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Colistin Resistant Rate among Gram's Negative Bacteria Isolated from Sharg Alnile and Yastabsheroon Hospitals from 2016 to 2017

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Abstract

Background: Gram negative bacteria is an organisms can cause different types of infections like UTI, Respiratory tract infections, Bacterimia etc… due to gram negative cell components become these organism resist to many types of antibiotics. Colistin one of the best antimicrobial agents that effect against multidrug-resistant Gram's negative bacteria. Multi drug resistance organisms have been considered as main cause of high morbidity and mortality rates in Sudan and worldwide.

Objectives: The aim of this study to determine the prevalence of Colistin resistant rate among Gram's negative bacteria.

Materials and Methods: One hundred and nineteen clinical isolated were collected during five months in 2016-2017 susceptability to the thirteen antibiotics was investigated using Kirby-Bauer.

Results: There are no isolated organisms that resist to Colistin. Two (2%) isolates were multi-drug resistant mainly resist to Meropenam and Amachcin and sensitive to the Colistin.

Conclusion: The colistin resistance rate in Sharg Alnile and Yastabsheroon hospitals is (0%) but the potential of colistin resistance in Sudan is gradually increase.

Keywords: Clistin; Gram's negative bacteria; Meropenam; Amachcin

Introduction

Multidrug resistance

The emergence of multidrug-resistant (MDR) Gram's negative pathogens has been increasingly described worldwide. The recovery of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates susceptible only to polymyxins from critically ill patients has led to the revival of Colistin, an antimicrobial forgotten for decades, which appears as the only treatment choice either empirically or as microbiologically documented therapy [1].

Gram's negative bacteria

The Gram's negative cell envelope contains an additional outer membrane composed by phospholipids and lipopolysaccharides which face the external environment. The highly charged nature of lipopolysaccharides confers an overall negative charge to the Gram's negative cell wall. The chemical structure of the outer membrane lipopolysaccharides is often unique to specific bacterial strains, and is responsible for many of the antigenic properties of these strains. Many species of Gram's negative bacteria are pathogenic. This pathogenicity is often associated with the lipopolysaccharide (LPS) layer of the Gram's negative cell envelope [2] (Figure 1).

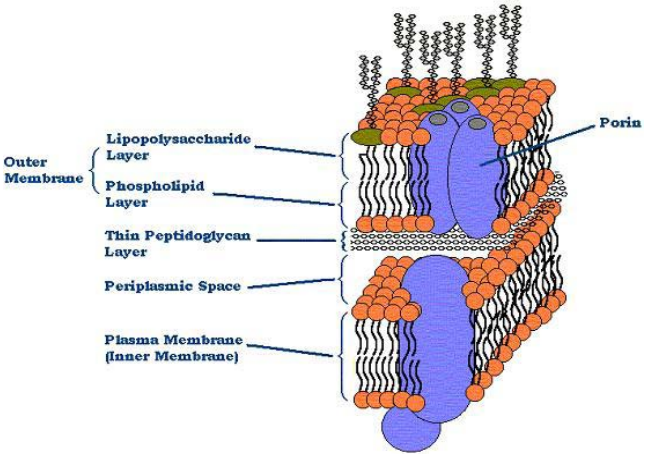

Figure 1: Cell wall components of gram's negative bacteria.

Infections caused by Gram's negative bacteria have features that are of particular concern. These organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure. Furthermore, they have available to them a increase of resistance mechanisms, often using multiple mechanisms against the same antibiotic or using a single mechanism to affect multiple antibiotics [3].

Hospital-acquired infections are most commonly associated with invasive medical devices or surgical procedures. Lower respiratory

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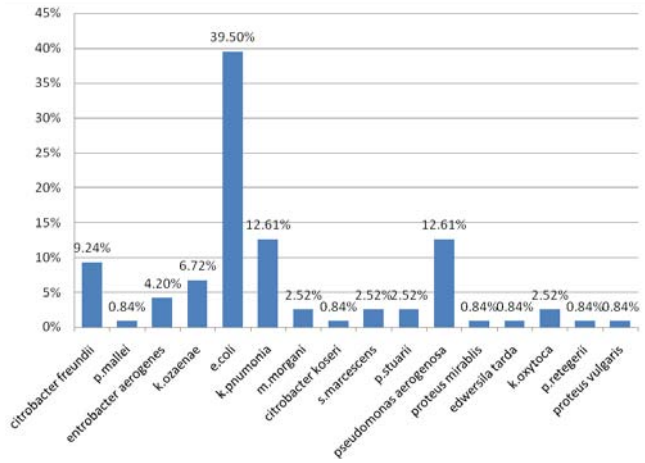

Figure 2: The percentage of the most common isolated organisms.

