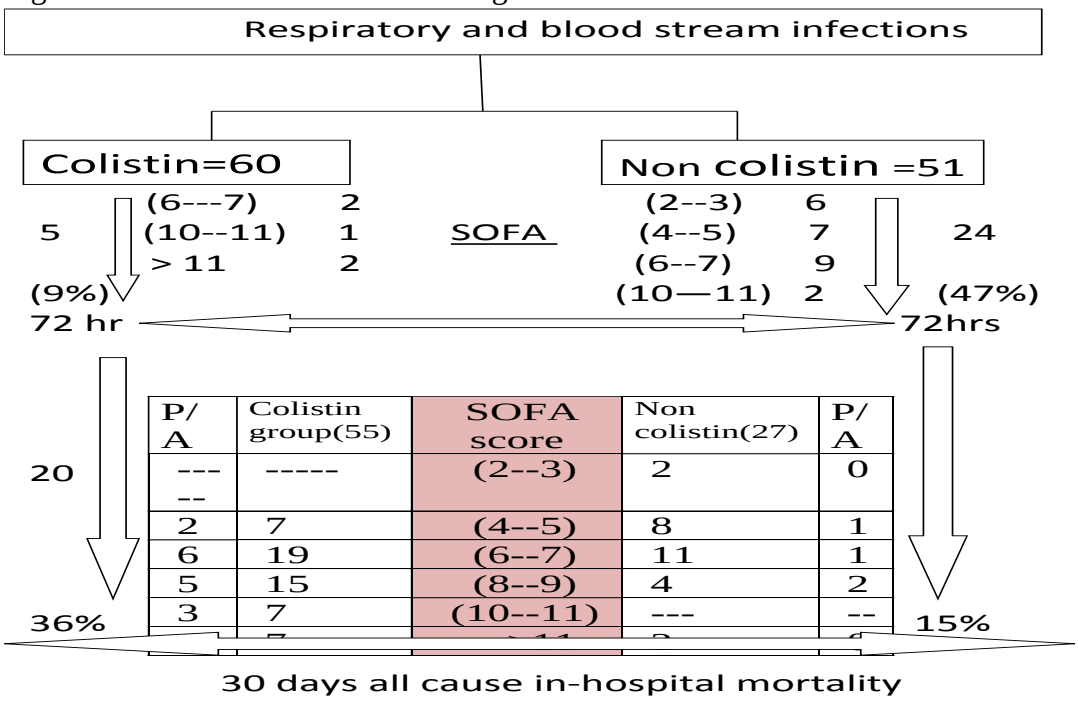
Our study population included all patients older than 18 years of age who were admitted to the Intensive Care Unit for more than 48 hours and acquired pulmonary or blood stream MDR acinetobacter species or both between January 1, 2007 and July 30, 2014.

Cases were identified through the infection control department and the micro-biology laboratory databases at AUBMC.A list of all patients who were hospitalized in the ICU and had positive blood or deep tracheal aspirate cultures with MDR acinetobacter species were obtained from databases that had collected information on nosocomial infection and type of microorganism contracted as a part of the hospital infection surveillance system.

3- Inclusion/Exclusion criteria

The colistin group consisted of patients who were treated with intravenous and/or inhaled colistin or both intravenous and inhaled with or without other antimicrobials for at least 72 hours for clinically diagnosed and/or microbiologically confirmed infections caused by MDR Acinetobacter. The non-colistin group included patients who were confirmed to have MDR Acinetobacter infections but were treated with antimicrobials other than colistin. Patients were excluded if they received less than72 hours of antimicrobial therapy, and also if colistin was initiated after 24hr from pathogen identification and completion of the susceptibility testing. Patients on dialysis replacement therapy and/or preexisting chronic renal dysfunction were also excluded. (Figure1).

Figure 1: Inclusion and Exclusion Diagram



P/A	Colistin group(55)	SOFA score	Non colistin(27)	P/A
------	-----	(2--3)	2	0
2	7	(4--5)	8	1
6	19	(6--7)	11	1
5	15	(8--9)	4	2
3	7	(10--11)	---	--

4- Collection and extraction of Data

The medical chart of each patient was retrieved and reviewed retrospectively to obtain demographic and clinical data. The average dose of colistin used and route of administration in each patient was calculated. Possible risk factors: Length of ICU stay, mechanical ventilation, invasive central lines, arterial line, Nasogastric tube and urinary indwelling catheter, previous antibiotic use, recent surgery, reason for admission, age, gender, severity of illness as indicated by SOFA score at the 24 hours of admission, Pittsburgh bacteremia score at the onset of bacteremia and Charlson's co-morbidity score were both calculated, co-morbidities not included in Charlson's score were recorded also, underlying disease, immunosuppression including neutropenia, Hypoalbumenia and concomitant use of other possible nephrotoxic agents and antimicrobials were also calculated and recorded.

5- Operational definitions

Ventilator associated pneumonia was defined after 48 hours of intubation as was proposed by the US centers for Disease control and prevention (CDC, 2013) as: radiographic chest X-ray with persistent infiltrates of unknown cause with the following parameters: a) Increase in min $FiO_2 \geq 20\%$ or an increase in PEEP $>3cmH_2O$ for two consecutive days .b) Temperature>38˚C or< 36˚C or WBC ≥12000 or≤4000 cells/mm^3.c) New antibiotic that started for more than four consecutive days. d) Purulent respiratory secretions and positive deep tracheal culture.

Blood stream infections were identified as common commensal from more than two blood cultures at the same period of time associated with a) fever>38˚C. b)Chills if applicable c)hypotension with systolic blood pressure less than 20mmHg

of baseline or Mean arterial pressure less than 70 mmHg or the use of any vasopressor. The onset of bacteremia is defined as the first day of sample collection. In patients who had more than one episode of acinetobacter bacteremia or pulmonary infection or both, only the first episode was considered.

In patients with normal renal function, nephrotoxicity is defined as serum creatinine concentration>2mg/dl or rise in creatinine level by >20% while in patients with preexisting renal impairment but not chronic >50%.The baseline creatinine level is defined as the creatinine level at the first day of administering the antimicrobial therapy. Immunosuppressant is defined as patients who received chemotherapy or other immunosuppressive therapy in the previous 30 days. Neutropenia is defined as absolute leukocyte count less than 1500 /mm^3.

B-Research Focus

1-Hypothesis and Objectives

Within the context of safety and efficacy, a number of questions remain unable to be rebutted concerning the clinical outcomes of using colistin to treat MDR A .baumanii, extending to the immediate present; no study result has proved conformity in colistin treatment that allows specialists to draw radical commutation in prescribed therapeutic regimens. The specific objectives of our study are to investigate whether the reintroduction of colistin (colisimethate sodium) into clinical practice has significantly improved the 30-day in hospital mortality of patients with MDR Acinetobacter pulmonary and/or blood stream infections compared to those who were not treated with colistin and to judge the nephrotoxicity status among both groups after adjusting for all confounding and interacting variables.

2- Dependent and independent variables

The primary dependent variable of the study is 30 days all-cause in-hospital mortality, which was coded as (0 for alive and 1 for dead).The independent variables included both continuous and categorical variables.

The continuous variables were: age, severity of illness scores calculated within 24 hours of admission that included sofa score and Charlson's co morbidity score, Pittsburgh bacteremia score at onset of bacteremia and duration antibiotic administration, because median is less sensitive to extreme values it was calculated to all continuous variables.

Categorical variables were :service, gender, other co morbidities not included or even specified precisely in Charlson's score as hypertension and coronary vascular diseases, recent surgery and antibiotic use within 30 days prior to hospitalization, previous hospitalization, immunosuppressant and corticosteroid usage, site of acinetobacter infections, presence of intensive care unit parameters: mechanical ventilation within 48 hours of admission and subsequent development of ventilator associated pneumonia, central venous line access, arterial line, urinary indwelling catheter, nasogastric tube, different abdominal drainage, tracheotomy, reason for intensive care admission, combination agents agent used in both the non colistin group and the colistin group, duration of each antibiotic administration, blood urea nitrogen and creatinine levels on admission then at the day of starting antibiotics and after 24 hours of antibiotic discontinuation.

The secondary dependent variable was nephrotoxicity while the independent variables were route of colistin administration, creatinine and blood urea nitrogen on

admission, at the day of starting antibiotics and after 24 hours of antibiotic and other nephrotoxic agents.

C-Statistical analysis

Bivariate analysis was conducted to examine the association between clinical and demographic variables and outcome. All the variables with p-value ≤ 0.2 at the bivariate level were included in the multivariate analysis. The Chi square test, the student's t test, Fisher's exact test were used to compare categorical variables, Mann Whitney U test was used for continuous variables respectively.

Stepwise regression procedure for model building was used based on setting 2 probabilities; the probability to remove=0.1001 and probability to enter=0.1, p-value less than 0.05 were considered significant. Adjusted and unadjusted odd ratios with their respective 95% confidence interval and the p-value for each variable were all reported. All the independent variables were tested for both multicollinearity and interaction between each other.

D-Ethical considerations

No direct patient contact neither follow up interviews were needed so informed consent was waived. A possible risk in the study was breach of confidentiality; all data collected from the medical charts remained confidential. Data entry was limited to one of the co-investigator. All of the data collection sheets were not included any personal identifiers but rather a code number, no social, psychological, legal and/or financial risks were associated with the study. However, the conclusions that were drawn about safety and efficacy of Colistin reuse allowed the benefits to outweigh the risks in this study. All team members were CITI certified and thus they were responsible for assuring confidentiality of data obtained at all stages. The data collected were kept in save and secure cabinet. The hard copy Data collection sheet

will be shredded after 5 years, the soft copy data sheet will be deleted three months after study completion and results publication.

CHAPTER IV

STATISTICAL ANALYSIS AND RESULTS

This study consisted of all patients who were admitted to the intensive care unit and acquired multi drug resistant acinetobacter baumanii between January 1, 2007 and July 30, 2014.Only 82 cases fit our inclusion criteria. Using stata 13, clinical characteristics of both colistin and non colistin groups were calculated in terms of percentages and frequencies, the main dependent and independent variables for the primary and secondary outcomes were regressed and presented in the appropriate tables and graphs.

Before conducting the analysis, the data were carefully examined to identify any missing or inconvenient labeling or coding, For example, gender was labeled as a string variable (male & female). Thus, encoding was done to convert it into numeric variable so as it is coded 0 for male and 1 for female by using the command "encode gender=gen (gender1)".After summarizing all the variables especially our dependent variable (0=alive,1=dead)by applying the command "codebook" we found no significant data missing that can affect our model.

