Sudan University of Science and Technology

College of Graduate Studies

Molecular Detection of Plasmids Mediated Colistin Resistance (*MCR-1*) Gene in *Enterobacteriaceae* from Clinical Specimens in Khartoum State

الكشف الجزيئي عن جين*MCR-1* البلازميدي المقاوم للكولستين فى العزلات المعوية المعزولة من عينات سريرية من ولاية الخرطوم

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الآيـة

بسم الله الرحمن الرحيم

قال تعالي :

وَهُوَ الَّذِي أَنْشَأَكُمْ مِنْ نَفْسٍ وَاحِدَةٍ فَمُسْتَقَرٌّ وَمُسْتَوْدَعٌ ۗ قَدْ فَصَّلْنَا الْآيَاتِ لِقَوْمٍ يَفْقَهُونَ ﴿٩٨﴾

صدق الله العظيم

سورة الأنعام الآية 98

Dedication

To soul of my father

To my loving mother

To unique wonderful husband

To supportive cheerful brothers

To sweet sisters

And those whom helped us

Acknowledgment

Firstly thanks to almighty Allah for helping me to finish this work.

I would like to express my sincere gratitude and thank fullness to my supervisor**Dr. HishamNooraldaymAltayeb Mohammed** who spared no effort giving me the maximum assistance to complete this thesis. I deeply grateful to him for long hours spent to correcting and advising me on this work.

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Abstract

The aim of this study was to detect the presence of *MCR-1* gene in clinical isolates of *Enterobacteriaceae* in Khartoum State, Sudan,at the period between February to July, 2016.

Fifty clinical samples were collected and cultured on MacConkey agar, the grown bacteria was identified by their growth criteria, Grams stain and biochemical tests. Antimicrobial susceptibility was done for them by using the following antibiotics; ciprofloxacin, gentamicin, co-trimoxazole, cefotaxime, cefuroxime and cefixime. DNA was extracted by using bacterial genomic extraction Kits. PCR was done by using *MCR-1* gene specific primer.

The most commonly isolated organism was *E. coli* 38 (76%), followed by *Proteus spp.* 8 (16%), and *K. pneumoniae* 4 (8%). Males (60%) weremore infected than females (40%), and elderly patients 61-90 years (42%) are found to be more susceptible to infection. We also reported highly resistant rate to ciprofloxacin (66%), gentamicin (68%) and cefuroxime (96%). *MCR-1* gene was detected in 7 (14%) isolates, mostly in *E. coli.*

We reported for the first time in Sudan the presence of *MCR-1* gene in clinical isolates, and the prevalence of this gene is higher when compared to other countries.

المستخلص

كان الهدف من هذه الدراسة هو الكشف الجزئيي عن جين *MCR – 1* في العزلات المعوية . أخذت عينات مختلفة من مجموعة من المرضى بمستشفى الزيتونة ومستشفى شرق النيل في الفترة ما بين فبراير إلى يوليو 2016 م . أخذت العينات من الإناث والذكور من اعمار مختلفة.تم زراعة العينات في وسط اجار الماكونكي للتعرف على البكتريا المعزولة من خلال خصائصها الظاهرية وبعض الإختبارات البيوكيميائية. تم أجراء إختبار الحساسية للمضادات الحيوية وهي السبروفلوكاسين والجنتامايسن، السيفيكيسم، سيفوروكسيم، وكوتراي مكسازول. وقد وجدنا ان الذكور (60%) أكثر عرضةللاصابة من الإناث (40%) .

وقد كانت الأشريكية القولونية أكثر انواع البكتريالمعزولة بنسبة 76% والكلبسيلية الرئوية بنسبة8% والبكتريا المتقلبة 16%. وقد لاحظنا وجود نسبة عالية من البكتريا المقاومة للمضادات الحيوية مثل السبروفلوكساسين 66% جنتامايسين 68% والسيفوروكسيم بنسبة96%.تم إجراء تفاعل البلمرة المتعدد للكشف عن جين *MCR – 1* وقد وجدنا ان العزلات الموجبة كان عددها 7 (14%) والتي لا تحتوي على الجين 43 (86%) . وقد كانت الاشريكية القولونية من أكثر البكتريا المعوية التي تحتوي على هذا الجين.

خلصت هذه الدراسة لان جين *MCR – 1* يوجد بنسبة عالية في السودان مقارنة بالدول الأخرى وهذا يستدعي من الجهات المختصة إجراء اللازم للحد من إنتشاره.