Table 1: Antibiotic used according to clinical laboratory standards institute.

Name of antibiotic	Concentration	Sensitive mm	Intermediate Mm	Resistant mm
URINE				
Piperacillin(PRL)	100mcg	21	18-20	17
Nitrofurantoin(F)	300mcg	17	15-16	14
Cefixime(CFM)	30mcg	19	16-18	15
Ciprofloxacin(CIP)	5mcg	22	20-21	19
Norfloxacin(NOR)	10mcg	22	20-21	19
WOUND				
Amoxicillin(AX)	10mcg	19-25	-	-
Gentamicin(GM)	10µg	15	13-14	12
Cefuroxime(CXM)	30mcg	18	15-17	14
Ceftazidime(CAZ)	30mcg	18	15-17	14
Ceftrixon(CRO)	30mcg	23	20-22	19
FOR MULTI DRUG RESISTANT				
Meropenem(MEM)	10mcg	23	20-22	19
Amikacin(AK)	30mcg	18	16-17	19-26
Colistin(cl)	10mcg	11	-	10

tract and bloodstream infections are the most lethal; however, urinary tract infections are the most common [3,4].

Gram's negative bacteria (*E.coli, proteus, K.pneumoniae, Citrobacter, Pseudomonas*) cause infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings [4].

Table 3: Frequency and the percentage of the isolated gram negative.

Organism	Frequency	Percentage
Citrobacter freundii	11	9.24%
Burkholderia mallei	1	0.84%
Entrobacter aerogenes	5	4.20%
Klebsiella ozaenae	8	6.72%
Eschrichia coli	47	39.50%
Klebsiella pnumoniae	15	12.61%
Morganella morgani	3	2.52%
Citrobacter koseri	1	0.84%
Serratia marcescens	3	2.52%
Providencia stuarii	3	2.52%
Pseudomonas aeruginosa	15	12.61%
Proteus mirablis	1	0.84%
edwersilatarda	1	0.84%
Klebsiella oxytoca	3	2.52%
Pretegerii	1	0.84%
proteus vulgaris	1	0.84%
TOTAL	119	100.00%

Colistin

Colistin is antibiotic that used to treat infections caused by multidrug-resistant Gram's negative bacteria (MDR-GNB). It is administered intravenously in the form of Colistin methane sulfonate (CMS), which is hydrolyzed in vivo to the active drug [5].

Polymyxins, a group of polypeptide antibiotics that consists of 5 chemically different compounds (polymyxins A-E), only polymyxin B and polymyxin E (colistin) have been used in clinical practice. Which it synthesized by *Bacillus polymyxa* subspecies *colistinus Koyama* [6].

Mechanism of action and resistance

The target of antimicrobial activity of Colistin is the bacterial cell membrane. The initial association of Colistin with the bacterial membrane occurs through electrostatic interactions between the cationic polypeptide (Colistin) and anionic lipopolysaccharide (LPS) molecules in the outer membrane of the Gram's negative bacteria, leading to derangement of the cell membrane. Colistin displaces magnesium (Mg^{+2}) and calcium (Ca^{+2}), which normally stabilize the LPS molecules, from the negatively charged LPS, a local disturbance of the outer membrane. The result of this process causes an increase in the permeability of the cell envelope, leakage of cell contents, and, subsequently, cell death [7,8].

Table 2: Frequency and the type of the specimens.

	the isolated gram's negative											
Type of sample	e.coli	klebsiella spp	proteus spp	Providancea	citrobacter spp	entrobacter spp	psudomonase	morganella	edwersila	Enterobacter spp	seritia	total
Wound swab	15	11	1	4	2	3	7	1	0	5	1	50
Urine	30	15	1	1	6	0	7	2	1	0	2	65
semen	1	0	0	0	0	0	0	0	0	0	0	1
sputum	0	1	0	0	0	0	0	0	0	0	0	1
ear swab	1	0	0	0	0	0	1	0	0	0	0	2

Table 4: Antimicrobial Susceptibility testing of *Escherichia coli.* (84%) resist to Piperacillin PRL (16%) is sensitive, Nitrofurantoin (F) resistance is (19%) and sensitive to (74%), Cefixime (CFM) resistance is (42%), Ciprofloxacin (CIP) resist to (52%) and sensitive to (48%), Norfloxacin (NOR) resist to (58%) and sensitive to (42%), Amoxicillin (AX) resist to (94%) and sensitive to (6%), Gentamicin (GM) resist to (19%) and sensitive to (81%), Cefuroxime (CXM) resist to (81%) and sensitive to (19%), Ceftrixon (CRO) resist to (69%) and sensitive to (31%), Ceftazidime (CAZ) resist to (56%) and sensitive to (44%), Meropenem (MEM) resist (6%) and sensitive (94%), Amikacin (AK) resistance (6%) and sensitive (88%) and colistin (CL) sensitive (100%).