Initially descriptive analysis was conducted for the colistin group and the non colistin group each alone (Table 1 & Table 2), then between treatment groups, percentages and frequencies were reported for the categorical variables, median and interquartile range for the continuous variables (Table 3). Moreover, Continuous variables were tested for normality by using the Q-Q test; those who had severely violated normality were analyzed using the Mann-Whitney U test. Interquartile ranges were calculated when appropriate for both groups, and antimicrobial combination agents were stratified and compared (tables 4&5).

Table 1: Colistin group characteristics

Table 1:	*Colistingroup (n= 55)*
Diagnostic Category:	
Medical,n= 30	*54.55 %*
Surgical,n=25	*45.45 %*
Age,Year,Mean ± Sd	*57.8 ± 22.51*
No.Of Males,n= 36	*65.45 %*
Sofa Score On Admission, Median (Range)	*9 (6—10)*
Pitts Bacteremia Score, Median (Range)	
Charlson's Comorbidity Score, Median (Range)	*5 (1—6)*
Other Co-Morbidities:	
Cardiovascular Disease, n=25	*45.45 %*
Hypertension, n=22	*44 %*
Rheumatogic Disease, n=5	*9.26%*
Immunosuppressed, n=25	*45.45%*
Recent Surgery, n=25	*45.45%*
Recent Antibiotic, N=36	*65.45%*
Reason For Admission:	
Respiratory Failure, n=28	*50.91%*
Sepsis, n=13	*23.64%*
Gastrointestinal, n=10	*18.18%*
Multiple Trauma, n=10	*18.18%*
Post Any Surgery, n=10	*18.18%*
Site Of Infection:	
Respiratory, n=50	*90 %*
Blood, n=2	*3.64 %*
Both Respiratory And Blood, n=3	*5.45 %*
Length Of Icu Stay: (Median)	
Prior To Infection,Days	*5 (1—60)*
Following Infection,Days	*11 (0—71)*
Hospital Los,After Icu,Days	*23 (3—1320)*

Medical History:	
Previous Surgery, n=31	*56.36 %*
Previous Hospitalization, n=47	*85.45 %*
Previous Antibiotic Use, n=36	*65.45 %*
Icu Parameters:	
Mechanical Ventilation, n=50	*90.91 %*
Central Venous Catheter, n=28	*50.91 %*
Urinary Catheter, n=54	*98.18 %*
Arterial Radial Line, n=12	*21.82 %*
Tracheotomy, n=6	*10.91 %*
Nasogastric Tube, n=43	*78.18 %*
Abdominal Drainage, n=6	*10.91 %*
Hypoalbuminemia,gr/dl, n=46	*86.79 %*
Ventilator Associated Pneumonia, n=38	*69.09 %*
Combination Therapy:	
Amikacin, n=11	*20 %*
Ceftazidime, n=7	*12.73 %*
Meropenem, n=29	*52.73 %*
Cefepime, n=4	*7.27 %*
Tazocine, n=3	*5.45 %*
Imipenem, n=2	*3.64 %*
Tigacyclin, n=43	*78.18 %*
Duration Of Antibiotic Administration, Days, ,Median	*14 (3—70)*
Primary Outcome	
30 Days In Hospital Mortality, n=20	*36.36 %*
Secondary Outcome	
Nephrotoxicity, n=8	*14.55 %*

1- Basic characteristics of the Colistin group:

The colistin group consisted mainly of 55 cases; the basic clinical and demographic characteristics were obtained in terms of frequencies, percentages, mean and medians. The mean age of the colistin group was 57.87 + 22.51 SD. The median scores of *SOFA* and Charlson's co- morbidity were 9 and 5 respectively. The type of service which patient admitted was 54.55% for surgical and 45.45% for medical. The male gender represented 65.45% of admissions in this treatment group. Other co morbidities and significant predictors for morbidity and mortality not included in Charlson's were also presented as: coronary vascular disease (45.45%), hypertension (44%), Immunosuppressed (45.45%), recent surgery (45.45%), and recent antibiotic use (65.45%) recent surgery ((45.45%).Reason for admissions varied from respiratory failure in both medical and surgical cases (50.91%) to multiple trauma (18.18%), sepsis(23.64%),post any surgery (18.18%) and both medical and surgical gastrointestinal failure(18.18%).Site of acinetobacter baumanii multidrug resistant were classified as respiratory (90%) causing pneumonia, blood stream infections(3.64%) causing bacteremia, and both respiratory and blood stream infection (5.45%).Intensive care unit and hospital stays were reported in terms of median and interquartile range in order to avoid extreme values, length of intensive unit stay was calculated pre and post acquiring infection with their respective medians of 5 and 11while the median length of hospital stay was 23. Past medical history was obtained and included previous surgery (56.36%), previous hospitalization (85.45 %) and previous antibiotic use (65.45%).

Intensive care parameters were presented as percentages and incorporated presence of mechanical ventilator (90.91%), central line catheter (50.91%), urinary indwelling catheter (98.18%), arterial line catheter (21.82%), tracheotomy (10.91%),

Nasogastric tube (78.18%), abdominal drainage (10.91%), occurrences of ventilator associated pneumonia 48 hours post admission and intubation (69.09%) and presence of hypoalbumenimia on admission (86.79%).Combined agents with colistin were stratified and recorded on each patient (table 5)with prominent use of Tigacycline (78.18%),Meropenem (52.73%),Imipenem (3.64%),and Amikacin as single shots or standing doses (20%),Ceftazidime (12.73%), Tazocine (5.45%)and Cefepime (7.27%).We should note that colistin route of administration was also identified with 31 nebulized (56.36%), six intravenous use (10.91%) and 18 patients nebulized + intravenous use (32.73%).

The primary outcome 30days in-hospital mortality represented (36.36%) of the colistin group, while nephrotoxicity the secondary outcome represented (14.55%).

Table 2:	Non –Colistin group (n= 27)
Diagnostic Category:	
Medical,n= 13	48.15 %
Surgical,n=14	51.85 %
Age,Year,Mean ± Sd	53.88 ± 27.04
No.Of Males,n= 18	66.67 %
Sofa Score On Admission, Median (Range)	6 (5—7)
Pitts Bacteremia Score, Median (Range)	
Charlson's Comorbidity Score, Median (Range)	5 (0—6)
Other Co-Morbidities:	
Cardiovascular Disease, n=10	37.04 %
Hypertension, n=12	44.44 %
Rheumatogic Disease, n=2	7.41 %
Immunosuppressed, n=12	44.44 %
Recent Surgery, n=12	44.44 %
Recent Antibiotic, n=17	62.96 %
Reason For Admission:	
Respiratory Failure, n=12	44.44 %
Sepsis, n=1	3.70 %
Gastrointestinal, n=2	7.41%
Multiple Trauma, n=7	25.93 %
Post Any Surgery, n=4	14.81 %
Site Of Infection:	
Respiratory, n=25	92.59 %
Blood, n=1	3.70 %
Both Respiratory And Blood, n=1	3.70 %
Length Of Icu Stay: (Median)	
Prior To Infection,Days	7 (1—90)
Following Infection,Days	8 (0—69)
Hospital Los,After Icu,Days	22 (3—150)

Medical History:	
Previous Surgery, n=14	51.85 %
Previous Hospitalization, n=21	77.78 %
Previous Antibiotic Use, n=17	62.96 %
Icu Parameters:	
Mechanical Ventilation, n=21	77.78 %
Central Venous Catheter, n=12	44.44 %
Urinary Catheter, n=26	96.30 %
Arterial Radial Line, n=11	40.74 %
Tracheotomy, n=4	14.81 %
Nasogastric Tube, n=24	88.89 %
Abdominal Drainage, n=7	25.93 %
Hypoalbuminemia,gr/dl, n=21	77.78 %
Ventilator Associated Pneumonia, n=11	40.74 %
Concurrent Therapy:	
Amikacin, n=10	37.04 %
Meropenem, n=7	25.93 %
Cefepime, n=1	3.70 %
Tazocine, n=2	7.41 %
Imipenem, n=10	37.04 %
Tigacyclin, n=15	55.56 %
Duration Of Antibiotic Administration, Days,	12 (3—42)
Primary Outcome	
30 Days In Hospital Mortality, n=4	14.81%
Secondary Outcome	
Nephrotoxicity, n=1	3.70 %

2- Basic clinical characteristics of the non-Colistin group:

The non colistin group consisted of 27 cases; the basic clinical and demographical characteristics were obtained through the mean, median, frequencies and percentages that used to describe different variables. The mean age was 53.88±27.04 SD. The median score of *SOFA* and Charlson's co morbidity were 6and 5 respectively. The male gender represented 66.67% of this treatment group while 51.85% of the admitted cases were surgery and 48.15%as medical cases.

Risk factors for morbidity and mortality not specified in Charlson's co morbidity score were analyzed with coronary vascular disease (37.04%), hypertension (44.44%), rheumatoid disease (7.41%), immunosuppressed (44.44%), recent surgery (44.44%), and recent antibiotic use(62.96%).Reason for admission was due to respiratory failure in both medical and surgical cases (44.44%), sepsis(3.70%), gastrointestinal problem in both services(7.41%),multiple trauma(25.93%)and post any surgery (14.81%).Site of infection was categorized as respiratory(92.59%), blood stream infection (3.70%) and both blood and respiratory infections (3.70%).

The median length of intensive unit stays pre and post infection was 7 and 8 respectively while the median length of hospital stay was 22.Past medical history was recorded as previous surgery (51.85%), previous hospitalization(77.78%) and previous antibiotic use (62.96%).Intensive care unit variables were identified as mechanical ventilation (77.78%),central venous catheter(44.44%),urinary catheter (96.30%), arterial line catheter(40.74%)tracheotomy(14.81%), nasogastric tube(88.89%), abdominal drainage(25.93%), acquisition of ventilator associated pneumonia 48 hours post intubation and admission(40.74%) and hypoalbumenimia on admission(77.78%).

Antibiotic agents used to treat acinetobacter baumanii infections were analyzed initially as single then combined (table 6) with Amikacin(37.04%), Meropenem

(25.93%), Cefepime (3.70%), Tazocine (7.41%), Imipenem (37.04%) and Tigacycline (55.56).In the non Colistin group, the 30 days in- hospital mortality represented 14.81% and nephrotoxicity 3.70%.

3- Comparison between the Colistin group and the non Colistin group

Further analysis was conducted to compare the differences between the two treatment groups, Pearson χ^2tests and the likelihood ratio tests were used to assess the significance between categorical variables, continuous variables were compared using Mann Whitney U test. The corresponding *P* –values of all variables were reported. (Table 3&4)

There was no significant difference between the types of service to which patients were admitted with a *P*-value of 0.52.Out of the medical cases 69.44% of admissions were in the colistin group compared to 30.23 % in the non colistin group, while the surgical cases were 64.10%in the colistin group compared to35.90% in the non colistin group.