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Introduction

1.1 Antibiotics resistance

Antibiotic resistance is the ability of bacteria to survive and multiply even if the person was treated by a given antibiotic (WHO, 2010).

In 2010, World Health Organization director General Dr. Margaret Chan warned that antibiotics resistance was developed to a post antibiotic era " means in effect an end to modern medicine as we know it ". Things as common as strep throat or child's scratched knee could once again kill.

Major causes of antibiotic resistance are using antibiotics when they are not needed and not taken at the time and doses that doctors prescribed it. This will allows bacteria to become resistant (WHO, 2010).

Bacterial resistance to antimicrobial drugs is an increasing health and economic problem. Bacteria may be innate or acquire resistant to one or few classes of antimicrobial agents. Acquire resistance arise from chromosomal mutations leading to cross-resistance or gene transfer from onemicroorganism to other by plasmids, transposons, and bacteriophages. Most drug resistance in *Enterobacteriaceae* is due to wide spread transmission of resistant plasmids among different genera. About half of *Shigellaspp*. nowbecame resistant to multiple types of drugs. The abundant use of antimicrobial drugs in hospitalized patients led to suppression of drug susceptible organisms in the gut flora and growth resistant bacteria including *Enterobacter*, *Klebsiella*,*proteus*,*Pseudomonas* and *Serratia spp*.(Brooks *et al*., 2004).

Bacteria can collect resistance genes and splices them together on strand of DNA that can move around between bacteria. When a bacterium acquires one of these pieces of mobile DNA with many resistant genes, it can transform bacterium from one posing little threat to a potentially lethal super that resist treatment by multiple antibiotics (Carmen, 2016).

MCR-1 is a genetic element by which *mcr-1* gene confers the first known plasmid-mediated resistance to colistin (Sara, 2015). Colistin is used for treatment of patients infected with the highly resistant bacteria such as carbapenem-resistant *Enterobacteriaceae* like *Klebsiellapneumoniae*carbapenemase (KPC-2) and New Delhi Metallo beta lactamase (NDM-1) (Falagas, 2011).The first description of plasmid-mediated colistin resistance gene reported in China on 18 November 2015 during a routine surveillance project on antimicrobial resistance in commensal from food and animals. The mechanism first described in *Escherichia coli* (strain SHP45) (Liu *et al.*, 2015).At least 17 countries have now reported the presence of*mcr-1* gene such as Denmark, Netherland, France and Thailand (Olaitan*et al*., 2015).

1.2 Enterobacteriaceae:

The *Enterobacteriaceae* are large family of gram negative bacteria that include more familiar pathogens such as *Escherichiacoli*, *Yerisniapestis, Salmonella*, *Klebsiella* and *Shigella*. Other disease causing bacteria in this family include *Proteus, Enterobacter, Serratia* and *Citrobacter*.

Enterobacteria are facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. Many members of this family are a normal flora found in intestines of human and other animals (Brenner, 2005).

Members of *Enterobacteriaceae* can cause different kinds of infections such as urinary tract infections, pneumonia, wound infections, infections of blood stream and diarrhea.

1.3Rationale

Antimicrobial resistance is one of the most serious problems that faced human health in 21s century. Plasmids mediated colistin resistance (*MCR-1* gene) is one of the mechanisms by which bacteria resist multiple antimicrobial agents. Bacteria that possess this gene are difficult to treat. *MCR-1* can transferred from one bacteria to another, so the combination of this gene with other resistance genes may occur which can lead to resistance of the most effective antibiotics. There are no previous studies were done in Sudan to detect *mcr-1* gene,so we conducted this study to detect and characterize *mcr-1* gene.

1.4 Objectives

The general objective of this study was to:

Investigate the presence of *mcr-1* gene in clinical isolates of *Enterobacteriaceae* in Khartoum State.

The specific objectives of this study were to

1- Investigate the occurrence of *mcr-1*gene in multidrug resistant *Enterobacteriaceae*.

2- Determine the most commonly species of *Enterobacteriaceae* that possessed*mcr-1*gene.

Chaptertwo

LiteratureReview

Chaptertwo

LiteratureReview

2-1 Antibiotic resistance

The spread of antibiotics resistance genes in *Enterobacteriaceae* is complicating the treatment of serious nosocomial infection.

Approximately 20% of *Klebsiellapeumoniae* infections and *Enterobacterspp.* infections in intensive care units in the United States now involve strains not susceptible to third generation cephalosporins.