Escherichia coli	Sensitive	Resist	Intermediate
CAZ(30mcg)	7(44%)	9(56%)	0(0%)
GM(10µg)	13(81%)	3(19%)	0(0%)
CXM(30mcg)	3(19%)	13(81%)	0(0%)
AX(10mcg)	1(6%)	15(94%)	0(0%)
CRO(30mcg)	5(31%)	11(69%)	0(0%)
NOR(10mcg)	13(42%)	18(58%)	0(0%)
CIP(5mcg)	15(48%)	16(52%)	0(0%)
F(300mcg)	23(74%)	6(19%)	2(6%)
PRL(100mcg)	5(16%)	26(84%)	0(0%)
CFM(30mcg)	18(58%)	13(42%)	0(0%)
MEM(10mcg)	16(94%)	1(6%)	0(0%)
AK(30mcg)	15(88%)	1(6%)	1(6%)
CL	1(100%)	0(0%)	0(0%)

Table 5: Antimicrobial Susceptibility testing of *proteus vulgeris*. (100%) Resist to Ceftazidime (CAZ), Gentamicin (GM), cefuroxime (CXM), Amoxicillin (AX) and Ceftrixon (CRO), (100%) sensitive to Meropenem (MEM) and Amikacin (AK).

proteus vulgaris	Sensitive	Resist	Intermediate
CAZ(30mcg)	0(0%)	1(100%)	0(0%)
GM(10µg)	0(0%)	1(100%)	0(0%)
CXM(30mcg)	0(0%)	1(100%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	0(0%)	1(100%)	0(0%)
MEM(10mcg)	1(100%)	0(0%)	0(0%)
AK(30mcg)	0(0%)	1(100%)	0(0%)

Increase in the prevalence of Gram's negative pathogens that are resistant to Fluoroquinolones and Aminoglycosides as well as all β-lactams, including Carbapenems, Monobactam, Cephalosporins and broad-spectrum Penicillin, has prompted the reconsideration of Colistin as a valid therapeutic option [8]. Gram's negative bacteria can develop resistance to Colistin through mutation or adaptation mechanisms. Mutation is inherited, low-level, and independent of the continuous presence of the antibiotic, whereas adaptation is the opposite. Studies of polymyxin-resistant *Pseudomonas aeruginosa* strains have suggested that alterations of the outer membrane of the bacterial cell (reduction in LPS, reduced levels of specific outer membrane proteins, reduction in cell envelope Mg^{+2} and Ca^{+2} contents, and lipid alterations) are related to the development of resistance. In addition, a recent study in *Yersinia* species demonstrated that an efflux pump/potassium system may be associated with resistance to polymyxin B. Although enzymatic resistance of bacteria to Colistin has not been reported, it is interesting that *Bacillus polymyxa* subspecies *colistinus* produces colistinase that inactivates Colistin [9].

Table 6: Results of Antimicrobial Susceptibility testing of *Pseudomonas aeruginosa.* Piperacillin (PRL) resist (63%) and sensitive (25%), Nitrofurantoin (F) resistance is (75%) and sensitive to (25%), Cefixime (CFM) resistance is (88%), Ciprofloxacin (CIP) resist to (63%), Norfloxacin (NOR) resist to (75%) and sensitive to (25%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) resist to (43%) and sensitive to (57%), Cefuroxime (CXM) resist to (100%), Ceftrixon (CRO) resist to (86%) and sensitive to (14%), Ceftazidime (CAZ) resist to (57%) and sensitive to (43%), Meropenem (MEM) resist (10%) and sensitive (90%), Amikacin (AK) resistance (20%) and sensitive (80%).

pseudomonas aeruginosa	Sensitive	Resistance	Intermediate
CAZ(30mcg)	3(43%)	4(57%)	0(0%)
GM(10µg)	4(57%)	3(43%)	0(0%)
CXM(30mcg)	0(0%)	7(100%)	0(0%)
AX(10mcg)	0(0%)	7(100%)	0(0%)
CRO(30mcg)	1(14%)	6(86%)	0(0%)
NOR(10mcg)	2(25%)	6(75%)	0(0%)
CIP(5mcg)	3(38%)	5(63%)	0(0%)
F(300mcg)	2(25%)	6(75%)	0(0%)
PRL(100mcg)	2(25%)	5(63%)	1(13%)
CFM(30mcg)	1(13%)	7(88%)	0(0%)
MEM(10mcg)	9(90%)	1(10%)	0(0%)
AK(30mcg)	8(80%)	2(20%)	0(0%)