The difference in the mean age between both treatment groups was not significant with a *P*- value of 0.47.Taking gender; it was not significant also with *P*-value of 0.913 by which male represented66.67% in the colistin group and 33.33% in the non colistin group. The severity of illness scores were compared between the two treatment groups excluding Pittsburgh bacteremia score due to the non-availability of cases that fitted our inclusion criteria. *SOFA* score was significant between the two groups with P – value of 0.009which indicates severity of illness in the colistin group even on admission and before acquiring infection, while the Charlson's co morbidity score was not significant with P –value of 0.86.

Comparison between other comorbidities not included or specified in the Charlson's score and risk factors for morbidity and mortality in both groups have shown no significant association with, coronary vascular disease(*P*-value =0.46), hypertension(*P*-value=0.7),rheumatoid disease(*P*-value= 0.78),Immunosuppressed (*P*-value=0.931),recent surgery(*P*-value=0.931),recent antibiotic use(*P*-value=0.82).

Reason for admission was assessed between the two groups. Significance was noted in sepsis with *P*-value of 0.02.Other admission reasons were not significant with *P*-value for respiratory failure and gastrointestinal failure in both services of 0.582 and 0.195 respectively, multiple traumas and post any surgery diagnosis were also not significant with *P*-value of 0.581 and 0.703 respectively. Respiratory site of infection and subsequent pneumonia represented 66.67% of cases in the colistin group and 33.33% of cases in the non colistin group with P- value of 0.75.Length of intensive unit stay pre and post infection and hospital stay were not significant with P-values of 0.98, 0.21& 0.67.

Past medical history was compared between the two treatment groups with no significant association found by which the *P*- values of recent surgery, recent hospitalization and recent antibiotic use were 0.70,0.38 and0.82 respectively.

Intensive care unit parameters were compared, their respective *P*- values obtained and which included: mechanical ventilation of *P*-value 0.10, central venous catheter of *P*-value of 0.52, urinary catheter of *P*-value 0.60, arterial line catheter of *P*- value0.07, tracheotomy of *P*-value 0.61, Nasogastric tube of *P*-value 0.23, abdominal drainage of *P*-value 0.08, with significant ventilator associated pneumonia after 48 hours of admission and mechanical ventilation of *P*-value 0.014 and hypoalbumenimia of *P*-value 0.30.

Combination of antimicrobial agents was significant for some antibiotics in both treatment groups with *P*-values of 0.02, 0.03 and 0.00 for Meropenem, Tigacycline and Imipenem. While the *P*- value was not significant for Ceftazidime, Cefepime and Tazocine with respective *P*-values of 0.79, 0.52 and 0.46.Duration of antibiotic administration was not significant between groups with *P*-value of 0.153.

The primary outcome in-hospital 30 days mortality was significant between groups with *P*-value of 0.004; the resultant nephrotoxicity was not significant between the treatment groups with *P*-value of 0.14.

Table 3: Comparison between Colistin and Non-Colistin group

Table:3	Colistin group, N =(55)	Non-Colistin Group N=(27)	P- value
Diagnostic Category			
Medical, n=43	30	13	
Surgical, n=39	25	14	0.52
Age,Year,Mean ± SD	57,87± 22.51	53,88 ± 27,04	0.47
No.Of Males, N=54	36	18	0.913
SOFA Score On Admission, Median (Range)	9 (6—10)	6 (5—7)	0.009
Charlson's Comorbidity Score, Median (Range)	5(1—6)	5 (0—6)	0.86
Other Co-Morbidities:			
Cardiovascular Disease, n=35	25	10	0.469
Hypertension, n=34	22	12	0.701
Rheumatogic Disease, n=7	5	2	0.78
Immunosuppressed, n=37	25	12	0.931
Recent Surgery, n=37	25	12	0.931
Recent Antibiotic Use, n=53	36	17	0.82
Reason For Admission:			
Respiratory Failure, n=40	28	12	0.582
Sepsis , n=14	13	1	0.02
Gastrointestinal, n=12	10	10	0.195
Multiple Trauma, n=17	10	7	0.581
Post Any Surgery, n=14	10	4	0.703
Site Of Infection:			
Respiratory, n=75	50	25	0.75
Blood, n=3	2	1	
Both Respiratory And Blood, n=4	3	1	
Length Of ICU Stay:			
Prior To Infection,Days, Median	5 (1—60)	7 (1—90)	0.981
Following Infection,Days, Median	11 (0—71)	8 (0—69)	0.21
Hospital LOS After ICU Days, Median	23 (3—1320)	22 (3—150)	0.67

Medical History:			
Previous Surgery, n=45	31	14	0.70
Previous Hospitalization, n=68	47	21	0.38
Previous Antibiotic Use, n=53	36	17	0.82
ICU Parameters:			
Mechanical Ventilation, n=71	50	20	0.10
Central Venous Catheter, n=40	28	12	0.52
Urinary Catheter, n=80	54	26	0.60
Arterial Radial Line, n=23	12	11	0.07
Tracheotomy, N=10	6	4	0.61
Nasogastric Tube, n=67	43	24	0.23
Abdominal Drainage, n=13	6	7	0.08
Hypoalbuminemia, gr/dl, n=67	46	21	0.30
Ventilator Associated Pneumonia, n=49	38	11	0.014
Combined Therapy:			
Amikacin, n=21	11	10	0.09
Ceftazidime, n=11	7	4	0.79
Meropenem, n=36	29	7	0.02
Cefepime ,n= 5	4	1	0.52
Tazocine, n=5	3	2	0.46
Imipenem, n=12	2	10	0.00
Tigacyclin, n=58	43	15	0.03
Duration Of Antibiotic Administration, Days, Median	14 (3—70)	12(3—42)	0.153
Primary Outcome:			
30 Days In Hospital Mortality, n=24	20	4	0.04
Secondary Outcome:			
Nephrotoxicity, n=9	8	1	0.14

Table 2: Percentage Difference between Colistin and Non-Colistin Group

Table:4	Colistin Group, N = (55)	Non-Colistin Group, N= (27)
Diagnostic Category		
Medical	69.44 %	30.23 %
Surgical	64.10 %	35.90 %
Age,Year,Mean ± SD	57,87± 22.51	53,88 ± 27,04
No.Of Males	66.67 %	33.33 %
SOFA Score On Admission, Median (Range)	9 (6—10)	6 (5—7)
Charlson's Comorbidity Score, Median (Range)	5(1—6)	5 (0—6)
Other Co-Morbidities:		
Cardiovascular Disease	71 %	29 %
Hypertension	64.71 %	35.29 %
Rheumatogic Disease	71.43 %	28.57 %
Immunosuppressed	67.57 %	32.43 %
Recent Surgery	67.57 %	32.43 %
Recent Antibiotic Use	67.92 %	32.08 %
Reason For Admission:		
Respiratory Failure	70 %	30 %
Sepsis	92.86 %	7.40 %
Gastrointestinal	83.33 %	16.67 %
Multiple Trauma	38.82 %	41.18 %
Post Any Surgery	71.43 %	28.57 %
Site Of Infection:		
Respiratory	66.67 %	33.33 %
Blood	66.67 %	33.33 %
Both Respiratory And Blood	75 %	25 %
Length Of ICU Stay(Median)		
Prior To Infection,Days	5 (1—60)	7 (1—90)
Following Infection,Days	11 (0—71)	8 (0—69)
Hospital LOS After ICU,Days	23 (3—1320)	22(3—150)

Medical History:		
Previous Surgery	68.89 %	31.11 %
Previous Hospitalization	31.11 %	30.88 %
Previous Antibiotic Use	67.92 %	32.08 %
ICU Parameters:		
Mechanical Ventilation	70.42 %	29.98 %
Central Venous Catheter	70 %	30 %
Urinary Catheter	67.50 %	32.50 %
Arterial Radial Line	52.17 %	47.83 %
Tracheotomy	60 %	40 %
Nasogastric Tube	64.18 %	35.82 %
Abdominal Drainage	46.18 %	53.85 %
Hypoalbuminemia, gr/dl	68.66 %	31.34 %
Ventilator Associated Pneumonia	77.55 %	22.45 %
Combined Therapy:		
Amikacin	52.38 %	47.62 %
Ceftazidime	63.64 %	36.36 %
Meropenem	80.56 %	19.44 %
Cefepime	80 %	20 %
Tazocine	60 %	40 %
Imipenem	16.67 %	83.33 %
Tigacyclin	74.14 %	25.86 %
Duration Of Antibiotic Administration, Days, Median	14 (3—70)	12 (3—42)
Primary Outcome:		
30 Days In Hospital Mortality,n=24	36.36 %	14.18 %
Secondary Outcome:		
Nephrotoxicity,n=9	88.89 %	11.11 %

Tables 3& 6: Comparison of the SOFA scores between groups

Table 5: SOFA score levels in both treatment groups				
Mortality	Colistin group	SOFA score levels	Non colistin group	Mortality
-----	-----	(2--3)	2	0
2	7	(4--5)	8	1
6	19	(6--7)	11	1
5	15	(8--9)	4	2
3	7	(10--11)	---	--
4	7	>11	2	0
20	55	Total	27	4

Table 6: Initial SOFA scores levels and predicted mortality rates			
colistin group mortality rates	SOFA score levels	Predicted mortality rates in the first 48 hrs	Non colistin group mortality rates
-----	(2--3)	7 %	0 %
28 %	(4--5)	21 %	12.5%
31 %	(6--7)	22%	9 %
33 %	(8--9)	33 %	50 %
42 %	(10--11)	50 %	-----
57 %	>11	95 %	0 %