*Klebsiellapeumoniae*resist to third generation cephalosporins due to the presence of plasmids that containing genes encode extended spectrum beta lactamases (ESBLs) and these plasmids often carry other resistance genes. ESBLS- producing *Klebsiellapneumoniae* and *Escherichiacoli* are common on health care. *Salmonella* and other *Enterobacteriaceae* that cause gastroenteritis may be also ESBLS producers. *Enterobactercloacae*strains are resist to both third and fourth generations cephalosporins (Paterson, 2006).

*MCR-1*gene is found on a circular bit of free-floating DNA called plasmid which can be easily shared between bacteria there by allowing more microorganisms become resist to antibiotics (Sheryl, 2016).

The most frightening thing about *mcr-1*gene is the easiness with which can spread resistance to other bacteria through a process known as horizontal gene transfer, which is a rapid process of sharing resistance exist among single celled organisms in which there is direct exchange of genes(Molin, 2003; Julie, 2015).

*MCR-1*is member of the phosphoethanolaminetransferase enzyme family it is expression in *E.coli* resulting in the addition of phosphoethanolamine to lipid A. (liuY*et al.*, 2015).

A few years ago there was similar scare about *NDM-1*(New Delhi Metallo beta lactamase) a gene with the ability to jump from one bacteria to other species making them highly resistant to all known drugs, except two including colistin. If the colistin resistance MCR-1gene combine with *NDM-1* then the bacteria having combined gene would be resistant to virtually all drugs (Julie, 2015).

Salmonellaspp. and *Escherichiacoli* are causing nosocomial infections and their antibiotic resistance is due to adaptation to wide spread antibiotic use (Davies *et al.*, 2010). Carbapenemase (KPC) producingbacteria are group of highly drug resistant gram negative bacilli causing infections associated with significant morbidity and mortality whose incidence is rapidly increased in a variety of clinical setting around the world.

K.pneumoniae includes numerous mechanisms for antibiotics resistance genes many of them are located on highly mobile genetic elements.

Carbapenem antibiotic which is used for treatment of last resort of resistant infections, are generally not effective against KPC producing organism (Arnold *et al*., 2011).

Although mutation alone plays a huge role in development of antimicrobial resistance, in 2008 a study found that high survival rates after exposure could not accounted by mutation alone. This study focused on resistance of *E. coli* to three antibiotics drugs ampicillin, tetracycline and nalidixic acid. The result of this study is *E.coli* developed resistance to some antibiotics due to epigenetic inheritance rather by direct inheritance of mutated gene. Epigenetic is type of inheritance in which gene expression is altered rather than genetic code itself. The alteration of the gene expression can occur by methylation of DNA and histone modification. The important point is that both inheritances of random and

epigenetic markers can result in the expression of antibiotic resistance gene (Adam *et al*., 2008).

2.2 Mechanismsofantimicrobialresistance

There are different mechanisms by which microorganisms resist the antimicrobial agents (Li,X and Naikadio, 2009)

1- Production of enzymes that destroy the active drug: Gram negative rods resist to aminoglycosides by production adenylyating, phosphorylating or acetylating enzyme that destroy the drug.

2- Changing their permeability to the drug: Polymxins are one of drugs that associated with change in permeability of the drug.

3- Alteration of the target site.

4- Developed an altered metabolic pass ways.

2.3 Origin of drug resistance

2.3.1 Non genetic origin

Microorganisms may lose the specific target structure for drug foe several generations. For example penicillin- susceptible organism may change to cell wall deficient L-forms which make them resist to penicillin and cephalosporin.Microorganisms may infect the host at the sites where antimicrobials drug are not active. Example: Aminoglycosides are not effective in treating *Salmonella enteric* fevers because *Salmonellae* are intracellular and aminoglycosides do not enter the cells.

2.3.2 Genetic origin of drug resistance

The genetic drug resistance occurs as result of genetic change and subsequent selection processes by antimicrobial drug.

2.3.3 Chromosomal resistance:

Chromosomal resistance develops as the result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial drug. The spontaneous mutation occurs with a frequency of 10-12 to10-7 and thus is

a frequent cause emergence of clinical resistance in a given patient. Chromosomal mutants are most commonly resistant by virtue of a change in a structural receptor for a drug.