Table 7: Results of Antimicrobial Susceptibility testing of *serratia marcescens.* Piperacillin (PRL) resist (40%) and sensitive (50%), Nitrofurantoin (F) resistance is (100%), Cefixime (CFM) resistance is (50%), Ciprofloxacin (CIP) resist to (50%), Norfloxacin (NOR) resist to (50%) and sensitive to (50%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) sensitive to (100%), Cefuroxime (CXM) sensitive (100%), Ceftrixon (CRO) sensitive to (100%), Ceftazidime (CAZ) sensitive (100%), Meropenem (MEM) sensitive (100%), Amikacin (AK) sensitive (100%).

Serratia marcescens	Sensitive	Resistance	Intermediate
CAZ(30mcg)	1(100%)	0(0%)	0(0%)
GM(10µg)	1(100%)	0(0%)	0(0%)
CXM(30mcg)	1(100%)	0(0%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	1(100%)	0(0%)	0(0%)
NOR(10mcg)	1(50%)	1(50%)	0(0%)
CIP(5mcg)	1(50%)	1(50%)	0(0%)
F(300mcg)	0(0%)	2(100%)	0(0%)
PRL(100mcg)	1(50%)	1(50%)	0(0%)
CFM(30mcg)	1(50%)	1(50%)	0(0%)
MEM(10mcg)	1(100%)	0(0%)	0(0%)
AK(30mcg)	1(100%)	0(0%)	0(0%)

Colistin is an old-generation antimicrobial agent; however, because it is one of the few agents that remain effective against multidrug-resistant Gram's negative bacteria (e.g., carbapenem-resistant *Pseudomonas aeruginosa* and *Enterobacteriaceae*), its clinical usefulness is being increasingly recognized [10], among the most clinically significant multidrug-resistant bacteria are carbapenemase-producing Enterobacteriaceae. Because these bacteria usually remain susceptible to polymyxins, because of their potential toxicity, interest in polymyxins (colistin and polymyxin B) has been renewed worldwide. However, the increasing use of colistin

Table 8: Results of Antimicrobial Susceptibility testing of *Providencia stuarii.* Piperacillin (PRL) (100%) is sensitive and resist, Nitrofurantoin (F) (100%) resist, Cefixime (CFM) (100%) sensitive, Ciprofloxacin (CIP) (100%) sensitive, Norfloxacin (NOR) (100%) sensitive, Amoxicillin (AX) (100) resist, Gentamicin (GM) (100%) was sensitive, Cefuroxime (CXM) resist to (100%), Ceftrixon (CRO) (100%) is resist, Ceftazidime (CAZ) (100%) sensitive.

Providencia stuarii	Sensitive	Resist	Intermediate
CAZ(30mcg)	1(100%)	0(0%)	0(0%)
GM(10µg)	1(100%)	0(0%)	0(0%)
CXM(30mcg)	0(0%)	1(100%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	0(0%)	1(100%)	0(0%)
NOR(10mcg)	2(100%)	0(0%)	0(0%)
CIP(5mcg)	2(100%)	0(0%)	0(0%)
F(300mcg)	0(0%)	2(100%)	0(0%)
PRL(100mcg)	1(100%)	1(100%)	0(0%)
CFM(30mcg)	2(100%)	0(0%)	0(0%)

Table 9: Results of Antimicrobial Susceptibility testing of *klebisella oxytoca.* (100%) Resist to Ceftazidime (CAZ), (100%) sensitive to Gentamicin (GM), (100%) resist to cefuroxime (CXM), Amoxicillin (AX) and Ceftrixon (CRO), (67%) sensitive and (33%) resist to Meropenem (MEM) and Amikacin (AK), and (100%) was sensitive to Colistin (CL).

Klebisella oxytoca	Sensitive	Resist	Intermediate
CAZ(30mcg)	0(0%)	3(100%)	0(0%)
GM(10µg)	3(100%)	0(0%)	0(0%)
CXM(30mcg)	0(0%)	3(100%)	0(0%)
AX(10mcg)	0(0%)	3(100%)	0(0%)
CRO(30mcg)	0(0%)	3(100%)	0(0%)
MEM(10mcg)	2(67%)	1(33%)	0(0%)
AK(30mcg)	2(67%)	1(33%)	0(0%)
COL	1(100%)	0(0%)	0(0%)

explains why acquired Colistin resistance may now be added to the carbapenem resistance trait in Enterobacteriaceae [11]. Previous reports have described the mechanisms of Colistin resistance as being chromosomally mediated and not associated with horizontal gene transfer. However, from 2011 through 2014, a plasmid-encoded Colistin-resistance gene was identified in Colistin-resistant *Escherichia coli* isolated in China, particularly from animals, and 1% of hospitalized human patients [11,12].