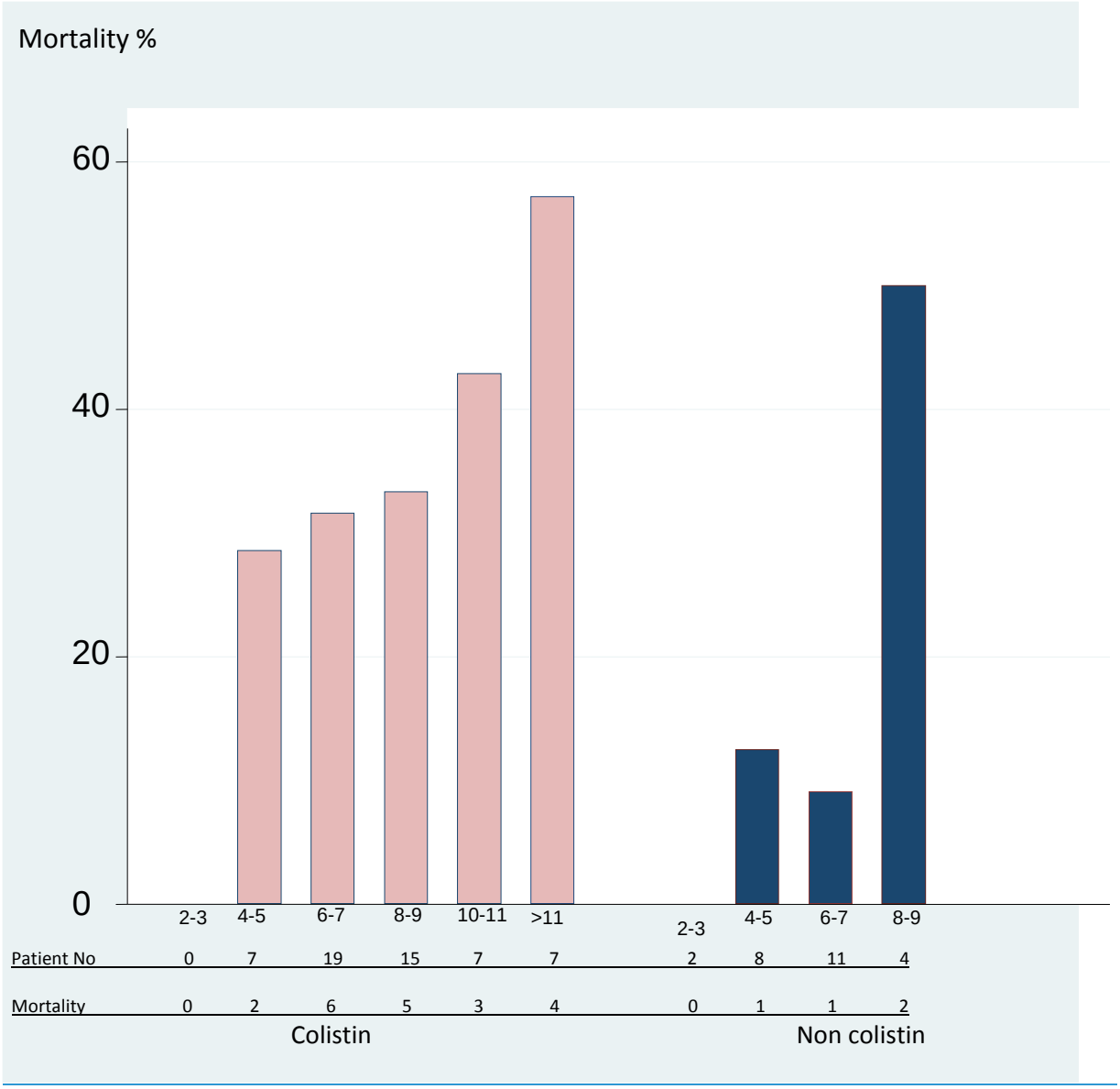


Figure 2: Initial SOFA score and 30 days mortality rates

In the colistin group patients with initial sofa score between2 and 7 the mortality rate was 30 % while in the non colistin group was 10%.
In SOFA score > 8, the mortality rate was in the colistin group 41 % compared to 50 % in the non colistin group.

Tables 7: Charlson's score level in both treatment groups

Mortality	Colistin group	Charlson's score levels	Non colistin group	Mortality
2	12	0	8	1
4	11	1	1	0
4	9	2	6	2
2	3	3	3	1
1	2	4	----	-----
1	3	5	2	0
2	7	6	1	0
1	4	7	3	0
1	2	8	1	0
1	1	9	1	0
---	---	10	----	------
1	1	11	----	----
---	---	12	-----	----
----	-----	13	1	0

Table 8: Charlson's predicted 1 year mortality rates

Charlson's predicted 1 year mortality rates					
Mortality	Colistin group	Charlson's score levels	One year predicted mortality	Non colistin group	Mortality
2	12	0	12 %	8	1
8	20	1---2	26 %	7	2
3	5	3---4	52 %	3	1
7	18	≥ 5	85 %	9	0

Table 9: Stratified combination agents of each colistin group patient, route of administration, mortality and nephrotoxicity

Colistin	Amikacin	Ceftazidime	Cefepime	Meropenem	Imipenem	Tazocine	Tigacycline	Nephrotoxicity	Mortality
				+			+	N	A
								N	A
	+			+				N	P
							+	N	A
				+			+	N	A
	+			+			+	Y	A
	+						+	Y	P
				+			+	Y	P
				+			+	N	A
	+						+	N	A
			+				+	N	P
	+						+	Y	P
Aerolized							+	N	P
							+	N	A
				+			+	N	P
				+			+	N	A
	+				+			N	A
	+						+	N	A
				+				N	P
							+	N	A
					+			N	A
				+				N	A
			+					N	A
				+				N	A
								N	P
								N	P
				+			+	N	P
							+	N	P
							+	N	A
						+		N	P
				+			+	N	A
Intravenous		+					+	N	P
							+	N	A
							+	N	A
				+		+		N	A
				+			+	N	P
							+	Y	A
				+			+	N	A
							+	Y	A
	+			+			+	N	A
				+			+	N	P
							+	N	A
		+				+	+	N	A
		+		+			+	N	A
				+			+	Y	P
				+			+	N	A
IV+ Neb				+			+	N	A
			+				+	N	A
				+			+	Y	P
	+			+			+	N	A
		+		+			+	N	A
			+	+			+	N	A
	+			+			+	N	P
	+	+		+			+	N	A
				+			+	N	P

Table 10: Stratified combination agents of each non-Colistin group patient

Amikacin	Ceftazidime	Cefepime	Meropenem	Imipenem	Tazocine	Tigacycline
			+			+
			+			+
			+			
						+
						+
						+
			+			+
			+			
						+
						+
+						+
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					+	+
			+		+	+

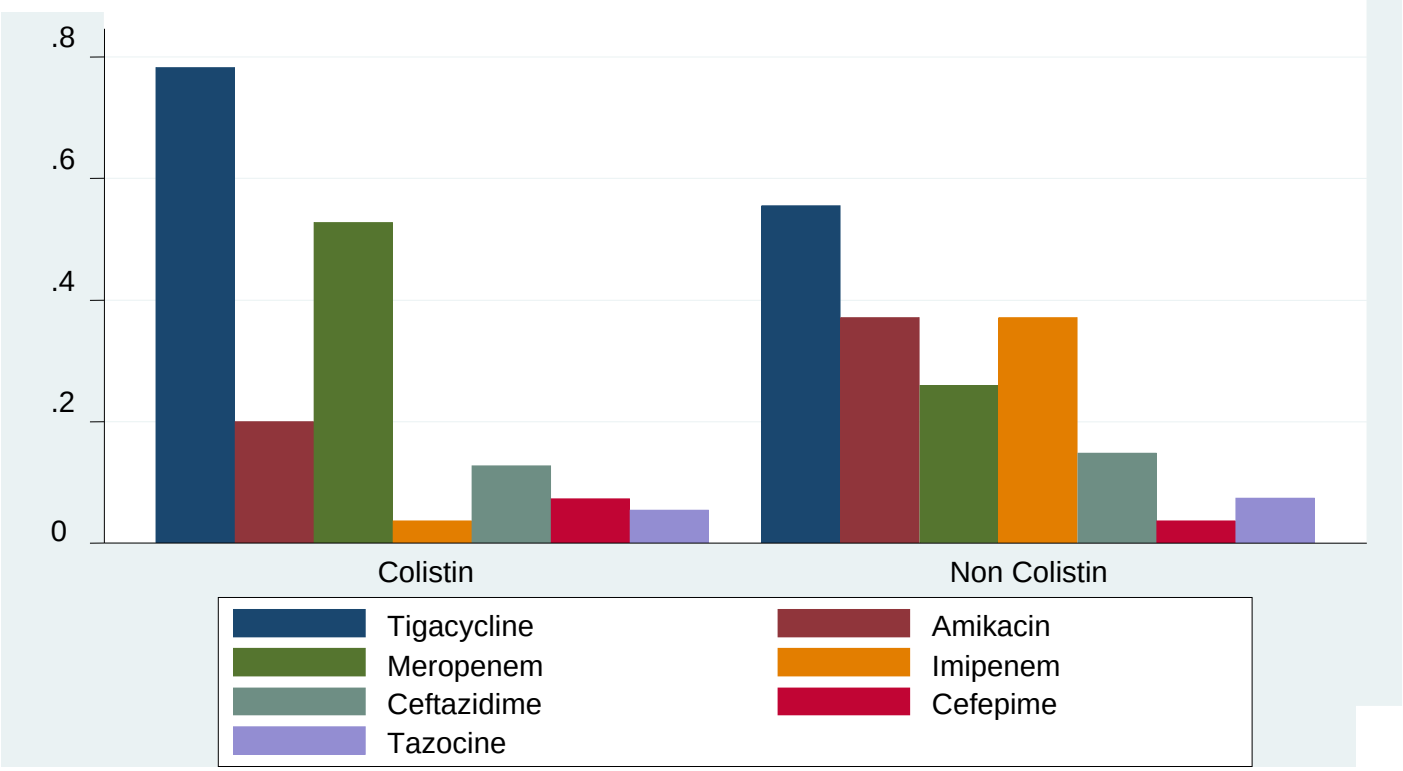


Figure 3: Antibiotic combination in both Colistin and Non Colistin group

A-Primary outcome analysis

Bivariate analyses:

At the bivariate level each independent variable was regressed on the primary outcome, 30 days in-hospital mortality, to assess its significance and association with the dependent variable. The unadjusted odd ratios, their 95% OR confidence intervals and the *P*-values were obtained for each explanatory variable (Table 11).From table 11; we will draw each variable with p-value ≤0.2 to be included in the multivariate analysis by which 17 variables with P- values≤0.2 were extracted to be included in the multiple logistic model.

The results obtained from our sample at the univariate level show that age of patients admitted to the intensive care unit is not a significant predictor of 30 days in-hospital mortality with a *P*-value of 0.32 and 95%CI (0.989--1.03).Patient's sex is not also a significant predictor for mortality with a *P*-value of 0.92 and 95%CI (0.347--2.599). we can find a significant association between mortality and type of service to which patient admitted with *P*-value of 0.011 and 95%CI(0.087--0.72).Severity of illness score analysis showed that SOFA score was significant with P- value of 0.06 and 95%CI(0.932--6.521)while Charlson's comorbidity score is not a significant predictors of 30days in-hospital mortality with *P*-value of 0.629 and 95%CI(0.89--1.21*)* respectively.