2.3.3.1 Extra chromosomal resistance

The extra chromosomal genetic element called plasmids which carry genes for resistance to one or several antimicrobial drugs. Plasmids genes for antimicrobial resistance often control the formation of the enzymes capable of destroying the antimicrobial drugs.

2.3.3.2Cross resistance

Microorganism that resistant to a certain drug may also be resistant to other drugs that share a mechanism of action. Such relationships exist mainly between agents that are closely related chemically or have a similar mode of binding or action (Brooks *et al*., 2004).

2.4 Colistin

Has a broad spectrum activity against the most species of bacteria and is considered one of the last treatments of gram negative pathogens. neurotoxic and nephrotoxic.Use in topical preparation and bowel decontamination regimens. If systemic use is contemplated a sluphomethylated is preferred.The two polymxins in clinical use are polymxin B and polymxin E (colistin) (Greenwood *et al*., 2002).

The mechanism of resistance to polymxins is modification of lipid A resulting in reduction of polymxin affinity. The resistance to polymxins is chromosomally mediated and involve modulation of two components of regulatory system (example *Pmr* AB, pho PQ and its negative regulation *mgr B* in case of *Klebsiellapneumoniae*) leading to modification of lipid A with moieties such as phosphoethanolamine or 4 arabinose, or in rare instances total loss of the lipopolysaccharides thus far the

polymxinsremain one of the last classes of antibiotics in which resistance plasmid is mediated (Kem*etal.*,2013, Cannatelli*etal.*,2013).

Colistin is widely used in farming particularly in China where farmers feed it to pigs and chickens to promote their growth. In the United States approximately 70% of antibiotics considered vitally important to human health are used in farm animals (Julie, 2015).

China is one of the world's largest users and producer of colistin for agriculture and veterinary use. Worldwide demand for the antibiotic in agriculture is expected to reach almost 12,000 tons per year by the end of 2015 rising to 16,000 tons by 2021 according to a 2015 report by QYResearch Medical Research Center (Kate, 2015).

2.5 Plasmids:

Are small DNA molecules within the cell that is physically separated from a chromosomal DNA and can replicate independently. They are found in the bacteria as circular double strand DNA molecules. Plasmids are sometimes present in eukaryotic organisms. Plasmids often carry genes that may benefit the survival of the organism, for example antibiotics resistance. They are usually very small and contain only additional information while chromosomes contain all the essential information for living. Plasmids are considered as replicons unit of DNA capable of replicating autonomously within the suitable host (Sinkovis*et al.*, 1999).

Plasmids transmitted from one bacterium to another via three mechanisms; transformation, transduction and conjugation. Some classes of plasmids encode conjugative sex pilus necessary for their own transfer. The size of plasmids varies from 1 to over 200 kbp (Thomas *et al*., 2008).

Plasmids may carry genes that provide resistance to natural occurring antibiotic, in competitive environment, or proteins produced may act as toxins under similar condition, or allow organism to utilize particular

organic compounds that would be advantageous in bad environment (Wolfgang, 2008).

Plasmids always carry at least one gene. Many genes carried by plasmids are beneficial for the host cell, they encode traits for antibiotic resistance or resistance to heavy metals, while other may produce virulence factors that enable a bacterium to colonize and overcome its defenses or have specific metabolic function that make a bacterium utilize a particular nutrient. Plasmids are generally circular but examples of liner plasmids are also known. The liner plasmid has special mechanism to replicate (Finbarr, 2003).

Plasmids in an individual cell are varying in number, ranging from one to several hundreds. The copy number is normal number of copies of plasmids that found in a single cell and it's determined by how replication initiation is regulated and the size of the molecule. Larger plasmids tend to have lower copy number (Brown, 2010).

2.5.1 Classification of plasmids

There are different classifications of plasmids. Firstly plasmids can be classified into conjugative plasmids and non-conjugative plasmids. Conjugative plasmids contain a set of transfer or *tra* genes which promote conjugation between different cells. Non-conjugative plasmids are incapable of initiating conjugation; they can be transferred only with assistance of conjugative plasmids.

Secondly plasmids can be classified into incompatibility group, in which a microbe can harbor different types of plasmids, however, different plasmids can only found in a single bacterium if they are compatible.

The third classification according to the function of plasmids can be classified in to five classes:

1- Fertility F plasmids which contain *tra*genes. They are able of conjugation and result in the expression of sex pili.