For identification of polymyxin resistance in *Enterobacteriaceae,* they used many tests either by Antibiotic susceptibility testing that performed per CLSI guidelines (dick diffusion methods, dilution methods and E test) or by genotypes (PCR Amplification and Sequencing) [13].

Prevention of antibiotic resistance

Antimicrobial agents have been greatly important cornerstones of clinical medicine since the second half of the 20th century and have saved a great number of people from life threatening bacterial infections. However, the last decade of the 20th century and the first decade of the 21th century have witnessed the emergence and spread of antibiotic resistance in pathogenic bacteria around the World, and

Table 10: Results of Antimicrobial Susceptibility testing of *klebisella pneumoniae.* (82%) resist to Piperacillin (PRL) (18%) is intermediate, Nitrofurantoin (F) resist to (27%) and sensitive to (64%), Cefixime (CFM) resist to(55%), Ciprofloxacin (CIP) resist to (9%) and sensitive to (91%), Norfloxacin (NOR) resist to (18%) and sensitive to (73%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) resist to (25%) and sensitive to (75%), Cefuroxime (CXM) resist to (75%) and sensitive to (25%), Ceftrixon (CRO) resist to (75%) and sensitive to (25%), Ceftazidime (CAZ) resist to (75%) and sensitive to (25%), Meropenem (MEM) resist (20%) and sensitive (80%), Amikacin (AK) sensitive (100%).

Klebsiella pneumoniae	Sensitive	Resist	Intermediate
CAZ(30mcg)	1(25%)	3(75%)	0(0%)
GM(10µg)	3(75%)	1(25%)	0(0%)
CXM(30mcg)	1(25%)	3(75%)	0(0%)
AX(10mcg)	0(0%)	4(100%)	0(0%)
CRO(30mcg)	1(25%)	3(75%)	0(0%)
NOR(10mcg)	8(73%)	2(18%)	1(9%)
CIP(5mcg)	10(91%)	1(9%)	0(0%)
F(300mcg)	7(64%)	3(27%)	1(9%)
PRL(100mcg)	0(0%)	9(82%)	2(18%)
CFM(30mcg)	5(45%)	6(55%)	0(0%)
MEM(10mcg)	4(80%)	1(20%)	0(0%)
AK(30mcg)	5(100%)	0(0%)	0(0%)

Table 11: Results of Antimicrobial Susceptibility testing of *klebisella ozaenae.* (83%) resist to Piperacillin (PRL), Nitrofurantoin (F) resist to (27%) and sensitive to (64%), Cefixime (CFM) resist to (55%), Ciprofloxacin (CIP) resist to (100%), Norfloxacin (NOR) resist to (50%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) resist to (50%) and sensitive to (50%), Cefuroxime (CXM) resist to (100%), Ceftrixon (CRO) resist to (100%), Ceftazidime (CAZ) resist to (100%), Meropenem (MEM) (100%)sensitive, Amikacin (AK) sensitive (100%).

Klebsiella ozaenae	Sensistive	Resist	Intermediate
CAZ(30mcg)	0(0%)	2(100%)	0(0%)
GM(10µg)	2(100%)	0(0%)	0(0%)
CXM(30mcg)	0(0%)	2(100%)	0(0%)
AX(10mcg)	0(0%)	2(100%)	0(0%)
CRO(30mcg)	0(0%)	2(100%)	0(0%)
NOR(10mcg)	3(50%)	3(50%)	0(0%)
CIP(5mcg)	3(50%)	3(50%)	0(0%)
F(300mcg)	3(50%)	2(33%)	1(17%)
PRL(100mcg)	1(17%)	5(83%)	0(0%)
CFM(30mcg)	3(50%)	3(50%)	0(0%)
MEM(10mcg)	4(100%)	0(0%)	0(0%)
AK(30mcg)	4(100%)	0(0%)	0(0%)

the consequent failure of antibiotic therapy, especially in intensive care units (ICUs), which has led to hundreds of thousands of deaths annually [14].

Antibiotic resistance occurs when bacteria outsmart drugs [15]. Everyone has a role in helping to prevent antibiotic resistance. Canadians and healthcare professionals must work together to reduce its impacts on our health and healthcare system [16].

The doctors, nurses, veterinarians and other health workers must be aware that do not prescribe or dispense antibiotics unless they

Table 12: Results of Antimicrobial Susceptibility testing of *Entrobacter aerogenes.* (100%)resist to Piperacillin (PRL), Nitrofurantoin (F) sensitive to (100%), Cefixime (CFM) resist to(100%), Ciprofloxacin (CIP) resist to (100%), Norfloxacin (NOR) resist to (100%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) sensitive to (100%), Cefuroxime (CXM) sensitive to (100%), Ceftrixon (CRO) sensitive to (100%), Ceftazidime (CAZ) (100%) sensitive, Meropenem (MEM) (100%)sensitive, Amikacin (AK) sensitive (100%).