Other co morbidities that are not included or indicated in Charlson's score and believed to play important role in patient outcome are also analyzed .A significant association indicated between 30 days in-hospital mortality, coronary vascular disease and immunosuppressed patients with *P*-values of 0.02, 95%CI(0.117--0.844)and 0.04, 95%(0.137--0.978),while no association is found with hypertension, rheumatoid disease, recent surgery and recent antibiotic use with respective *P*-values and confidence

intervals of 0.981,95%CI(0.376--2.594),0.401,95%(0.288--22.34),0.171, 95%CI (0.741--5.396), 0.451,95%CI(0.241--1.882).It is found that sepsis was significantly associated with 30 days in hospital mortality with *P*-value and confidence interval of 0.016, 95%CI (0.069--0.764).Other admitting diagnosis like respiratory failure, gastrointestinal problems and multiple trauma are not significantly associated with 30 days in hospital mortality with *P*-values and confidence intervals of 0.113 ,95% CI (0.171---1.206),0.738,95%CI(0.21--2.95) and 0.092,95%CI (0.80--18.30).

Moving forward in analyzing the results obtained at the univariate level, it is found that length of intensive care stay pre and post infection are not associated with 30 days in hospital mortality with respective P-values and odd ratio confidence intervals of 0.486 ,95%CI(0.936--1.03)and 0.147 ,95%CI(0.934--1.01),while length of hospital stay is significantly associated with P-value of 0.002 and 95%CI(0.879--0.962*).*On other hand, site of infection is also not significant with *P*- value of 0.530 and 95%CI(0.19--2.29).

Past medical history which includes previous surgery, previous hospitalization and previous antibiotic use show no significant association with our dependent variable at the univariate level with *P*-values and confidence intervals of 0.934, 95%CI(0.40--2.70),0.482,95%CI(0.154--2.41*)*and0.45,*95%*CI(0.24--1.88). None of the intensive care unit parameters have significant association except for arterial line catheter with P-value of 0.02 and 95%CI(1.33--29.22),other ICU parameters including mechanical ventilation, central venous catheter, nasogastric tube and abdominal drainage are not significantly associated with 30 days in hospital mortality with *P* values and confidence intervals of 0.876,95%CI(0.21--3.69), 0.268,95%CI(0.221--1.51),0.80,95%(0.242--3)and0.244, 95% CI(0.525--12.61).

Ventilator associated pneumonia and hypoalbumenimia are both also not significant with respective *P*-values and confidence intervals of 0.866, 95%CI (0.413--2.859) and 0.697, 95%CI (0.187--3.05).Colistin group, the treatment under investigation is not significant predictor for 30 days in-hospital mortality with P- value of 0.05 and 95%CI OR (0.09--1.005), also none of the combination agents was significant at the univariate level. Regarding duration of antibiotic administration, it is not also significant at the univariate level with P-value of 0.09 and 95%CI (0.87--1.01).

Nephrotoxicity our secondary outcome show a significant association with *P*-value 0.017 and 95%CI (0.03--0.72).

It is worth to mention that we know from clinical background that some variables may not be significant at the univariate level, but when combined or interact with each other they may affect our dependent variable positively or negatively. For example, the interaction that could take place between severity of illness, co morbidities and age, also interaction between mechanical ventilation, ventilator associated pneumonia and length of ICU post infection may affect patient prognosis and outcome. So generating interaction terms is important to predict mortality. Interaction terms were created by applying the command generate interaction 1=Sofa* Charlson's*coronary vascular disease* hypertension*age, then these variables regressed together to see its synergistic effect on 30 days in-hospital mortality. (Model 1)

Another interaction term was created between mechanical ventilator and ventilator associated pneumonia, no effect was noticed in terms of 30 days in-hospital mortality (Model 2) .But, we should note that these interactions may predicts length of intensive and hospital stays rather than 30 days in-hospital mortality.

Table 4: Model 1 Interaction term

Model: 1	Odd ratio	P-value	95%Confidence interval
SOFA	1.10	0.372	(0.885--1.38)
Charlson's	1.042	0.720	(0.831--1.306)
Coronary vascular disease	0.027	0.006	(0.0021--0.351)
Hypertension	13.47	0.029	(1.311--138.47)
Age	0.993	0.726	(0.956--1.03)
Interaction 1	1.001	0.623	(0.999--1.0007)

Model 1: From the model generated we can deduce that the interaction between variables is not significant with P-value of 0.623, odd ratio 1.005 and 95%CI for OR (0.99--1.0007).

Table 5: Model 2 Interaction term

Model :2	Coefficient	P-value	95%Confidence interval
Mechanical ventilator	15.68	0.99	(-3031.95--3063.33)
Ventilator associated pneumonia	0.361	0.507	(-0.7068--1.43)
Interaction 2	16.44	0.992	(-3064.09--3031.199)
Constant	-0.99	0.002	(-1.62--0.352)

Model 2: Interaction 2 was not significant also with P-value of 0.999

Table 11	Bivariate analysis on 30 days in-hospital mortality		
	Unadjusted Odd ratios	P-value	95% OR Confidence Interval
Demographics			
Age (years)	1.01	0.323	(0.989 ---1.03)
Gender (male)	0.95	0.920	(0.347---2.599)
Service	0.252	0.011	(0.087—0.728)
Severity of illness scores			
SOFA	2.46	0.06	(0.932---6.521)
Pitts bacteremia score			
Charlson's co morbidity score	1.03	0.629	(0.89---1.21)
Other co morbidities:			
Coronary vascular disease	0.31	0.024	(0.117---0.844)
Hypertension	0.988	0.981	(0.376---2.594)
Rheumatoid disease	2.538	0.401	(0.288---22.34)
immunosuppressed	0.366	0.04	(0.137---0.978)
Recent surgery	2	0.171	(0.741---5.396)
Recent antibiotic use	0.673	0.451	(0.241---1.882)
Reason for admission:			
Respiratory failure	0.454	0.113	(0.171---1.206)
Sepsis	0.230	0.016	(0.069---0.764)
Gastrointestinal	0.8	0.738	(0.216---2.957)
Multiple traumas	3.837	0.092	(0.804---18.30)
Length of ICU stay:			
Prior to infection	0.982	0.486	(0.936---1.03)
Following infection	0.971	0.147	(0.934---1.01)
Hospital length of stay	0.92	0.002	(0.879---0.962)
Site of infection	0.67	0.530	(0.19---2.29)

Medical history:			
Previous surgery	1.041	0.934	(0.40---2.70)
Previous hospitalization	0.610	0.482	(0.154---2.41)
Previous antibiotic use	0.673	0.451	(0.241---1.881)
ICU Parameters:			
Mechanical ventilation	0.892	0.876	(0.215---3.699)
Central venous catheter	0.580	0.268	(0.221---1.51)
Urinary catheter	0	0.38	(0----4.71)
Arterial radial line	6.24	0.02	(1.33---29.22)
Nasogastric tube	0.854	0.807	(0.242---3)
Abdominal drainage	2.57	0.244	(0.525---12.61)
Hypoalbumenimia	0.757	0.697	(0.187---3.05)
Ventilator associated pneumonia	1.08	0.866	(0.413---2.859)
Colistin group	0.304	0.05	(0.09---1.005)
Route ofadministration:			
Nebulized	(Reference)		
Intravenous	0.69	0.532	(0.34---1.36)
Nebulized+ Intravenous	0.53	0.249	(0.24---1.17)
Concurrent agents			
Amikacin	1.04	0.935	(0.35---3.12)
Ceftazidime	4.79	0.146	(0.57---39.71)
Cefepime	1.70	0.642	(0.18---16.08)
Tazocine	1.70	0.642	(0.18---16.08)
Meropenem	0.70	0.475	(0.27---1.83)
Imipenem	2.29	0.310	(0.462---11.34)
Tigacycline	1.31	0.603	(0.470---3.66)
Duration of antibiotic administration	0.94	0.09	(0.87---1.01)
Nephrotoxicity	0.163	0.017	(0.037---0.722)

Multiple logistic regressions (model building)

Seventeen variables were extracted to be included in the multi logistic regression by which four continuous variables and twelve categorical variables. Some of these variables, tracheotomy and post any surgery, predict their failure perfectly and thus the regression model dropped these variables that resulted in decreasing the sample size. In order not to lose sample size, the decision was not to include these variables on model building despite its significance at the bivariate level. (Table 12).

Table 12: Multivariate analysis on 30 days in-hospital mortality

	Unadjusted Odd ratio	Adjusted Odd ratio	P-value	95%Confidence Interval
Service	0.252	1.67	0.717	(0.102--27.52)
SOFA	2.46	2.24	0.433	(0.298--16.84)
Coronary vascular disease	0.31	0.08	0.023	(0.0094--0.707)
Immunosuppressed	0.366	0.142	0.056	(0.019--1.052)
Recent surgery	2	7.52	0.112	(0.62--90.61)
Respiratory failure	0.454	0.560	0.649	(0.04--6.75)
Sepsis	0.230	0.404	0.545	(0.021--7.56)
Multiple Traumas	3.837	0.04	0.142	(0.00072--2.82)
Post infection ICU stay	0.971	1.11	0.155	(0.96--1.28)
Post infection LOH stay	0.92	0.818	0.009	(0.70--0.951)
Arterial line	6.24	15.09	0.106	(0.563--404.05)
Colistin group	0.304	0.141	0.103	(0.01--1.48)
Ceftazidime	4.79	1.02	0.891	(0.74--1.39)
Duration of antibiotic Administration	0.94	1.05	0.503	(0.90--1.21)
Nephrotoxicity	0.163	0.28	0.320	(0.02--3.35)

So far we have built our model according to certain criteria that suit our research question. However, we are in need to apply an advanced statistical technique to build our model.

Stepwise regression for Model Building:

Stepwise regression procedure for model building will be used based on setting 2 probabilities; the probability to remove=0.1001 and probability to enter=0.1, p-value less than 0.05 will be considered significant. (Table 13)

Because both SOFA score and colistin group were confounder for our outcome, so both variables were introduced in to the regression despite their significance by using lock term order.

Table 13:	Unadjusted Odd ratio	Adjusted Odd ratio	P-value	95%Confidence Interval
SOFA score	2.46	3.95	0.069	(0.89—17.44)
Colistin group	0.304	0.198	0.053	(0.038—1.01)
Coronary vascular disease	0.31	0.179	0.016	(0.044—0.72)
Immunosuppressed	0.366	0.161	0.019	(0.035---0.744)
Post infection LOH stay	0.92	0.88	0.001	(0.824---0.953)

In table 14, we ended with model that contains 5 variables at significance level of 10%,Furthermore; we wanted to use a stricter level of significance, i.e. 0.05, in order to reduce type 1 error, so we regressed the variables again with probability to enter pe=0.05 and probability to remove pr =0.05001.(Table 14)

Table 14:	Unadjusted Odd ratio	Adjusted Odd ratio	P-value	95%Confidence Interval
SOFA score	2.46	3.95	0.069	(0.89—17.44)
Colistin group	0.304	0.198	0.053	(0.038—1.01)
Coronary vascular disease	0.31	0.179	0.016	(0.044—0.72)
Immunosuppressed	0.366	0.161	0.019	(0.035---0.744)
Post infection LOH stay	0.92	0.88	0.001	(0.824---0.953)

After running the full model with level of significance 0.05, we got three independent variables that are significantly predictors for 30 days in-hospital mortality.