2- Resistance R plasmids which contain genes that provide resistance against antibiotics or poisons.
3- Col plasmids which contain genes that code bacteriocins, proteins that can kill other bacteria.
4- Degradative plasmids which enable the digestion of unusual substance e.g. toluene and salicylic acid.
5- Virulence plasmids which turn the bacterium in to a pathogen. (Margeret and Elizabeth, 1999).

2.5.2 Preparation of the plasmids

Plasmids preparation is method used to extract and purify plasmid DNA. There are many methods have been developed to purify plasmid DNA from bacteria. These methods are growth of bacterial culture, harvesting and lysis of the bacteria and purification of plasmid DNA. Plasmids are always purified from liquid bacterial culture. Virtually all plasmid vectors in common use encode one or more antibiotics resistance genes as selectable marker. Bacteria that have not taken up the plasmid vector re assumed to lack the resistance gene.

Harvesting and lysis of the bacteria were done under alkaline conditions (pH 12-12.5) to denature both chromosomal DNA and protein, however plasmid DNA remain stable. Some scientists reduce the NaoH concentration to 0.1 M in order to reduce the occurrence of ssDNA. After the addition of acetate containing neutralizing buffer the large and less supercoiled chromosomal DNA and protein precipitate but the small bacterial DNA plasmids stay in the solution.

Kits are available to purify plasmid DNA which is named by the size of bacterial culture and corresponding plasmids yield. In increasing order these are miniprep, midiprep, maxiprep, megaprep and gigaprep. The plasmids DNA yield will vary depending on the plasmids copy number,

type and size of bacterial strain, the growth condition and the kit (Cormier and Catherine, 2012).

Purification of plasmid DNA can be done by the addition of phenol/ chloroform which is dissolve and denature proteins like Dnase. This is important if the plasmids to be for enzyme digestion otherwise, smearing may occur in enzyme restricted from plasmid DNA (Birnboim and Dolyj, 1979).

2.6 MCR-1 gene

In the lancet Infectious Diseases Yi-yun Liu and colleagues described *mcr-1*a plasmid mediated gene that confers colistin resistance in *Escherichiacoli* and *Klebsiellapneumoniae*isolates cultured from animal and patients in China. Following this initial finding, several reports showed *mcr-1*has spread from China to South Asia and to other countries. These reports confirm the finding that *mcr-1*gene is mobilized on the plasmids that have spread to different *Enterobacteriaceaespp*. Of clinical concern is the inevitable spread of a plasmid harboring the *mcr-1*gene in to carbapenem resistant *Enterobacteriaceae,* creating a multidrug resistant isolate approaches pan drug resistance (Hong Du *etal*., 2016).

A study from Netherlands found that the antibiotic resistance gene *mcr-1*was not identified in human *Salmonella* but retrospectively detected at low levels in *Salmonella* from poultry (1%), as well *E. coli* from faces of Dutch livestock (0.3%) and retail meat (1.7%). The authors concluded retrospective screening with selective isolation procedures is needed to assess the current spread of the gene in bacterial population from animal sources. Researchers undertook whole genome sequencing from 600 *Enterobacteriaceae* isolates from different sources including humans in Germany. They found that resistance gene *mcr-1*has been present since at least 2010.

A global study evaluated the prevalence of the *mcr-1*gene in *Enterobacteriaceae* and the diversity of its isolates the later gives an indication of the rapidity of the gene's spread. The researchers found ESBL isolates carrying the *mcr-1*gene in travellers returning from three different continents. The findings suggest that a horizontal transfer is likely a cross many clones. The variety of plasmids backbones carrying the *mcr-1*gene indicates efficient transposition mechanisms. These observations indicate a promiscuous spread of the *mcr-1*gene which likely facilitated the observed dissemination across globe.

The researchers validated a newly developed real- time PCR assay specifically detects the *mcr-1*gene and was validated to accurately confirm the presence of this new gene in colistin- resistant clinical isolates. The assay may facilitate rapid *mcr-1*identification in stool specimens and can therefore be important to further improve clinical management and infection control.

A study from India evaluated the molecular characteristic of colistin resistance in *K. pneumoniae*. The researchers found that mutations in the *mgB* gene, and not the mobile *mcr-1*gene were responsible for resistance in the hospital. Due to the emergence of resistance to reserve drugs, there is a need for combination therapies for carbapenem resistant *K.pneumoniae* and colistin must be judiciously used.