Entrobacter aerogenes	Sensitive	Resist	Intermediate
CAZ(30mcg)	4(100%)	0(0%)	0(0%)
GM(10µg)	4(100%)	0(0%)	0(0%)
CXM(30mcg)	4(100%)	0(0%)	0(0%)
AX(10mcg)	0(0%)	4(100%)	0(0%)
CRO(30mcg)	4(100%)	0(0%)	0(0%)
NOR(10mcg)	0(0%)	1(100%)	0(0%)
CIP(5mcg)	0(0%)	1(100%)	0(0%)
F(300mcg)	1(100%)	0(0%)	0(0%)
PRL(100mcg)	0(0%)	1(100%)	0(0%)
CFM(30mcg)	0(0%)	1(100%)	0(0%)
MEM(10mcg)	1(100%)	0(0%)	0(0%)
AK(30mcg)	1(100%)	0(0%)	0(0%)

Table 13: Results of Antimicrobial Susceptibility testing of Citrobacter freundii. (75%) resist to Piperacillin (PRL), Nitrofurantoin (F) resist to (44%), Cefixime (CFM) resist to(56%), Ciprofloxacin (CIP) resist to (22%), Norfloxacin (NOR) resist to (44%), Amoxicillin (AX) resist to (100%), Gentamicin (GM) sensitive to (100%), Cefuroxime (CXM) sensitive to (100%), Ceftrixon (CRO) sensitive to (100%), Ceftazidime (CAZ) (100%)sensitive, Meropenem (MEM) (100%) sensitive, Amikacin (AK) sensitive (100%).

Citrobacter freundii	Sensitive	Resist	Intermediate
CAZ(30mcg)	2(100%)	0(0%)	0(0%)
GM(10µg)	2(100%)	0(0%)	0(0%)
CXM(30mcg)	2(100%)	0(0%)	0(0%)
AX(10mcg)	0(0%)	2(100%)	0(0%)
CRO(30mcg)	2(100%)	0(0%)	0(0%)
NOR(10mcg)	5(56%)	4(44%)	0(0%)
CIP(5mcg)	7(78%)	2(22%)	0(0%)
F(300mcg)	5(56%)	4(44%)	0(0%)
PRL(100mcg)	1(11%)	7(78%)	1(11%)
CFM(30mcg)	4(44%)	5(56%)	0(0%)
MEM(10mcg)	1(100%)	0(0%)	0(0%)
AK(30mcg)	1(100%)	0(0%)	0(0%)

are truly necessary and you have made all efforts to test and confirm which antibiotic your human patient or the animal you are treating should have [17].

So if you are sick and doctor give you antibiotics you should take antibiotics exactly as directed by your health care professional; make sure you know how much to take (the right dosage),when to take your antibiotics, and how many days you should take them, Even if you feel better, finish your antibiotics as directed to make sure that all of the bacteria are destroyed, Do not share your antibiotics with anyone, use leftover antibiotics or use antibiotics prescribed for someone other than yourself [18].

Table 14: Results of Antimicrobial Susceptibility testing of Morganella morgani. (100%) sensitive to Piperacillin (PRL), Nitrofurantoin (F) resist to (50%), Cefixime (CFM) sensitive to (100%), Ciprofloxacin (CIP) sensitive to (100%), Norfloxacin (NOR) sensitive to (100%), Amoxicillin(AX) resist to (100%), Gentamicin (GM) sensitive to (100%), Cefuroxime (CXM) resist to (100%), Ceftrixon (CRO) sensitive to (100%), Ceftazidime (CAZ) (100%) sensitive, Meropenem (MEM) (100%) sensitive, Amikacin (AK) sensitive (100%).

Morganella morgani	Sensitive	Resist	Intermediate
CAZ(30mcg)	1(100%)	0(0%)	0(0%)
GM(10µg)	1(100%)	0(0%)	0(0%)
CXM(30mcg)	0(0%)	1(100%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	1(100%)	0(0%)	0(0%)
NOR(10mcg)	2(100%)	0(0%)	0(0%)
CIP(5mcg)	2(100%)	0(0%)	0(0%)
F(300mcg)	1(50%)	1(50%)	0(0%)
PRL(100mcg)	2(100%)	0(0%)	0(0%)
CFM(30mcg)	2(100%)	0(0%)	0(0%)
MEM(10mcg)	2(100%)	0(0%)	0(0%)
AK(30mcg)	2(100%)	0(0%)	0(0%)

Table 15: Results of Antimicrobial Susceptibility testing of *Burkholderia mallei*. (100%) sensitive to Ceftazidime, (100%) sensitive to Gentamicin, (100%) sensitive to cefuroxime, (100%) resist to Amoxicillin and (100%) sensitive to Ceftrixon.