We should note the coding of the variables before interpreting the results where:

Treatment: (colistin group=0→1, non colistin group=1→0)

Coronary vascular disease: (yes=0→1, No=1→0)

Immunosuppressant: (yes=0→1, No =1→0)

Rerun the model after recoding:

Table 15:	Unadjusted Odd ratio	Adjusted Odd ratio	P-value	95%Confidence Interval
SOFA score	2.46	3.95	0.069	(0.89--17.44)
Colistin group	3.28	5.04	0.053	(0.98--25.94)
Coronary vascular disease	3.22	5.55	0.016	(1.37--22.45)
Immunosuppressed	2.73	6.18	0.019	(1.34--28.47)
Post infection LOH stay	0.92	0.88	0.001	(0.824--0.953)

From the obtained model, we can deduce, after adjusting for other covariates for each interpreted variable, that those patients who admitted to the intensive care unit and have history of coronary vascular disease will have 5.55 times the odds of patient with no coronary disease for 30 days in-hospital mortality with P-value of 0.016 and 95%CI OR(1.37--22.45). In simpler terms, coronary vascular disease is a risk factor for 30 days in-hospital mortality.

Adjusting for other covariates, the odds of 30 days in hospital mortality among immunosuppressed patients is 6.18 times of the non-immunosuppressed patients with P-value 0.019 and 95%CI(1.34--28.47).

Adjusting for other covariates, as post infection length of hospital stays increases by 1 day the odds for 30 days in-hospital mortality increases by 0.88.

Interpreting colistin group, the treatment under investigation, adjusting for other covariates in the model, we can conclude that those who treated with colistin have 5.04times the odds for30 days in-hospital mortality than the non colistin group with P-value of 0.053 and 95%CI OR (0.98--25.94).In other words, the colistin group treatment is not an independent risk factor for 30 days in hospital mortality after adjusting for other covariates.

B-Secondary outcome analysis

Nephrotoxicity is the most cited and notorious side effect resulted from colistin use. It was the main reason behind medication withdrawal and disinterest 50 years ago. In our study, we will assess certain risk factors that are believed to be associated with increased risk for toxicity with some physiological and clinical variables that allow us to compare renal status at different stages of intensive care unit stay and during the course of treatment. These variables will include: age, gender, Sofa score on admission, colistin route of administration, creatinine and blood urea nitrogen levels at day of intensive care admission, at day of starting antibiotic therapy and 24 hours of antibiotic discontinuation, nephrotoxic agents such as aminoglycosides, glycopeptides, diuretics, chemotherapeutics, analgesics and intravenous contrast use. (Tables 17 &18).

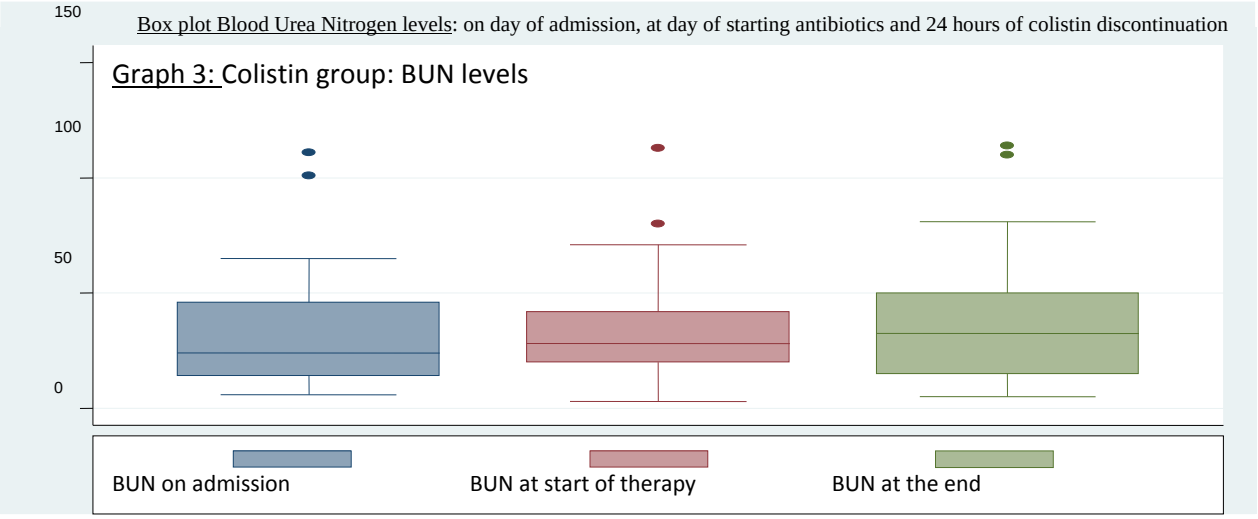
Basic characteristics of the Colistin group:

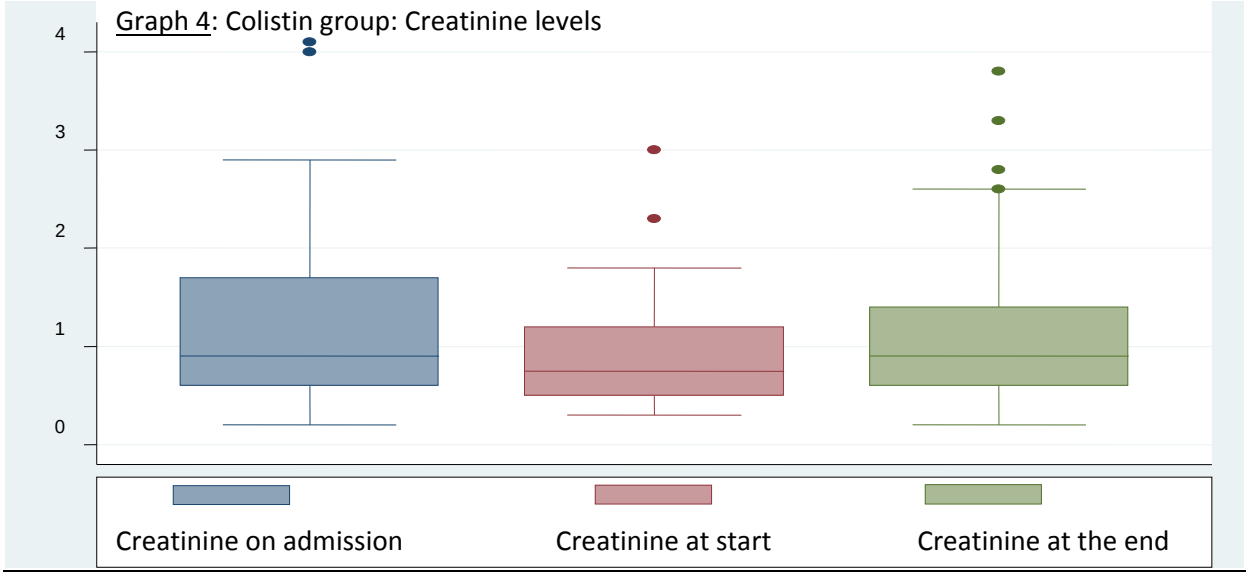
The mean age of the Colistin group patients is 57$\pm$22.51 SD, with 65.45% of the sample size are males, the median sofa score is 9 with interquartile range 6 to 10. Colistin route of administration was divided into intravenous (10.91%), inhaled (56.36%) and intravenous +inhaled (32.73%).

Creatinine levels were recorded at the day of intensive care unit admission, on the day of starting antibiotic therapy and 24 hours post medication discontinuation with respective medians of 1.4, 1.1 and 1.3.Blood urea nitrogen was also recorded as the same manner of creatinine with resultant medians of 28,31 and 36 respectively (graph 3&4).The use of nephrotoxic agents was also identified with respective representation of aminoglycosides(20%) ,glycosides(72.72%), diuretics(43.24%),chemotherapeutics (5.45%),analgesics(65.45%) and intravenous contrast(5.45%).(Table 16)

Table 16: Basic characteristics of the colistin group	
	Colistin group (n=55)
Demographics	
Age, years, mean	57.87±22.51
Gender (% males)	36 (65.45%)
Sofa on admission, median	9 (6—10
Colistin route of administration	
Intravenous	6 (10.91%)
Inhaled	31 (56.36%)
Intravenous +inhaled	18(32.73%)
Creatinine levels:(median)	
On admission	1.4 (0.6---1.7)
At the day of starting therapy	1.1 (0.5----1.2)
At the end of therapy	1.3 (0.6---1.4)
Blood urea nitrogen:(median)	
On admission	28 (14---46)
At the day of starting therapy	31 (20---42)
At the end of therapy	36 (15---50)
Duration of antibiotic admin-istration	16 (7---19)
Use of nephrotoxic agents	
Amino glycosides	11 (20%)
Glycopeptides	40 (72.72%)
Diuretics	24 (43.24%)
Chemotherapeutics	3 (5.45%)
Analgesics	36 (65.45%)
Intravenous contrast	3 (5.45%)

Colistin group: BUN and Creatinine levels on three occasions





Basic characteristics of the non Colistin group

The mean age of this treatment group is 53.88+ 27.04 SD, by which males represent 66.67% of the sample. The median sofa score calculated on admission is 6 with interquartile range 5 to 7. Creatinine and blood urea levels were both calculated on three different occasions, upon admission, at the day of starting antibiotic and after 24 hours of antibiotic discontinuation, graph (5 &6) the respective median values of creatinine 0.9(0.5--1),0.7(0.4--0.7), 0.7(0.4--0.9)and BUN simultaneously of 28 (14--46),35(20--42) and 36(15--50).

(Table 17)

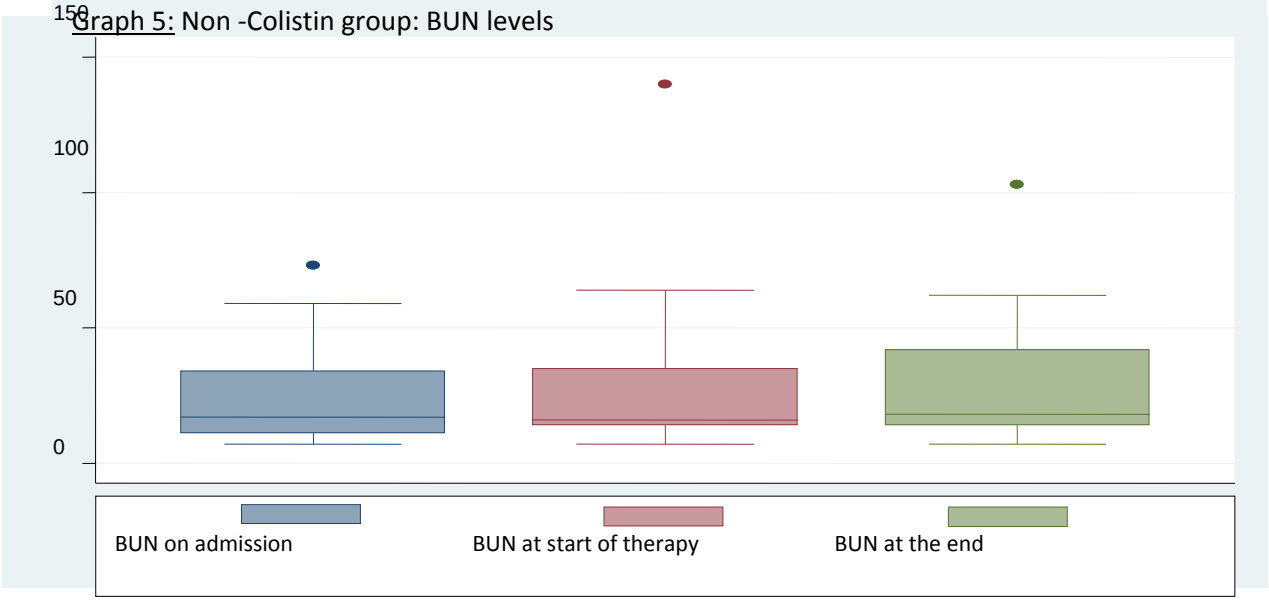
Table 17: Basic characteristics of the Non Colistin group

	Non Colistin group (n=27)
Demographics	
Age, years, mean	53.88±27.04
Gender (% males)	18 (66.67%)
Sofa on admission, median	6 (5--7)
Creatinine levels:(median)	
On admission	0.9 (0.5--1)
At the day of starting therapy	0.7 (0.4--0.7)
At the end of therapy	0.7 (0.4--0.9)
Blood urea nitrogen:(median)	
On admission	28 (14--46)
At the day of starting therapy	35 (20--42)
At the end of therapy	36 (15--50)
Duration of antibiotic administration	11 (6--14)
Use of nephrotoxic agents	
Amino glycosides	10 (37.04%)
Glycopeptides	10 (37.03%)
Diuretics	15(55.56%)
Chemotherapeutics	2 (7.41%)
Analgesics	12 (44.44%)
Intravenous contrast	0

Non -Colistin group: BUN and Creatinine levels on three occasions

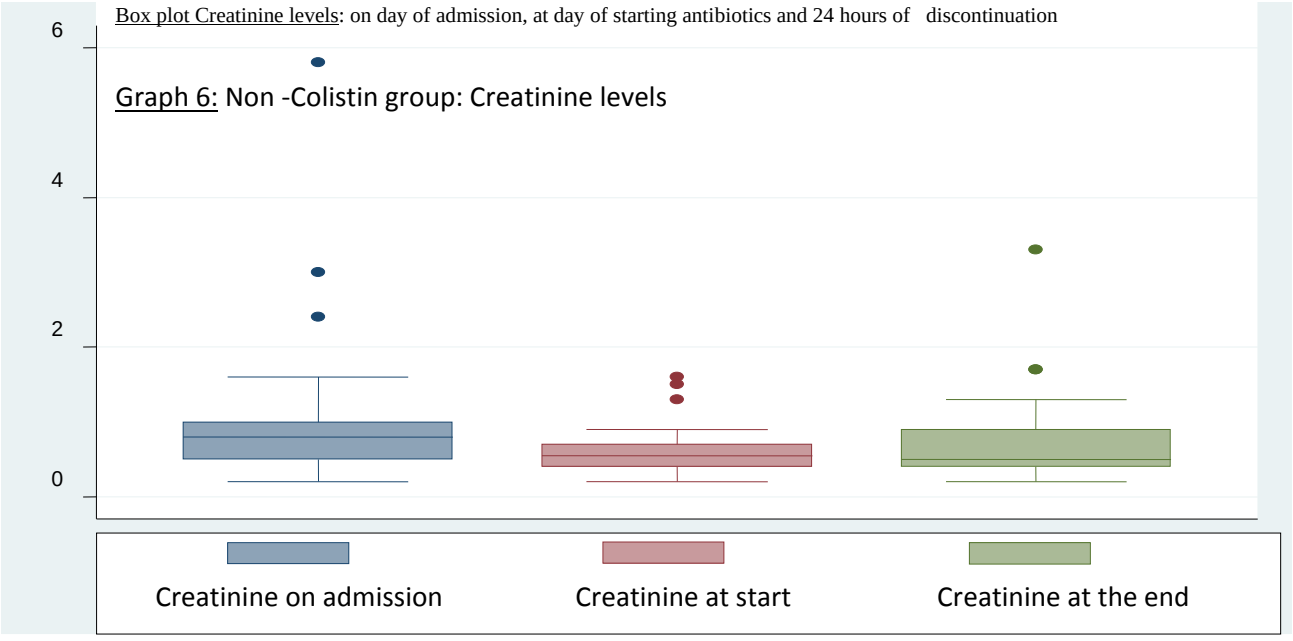
Box plot Blood Urea nitrogen levels: on day of admission, at day of starting antibiotics and 24 hours of discontinuation

Graph 5: Non -Colistin group: BUN levels



Box plot Creatinine levels: on day of admission, at day of starting antibiotics and 24 hours of discontinuation

Graph 6: Non -Colistin group: Creatinine levels



Between group's comparison:

Table 18:Basic characteristics of the Colistin group and non Colistin group			
	Colistin group(n=55)	Non Colistin group (n=27)	P-value
Demographics			
Age, years, mean	57.87+ 22.51	53.88 + 27	0.47
Gender (% males)	36	18	0.913
Sofa on admission, median	9 (6--10)	6 (5--7)	0.009
Creatinine levels(Median)			
On admission	1.4 (0.6--1.7)	0.9 (0.5--1)	0.608
At the day of starting therapy	1.1 (0.5--1.2)	0.7 (0.4--0.7)	0.03
At the end of therapy	1.3 (0.6--1.4)	0.7 (0.4--0.9)	0.05
Blood urea nitrogen: (median)			
On admission	28 (14--46)	28 (14--46)	0.249
At the day of starting therapy	31 (20--42)	35 (20--42)	0.344
At the end of therapy	36 (15--50)	36 (15--50)	0.119
Duration of antibiotic administration	16 (7--19)	11 (6--14)	0.188
Use of nephrotoxic agents:			
Amino glycosides	11	10	0.101
Glycopeptides	40	10	0.356
Diuretics	24	15	0.311
Chemotherapeutics	3	2	0.72
Analgesics	36	12	0.07

Between groups comparisons showed no significant association except for sofa score of p-value 0.009 which indicates more severe cases of the Colistin group on admission, and creatinine levels at the day of starting antibiotic therapy of p-value 0.03.(Table 18)

The correlation between Creatinine levels in both groups at the day of staring antibiotic and at the end of therapy was 0.3938 for the Colistin group and 0.4583 for the non Colistin group respectively.

```
. corr  creatatdayofstartantibx creatatendoftherapy if colistingroup==0
(obs=54)

             | creata~x creata~y
-------------+------------------
creatatday~x |   1.0000
creatatend~y |   0.3938   1.0000
```

```
. corr  creatatdayofstartantibx creatatendoftherapy if colistingroup==1
(obs=26)

             | creata~x creata~y
-------------+------------------
creatatday~x |   1.0000
creatatend~y |   0.4583   1.0000
```

Table 19: Nephrotoxic agents, Creatinine levels and duration of antibiotic administration

Colistin	Amikacin	Glycosides	Diuretics	Chemotherapeutics	Analgesics	Creatinine levels		Duration of colistin administration
						At the day of starting antibiotics	At the end of therapy	
		+				0.9	0.8	16
		+			+	0.9	0.8	4
	+	+				0.6	0.8	4
						0.5	0.7	9
		+	+		+	0.9	0.7	9
	+		+	+		1.5	3.8	19
	+	+	+		+	1.4	2.6	15
		+		+		1.5	2.1	6
Aerolized		+	+		+	0.7	0.3	25
	+	+			+	0.4	0.2	21
		+	+			0.4	0.3	20
	+		+		+	1.6	2.3	3
		+			+	0.8	1	14
		+			+	1	1.2	9
		+			+	0.6	0.7	16
					+	1.2	1.4	14
	+	+	+		+	0.9	0.5	14
	+	+	+		+	2.3	1	22
		+			+	0.5	1.1	3
					+	0.4	0.6	8
						0.6	0.6	4
		+	+			0.3	0.2	7
		+			+	0.4	0.5	13
			+		+	1.6	1.8	9
		+	+			0.5	0.6	5
		+	+		+	1.8	1.7	23
		+			+	0.9	1.2	5
					+	0.6	0.7	6
		+			+	0.5	0.9	14
		+	+		+	1.2	1.3	6
			+			0.3	0.2	26
Intravenous			+			1.3	1.5	4
		+			+	1	0.6	14
						1.2	1.1	7
		+			+	1.2	0.6	12
					+	1.4	1.5	12
					+	0.7	3.3	12
IV +Neb								
	+							
		+			+	0.4	0.4	70
		+				0.9	2.4	16
		+	+		+	0.6	0.6	48
			+			0.3	0.2	11
		+	+		+	0.3	0.5	18
		+				0.7	0.4	38
		+			+	0.6	2.8	24
		+	+			0.4	0.8	16
	+	+			+	0.6	1	14
			+			0.4	0.7	11
	+		+	+		0.7	2.1	6
	+	+			+	0.7	0.9	12
	+	+			+	3	1	16
		+			+	0.7	1.1	21
		+	+		+	1	1.4	26
		+			+	0.8	1.1	23
			+		+	1.1	1.4	8

Bivariate analysis of the secondary outcome:

Each of the specified variables was regressed on our secondary outcome, nephero-toxicity, by which the unadjusted odd ratios, p-values and their respective confidence inter-valswere obtained. (Table 20)

As shown in table 20, none of the independent variables in both the colistin and colistin groups that thought to be associated with nephrotoxicity was statistically significant. The use of chemotherapeutic agents was only the significant predictor with P- value of 0.04, odd ratio 17.75 and 95%CI OR(2.46--127.75).We should note that chemotherapeutics were used ap-proximately equally between the two treatment groups with 5.45% among the colistin group and 7.40% among the non colistin group.