Burkholderia mallei	Sensitive	Resist	Intermediate
CAZ(30mcg)	1(100%)	0(0%)	0(0%)
GM(10µg)	1(100%)	0(0%)	0(0%)
CXM(30mcg)	1(100%)	0(0%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	1(100%)	0(0%)	0(0%)

The development of quick, effective molecular techniques for identifying resistance genes and the search of diagnostic biomarkers such as procalcitonin for using as a guide to cessation of antibiotics treatment are useful for reducing the use of antibiotics. Ultimately, if all members of society take on responsibility for maintaining the effectiveness of antibiotics and perform their role, minimization of antibiotic resistance can be successful [19].

Materials and Methods

Study design

Cross sectional hospital based study.

Study area

This study was carried out in Sharg Alnile and yastabsheroon hospitals.

Study population

Isolated Gram's negative bacteria from different samples.

Sample size

According to duration between November 2016 and March 2017

Included criteria

Isolated Gram's negative bacteria.

Table 16: Results of Antimicrobial Susceptibility testing of Citrobacter koseri. (100%) resist to Norfloxacin (NOR), (100%) resist to Ciprofloxacin (CIP), (100%) resist to Nitrofurantoin (F), (100%) resist to Piperacillin (PRL), (100) resist to Cefixime (CFM), (100%) sensitive to Meropenem (MEM) and to Amikacin (AK).

Citrobacter koseri	Sensitive	Resist	Intermediate
NOR(10mcg)	0(0%)	1(100%)	0(0%)
CIP(5mcg)	0(0%)	1(100%)	0(0%)
F(300mcg)	0(0%)	1(100%)	0(0%)
PRL(100mcg)	0(0%)	1(100%)	0(0%)
CFM(30mcg)	0(0%)	1(100%)	0(0%)
MEM(10mcg)	1(100%)	0(0%)	0(0%)
AK(30mcg)	1(100%)	0(0%)	0(0%)

Table 17: Results of Antimicrobial Susceptibility testing of *Edwardsiella tarda.* (100%) Norfloxacin (NOR), (100%) Ciprofloxacin (CIP), (100%) Nitrofurantoin (F), (100%) Piperacillin (PRL), (100) Cefixime (CFM), (100%) Meropenem (MEM) are resistance and (100%) sensitive to Amikacin (AK).

Edwardsiella tarda	Sensitive	Resist	Intermediate
NOR(10mcg)	0(0%)	1(100%)	0(0%)
CIP(5mcg)	0(0%)	1(100%)	0(0%)
F(300mcg)	0(0%)	1(100%)	0(0%)
PRL(100mcg)	0(0%)	1(100%)	0(0%)
CFM(30mcg)	0(0%)	1(100%)	0(0%)
MEM(10mcg)	0(0%)	1(100%)	0(0%)
AK(30mcg)	1(100%)	0(0%)	0(0%)

Excluded criteria

Gram's positive bacteria.

Methods

The isolated Gram's negative organisms were collected from different clinical samples, and sub cultured on MacConkey Agar for purification, incubate aerobically at 37 ^{0}C for 24 hours [20-27].

MacConkey (Appendix 1) media which was used for cultivation of *enterobacteria,* contain a bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose-fermenting (pink) from non lactose-fermenting (yellow) [28].

Culture method

The organisms were isolated and subculture on MacConkey, a colony was taken by using a wire loop sterilized by holding it in Bunsen flame so that the whole length becomes red-hot and waits until cooled. The inoculums were streaked thoroughly over area A to give a well-inoculums. The loop was re-sterilized and then drawn from the well in two or three parallel lines on to the fresh surface of the medium this process was repeated [27].

Preparation of smear

On sterile microscopic slide by sterile wire loop few drops of sterile normal saline was putted and re-sterile the loop and cooled, touch the colony from microorganisms grown in culture. And emulsify on the normal saline and the smear dry by air and fixed by passing three times through the flame [27].

Gram's stain

The Gram's stain and microscopic evaluation of cultured bacteria were used with colony morphology to decide which identification steps are needed.

Table 18: Results of Antimicrobial Susceptibility testing of *Proteus mirablis.* (100%) Norfloxacin (NOR), (100%) Ciprofloxacin (CIP) are sensitive, (100%) Nitrofurantoin (F) is resist, (100%) sensitive to Piperacillin (PRL), (100%) resist to Cefixime (CFM).