Table 20: Bivariate analysis on Nephrotoxicity			
	Unadjusted odd ratio	P-value	95%Confidence interval
Age	0.98	0.396	(0.956---1.01)
Gender (% males)	1.04	0.957	(0.24—4.52)
Sofa on admission	0.32	0.137	(0.07—1.42)
Creatinine levels:			
On admission	0.74	0.329	(0.40—1.35)
At the day of starting therapy	0.40	0.135	(0.12---1.32)
Blood urea nitrogen:			
On admission	0.98	0.510	(0.96—1.02)
At the day of starting therapy	0.98	0.195	(0.96—1.008)
Colistin group	4.42	0.172	(0.52—37.36)
Colistin route of administration	0.61	0.450	(0.17—2.15)
Duration of antibiotic admin-istration	1.03	0.427	(0.94---1.14)
Use of nephrotoxic agents:			
Amino glycosides	2.63	0.182	(0.63—10.92)
Glycopeptides	1.04	0.596	(0.89—1.21)
Diuretics	0.86	0.84	(0.21---3.49)
Chemotherapeutics	17.75	0.004	(2.46—127.75)
Analgesics	0.87	0.848	(0.21—3.51)

Testing for interactions:

Colistin group	P-value
Interaction 1:Creatinine at the day of starting antibiotic + Amikacin+ vancomycin	0.59
Interaction 2:Creatinine at the day of starting antibiotic+ Amikacin+ diuretics	0.97
Interaction 3: Creatinine at the day of starting antibiotic+ Amikacin+ chemotherapeutics	0.163
Interaction 4:Creatinine at the day of starting antibiotic +Amikacin + analgesics	0.159
Interaction 5:Creatinine at the day of starting antibiotic +Amikacin+ vancomycin+ diuretics	0.205
Interaction 6:Creatinine at the day of starting antibiotic +Amikacin + vancomycin +Diuretics+ chemotherapeutics	0.197
Interaction 7:Creatinine at the day of starting antibiotic +Amikacin +vancomycin + Diuretics +chemotherapeutics + analgesics	0.09

None of the nephrotoxic agents was significantly associated with nephrotoxicity when they interacted with each other. We will draw every variable with p-value ≤0.2 to include in the multivariate analysis (Table 21)

Table 21:	Unadjusted odd ratio	adjusted odd ratio	P-value	95%Confidence interval
Sofa on admission	0.32	0.58	0.54	(0.10—3.31)
Colistin group	4.42	7.94	0.163	(0.43—145.93)
Amino glycosides	2.63	2.77	0.255	(0.478—16.10)
Chemotherapeutics	17.75	21.52	0.017	(1.71—270.30)
Creatinine at the day of starting therapy	0.40	0.65	0.67	(0.09—4.64)
BUN at the day of starting therapy	0.98	0.99	0.98	(0.95—1.04)

In our sample none of the believed treatments to cause renal impairment were significantly associated with nephrotoxicity except for chemotherapeutic agents with OR 21.52, 95 %CI (1.71--270.30) and P-value of 0.017.

Discussion:

This study assessed the risk factors associated with 30 days in-hospital mortality among critically ill patients with acinetobacter baumanii pulmonary and or blood stream infection. In the present study two types of treatments were compared in terms of 30 days in-hospital mortality and nephrotoxicity. Our major findings are :(i) Among ICU admitted patients ,the presence of coronary vascular disease history is independently associated with 30-days in-hospital mortality.(ii)Immunosuppressed patients admitted to the ICU are independently associated with 30 days in-hospital mortality.(iii)Infection with multidrug resistant acinetobacter baumanii is independently associated with length of hospital stay and 30 days in-hospital mortality.(iv)treatment with colistin group was not an independent risk factor for 30 days in-hospital mortality. We should note that in the present study, colistin group patients were more severely ill on admission and before contracting infection than the non colistin group as indicated by sofa score, added to that the differences in the sample size between the two groups is approximately two folds. This study design was used to avoid the over- estimation of the association between colistin use, 30 days in-hospital mortality and nephrotoxicity.

Extending to the present time, few studies were able to draw definitive conclusions about the impact of reintroducing colistin into clinical practice regarding optimal dosing and patient outcome (Basseti et al, 2008).

Several studies have evaluated colistin use in the treatment of critically ill patients and showed that colistin is safe and effective treatment with minimal side effects mainly nephrotoxicity (Michalopoluos et al, 2005).In the present study, none of the risk factors for nephrotoxicity and usually found on ICU settings as treatments were significantly associated with nephrotoxicity and thus renal impairment should not be attributed only to colistin toxicity as much as other cofactors such as creatinine levels at the day of starting therapy, concur-

rent and combined antibiotics ,underlying disease and nephrotoxic agents used .It is still we recommend close monitoring of renal function and proper dose adjustments in all different routes of colistin administration.

Although our study had retrospective design, the presence of comparison group and relatively large sample size in addition to the control of other co-administered nephrotoxic agents and severity of illness had resulted in less exaggeration of colistin use on both 30 days in hospital mortality and nephrotoxicity. All the interactions that may affect our both primary and secondary outcomes were also tested. All the concurrent and combined antibiotics administered with colistin treatment and duration of antibiotic administration were calculated and recorded

This study has several limitations, it is a retrospective in nature, but yet it is one of the few studies in terms of missing variables and sample size. Severity of illness scores were calculated only at the first 48 hours of admission and thus the progression of disease process following intensive care unit stay was not adjusted for. Cure of infection and microorganism eradication was not able to be assessed, repeated deep tracheal aspirate cultures needed at least every 3 days. Colistin doses were adjusted according to renal function on daily basis, no optimal dose treatment could be concluded. The single center design and the high proportion of respiratory infections treated with inhaled colistin probably limit the generalizability of our findings.

Conclusion:

Colistin treatment is not an independent risk factor for 30 days in hospital mortality and nephrotoxicity, but we need to conduct clinical trial in order to assess accurately the safety and efficacy of this treatment. Preexisting renal impairment is believed to be associated with increased creatinine levels during the course of treatment. Severity of illness and the underlying disease process are both confounders for 30 days in hospital mortality. Our results suggest that the presence of coronary vascular disease, use of immunosuppressant and length of hospital stay are independently associated with 30 days in hospital mortality.

Appendix A

Data collection sheet

ID: _____

Service: Medical ☐

Surgical ☐

Demographic characteristics:

Age (years): _____

Gender: Male ☐

Female ☐

Physiologic parameters first 48 hours of bacteremia: (SOFA &Pitt bacteremia scores) *		
Vitals	*Blood Gases:*	CHEM and CBC:
Rec , AX, Oral Highest temp: Lowest temp:	PaO_2/FiO_2:	Creatinine: GFR: BUN:
MAP: Highest SBP/DBP: Lowest SBP/DBP :	PCO_2: PH:	Bilirubin:
GCS:		Platelets count:
Others: Urine output:		%ANC :

Highest values of all parameters will be reported. MAP = [(2 x diastolic) +systolic] / 3

Extra for Pitt Bacteremia Score only :

Does the patient have cardiac arrest?

Yes: ☐ ☐

Extra for SOFA score only:

Dopamine:	☐	≤ 5micr/kg/min
	☐	> 5mic/kg/min
	☐	>15mic/kg/min
Epinephrine:	☐	≤0.1mic/kg/min
	☐	>0.1mic/kg/min
Norepinephrine:	☐	≤0.1mic/kg/min
	☐	>0.1mic/kg/min

Co morbidities:

Renal disease	☐	Hypertension	☐
Rheumatologic disease	☐	Hematologic malignancy	☐
Solid malignancy	☐	cardiovascular disease	☐
Pulmonary disease	☐	recent surgery	☐
Cerebrovascular disease	☐	Peripheral vascular disease	☐
Others	_____	Immunosuppressed	☐

Extra for Charlson's co morbidity score only:

Myocardial infarction	☐	Dementia	☐
Connective tissue disease	☐	Peptic ulcer disease	☐
Hemiplegia from any cause	☐	AIDS	☐

Diabetes Mellitus		Liver disease	
With end- organ damage:	☐	Mild:	☐
With no end -organ damage:	☐	Moderate:	☐
		Severe:	☐

Reason for admission

Respiratory failure	☐	Gastrointestinal	☐
Hemorrhage	☐	hepatic failure	☐
Cardiologic failure	☐	sepsis	☐
Multiple trauma	☐	vascular surgery	☐
Post any surgery	☐	others	_____

Site of infection

Respiratory tract infection	☐
Blood stream infection	☐
Respiratory and blood stream infection	☐

Other variables

Critical care stays at the day of suffering bacteria (Days)				☐
Post infection length of ICU stays (days)				☐
Post infection length of hospital stays (days)				☐
Previous hospitalization	yes:	☐	NO:	☐
Previous surgery	yes:	☐	NO:	☐
Previous antibiotic use:	yes:	☐	NO:	☐
Colistin group:	yes:	☐	NO:	☐

Average Colistin dose/24hrs (mg)

Route of Colistin administration:

Intravenous ☐ inhaled ☐ intravenous/inhaled ☐

Duration of antibiotic administration (days) ☐ Creatinine: ☐

Combination agents:

Amikacin	yes:	☐	NO:	☐
Sulbactam	yes:	☐	NO:	☐
Ceftazidime	yes:	☐	NO	☐
Levofloxacin	yes:	☐	NO:	☐
Tigacycline	yes:	☐	NO:	☐
Others	yes:	☐	NO:	☐

Nephrotoxic agents: yes: ☐ NO: ☐

Analgesics ☐ Antidepressants ☐ Antihistamine ☐

Antimicrobials ☐ Cardiovascular agents ☐ Chemotherapeutics ☐

Diuretics ☐ Contrast dye ☐ Proton pump inhibitors ☐

Others ☐

Non-Colistin group:

Meropenem ☐

Imipenem ☐

Tigacycline ☐

Sulbactam ☐

Others ☐

Invasive lines and tubes

Mechanical ventilation	yes: ☐	NO: ☐
Central venous catheters	yes: ☐	NO: ☐
Urinary catheter:	yes: ☐	NO: ☐
Arterial line catheter:	yes: ☐	NO: ☐
Tracheotomy:	yes: ☐	NO: ☐
Nasogastric tube:	yes: ☐	NO: ☐
Abdominal drainage:	yes: ☐	NO: ☐

30 Days in hospital mortality :(primary outcome)

Alive: ☐ Dead: ☐

Nephrotoxicity: (secondary outcome)

Yes: ☐ NO: ☐

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