Proteus mirablis	Sensitive	Resist	Intermedate
NOR(10mcg)	1(100%)	0(0%)	0(0%)
CIP(5mcg)	1(100%)	0(0%)	0(0%)
F(300mcg)	0(0%)	1(100%)	0(0%)
PRL(100mcg)	1(100%)	0(0%)	0(0%)
CFM(30mcg)	0(0%)	1(100%)	0(0%)

Table 19: Results of Antimicrobial Susceptibility testing of *Providencia retegerii.* (100%) Ceftazidime (CAZ), (100%) Gentamicin (GM), (100%) Cefuroxime (CXM) are sensitive, (100%) resist to Amoxicillin (AX), (100%) is sensitive to Ceftrixon (CRO).

Providencia retegerii	Sensitive	Resist	Intermediate
CAZ(30mcg)	1(100%)	0(0%)	0(0%)
GM(10µg)	1(100%)	0(0%)	0(0%)
CXM(30mcg)	1(100%)	0(0%)	0(0%)
AX(10mcg)	0(0%)	1(100%)	0(0%)
CRO(30mcg)	1(100%)	0(0%)	0(0%)

The dry and fixed smear was flood by crystal violet (Appendix 2) for few second and washed with tap water, and flood by iodine (Appendix 3) for few second and washed with tap water, the slide was flood on decolorize (Appendix 4) for second and washed with tap water, the slide was flood by counter stain (safranine) (Appendix 5) for 30 second and washed with tap water, then the slide was examined under the microscope [27].

Results

Antimicrobial susceptibility test

Standard antimicrobial susceptibility testing was done for isolated organism, and we used polymxin family (Colistin), for Multi-Drug Resistance (MDR) organism and was performed for all Gram's negative bacteria isolated by Kirby –Bauer disk diffusion method using Muller-Hinton agar media according to the clinical laboratory standards institute (CLSI) guidelines [28].

Method

Inoculation preparation: Used of pure cultured Gram's negative bacteria, by inoculated 4-5 colonies have same morphology in broth media and the suspension was standardized by Macfarland (Appendix 12) turbidity (concentration equaled to $1.5*10^8$) [27].

Sterile cotton swab was dipped into broth media and the excess was removed by rotation of the swab against the side of the tube and streaked on the Muller-Hinton agar (Appendix 13), the disc of the antibiotic was applied with sterile forceps into the surface of the media and incubated aerobically at 3^{0}C overnight [27].

Antibiotic used

After incubation the zone of inhibition was measured and compared to the sheet provided by manufacture [27] (Table 1 & Figure 2).

Table 20: Frequency and the percentage of the Merobenem and Amickacin resistance organisms.

Type of organism	Frequency	%Percentage
MEM&AK sensitive	117	98%
MEM&AK resistance	2	2%
Total	119	100%

MEM: Merobenem; AK: Amickacin

Table 21: Frequency and the Percentage of gram negative according to susceptibility to colistin.

COLISTIN	Frequency	%Percentage
Resistance	0	0%
Sensitive	2	100%
	2	

Control organisms

The control organisms were applied to checked Gram's stain, culture media, biochemical test and sensitivity test by standard organisms of *Staphylococcus aureus* American type culture collection (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), obtained from department of Microbiology in central lab (Tables 2-21).

Discussion

Development of antimicrobial resistance is a phenomenon inevitably related to microbial evolution and antibiotic use. This study to isolate and identify the Colistin resistant Gram's negative bacteria.

The resistance to colistin antibiotic in this study was 0%, which is not agree with study that conducted in Central Greece (2015) by Oikonomou O, and et al, Sassera D, and et al in Nigeria (2011), and Matthaiou DK et al in Athens, Greece (2008). Which showed that resistance to colistin antibiotic was 21.1%, 17.6% and 41% respectively, this may be due to difference in the sample size, study area and method that applied [21,24,26].

In this research the isolated MDR E.coli is sensitive to colistin was inconsistence with study done by Hua Yu et al. which show that there is detection of colistin resistant *Eschriachia coli: it may be* due to different in sample size, study area [27].

A research done by Goli HR et al, in Iran (2016), and Sassera D, et al. in Italy (2014) which the colistin resistance rate is 2% from 100 sample, this result Differs from the presented study result there is no colistin resistance detected from 119 sample. May be due to the different area [22,23].

A study conducted by Antoniadou A et al, they found that the isolated *K. pneumoniae* were resistant to colistin. Their results inconsistence with this research result, since the isolated *K. pneumoniae* was resistant to Meropenem and Amickacin and sensitive to Colistin [1].

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