Malaysian study evaluated *mcr-1*detection in *Enterobacteriaceae*. The researchers support the evidence that the detection of *mcr-1*gene in colistin resistant *Enterobacteriaceae* may be useful for rapid detection in clinical isolates. Rapid detection is needed for better patient management and to promptly implement effective infection control measures. They also suggest that the detection methods should include other molecular mechanisms that may contribute to the resistance of colistin in CR-KP. (Winfried, 2016).

In one Chinese study researchers examined a collection of 17 colistin resistant *Enterbacteriaceae* samples collected from hospital in Suzhou, China at 2013-2015. They found *mcr-1*gene in four isolate two *E. coli* and two *K. pneumoniae*. The two *K. pneumoniae* were resistant to all nearly drugs tested, and both carried *NDM-5* resistance gene, variant of *NDM-1* that has increased carbapenmase activity. The conclusion of this study was that mcr-1has already established itself in highly resistant *Enterobacteriaceaespecies*, including into Carbapenemase activity.

In Japan, researchers looked for database of gram negative bacteria isolates from hospitalized patients, animals and environment. They found the *mcr-1*in five animal gram negative bacterial isolates and two pig *E. coli* isolates from 2008 and 2010.

German researchers looked for the gene in a database of 577 whole genome sequences collected from people, animal and environment in Germany since 2009. They found *mcr-1*gene in four *E. coli* samples (Lisa, 2016).

Chapter three

Materials and methods

Chapter three

Materials and methods

3.1 Study design:

This study was a cross-sectional hospitals based study.

3.2 Study area

The study was done in patients attending to Alzaytona and ShargElneel hospitals, to investigate the presence of *mcr-1*gene in *Enterobacteriaceae* clinical isolates in Khartoum State.

3.3 Inclusion criteria

All patients attended to these two hospitals, of different ages suffering from bacterial suspected infections at the period of the study were included in this study.

3.4 Exclusion criteria

Fastidious and difficult growing organisms were excluded from this study.

3.5 Ethical consideration

Permission to carry out this study was taken from the College Of Graduates Studies, Sudan University for Sciences and Technology; all patients were informed for the purpose of the study before samples collections.

3.6 Data collections:

A questionnaire (personal information includes name, age,and sex and isolation site) was designed and used in the study (Appendix IV).

3.7 Data presentation:

Data was presented in a form of tables and figures.

3.8 Data analysis

The collected data was recorded and analyzed by SPSS statistical analysis software, version 16.0.

3.9 Sample collection and preservation:

A total of 50 *Enterobacteriaceae* samples were collected and preserved in glycerol peptone water. Clinical samples were cultured on a suitable culture media and the identification of these isolates was based on colonial morphology, Gram's stain reaction, lactose fermenting, and standard biochemical reactions.

The Suitable culture media are:

3.9.1 MacConkey agar:

MacConkey agar medium was prepared(Appendix 2) and Mixed well then dispensed aaseptically in sterile petridishes, after the surface of culture media have been dried, by using of sterilized wire loop asmall area of the plate have been inoculated, after that well streaked out three times by a sterile wire loop, then the plates were incubated over night at 37 ^{o}C.

3.9.2 Blood agar:

The blood medium (Appendix 2) after prepared, and dispensed was inoculated as above and incubated over night.

3.9.3 Colonial characterization:

The colonial morphology was reported as small or large, mucoid or not, flatted or not, the size, hemolysis on blood agar and lactose fermentation on MacConkey agar. On blood agar *E. coli* produce 1-4 mm and the colonies appear mucoid, some strains showed hemolysis. *Klebsiella spp*. gave white grey mucoid colony. *Proteus spp.* produced a characteristic fishy odor smell and swarmed over entire surface of blood agar medium.

3.9.4 Gram stain

Abacterial colony was suspended in normal saline , smeared, left to dry, fixed with heat by passing slide three times over flamed heat and then stained with crystal violet stain(see appendix 2) for 30–60 seconds, washed , covered with Lugol's iodine(see appendix 2) for 30–60 seconds, Decolorize rapidly (few seconds) with acetone–alcohol and washed and finally covered with neutral red stain for 2 minutes, washed and dried . The dried smear examined microscopically by oil immersion (Madigan *et al.*, 2004). Reporting of gram smear include the morphology of bacteria whether cocci or rods.

3.9.5. Biochemical tests

3.9.5.1. Oxidase test

A piece of filter paper is soaked with a few drops of oxidase reagent (see appendix 2). A colony of the test organism was then smeared on the filter paper by using a wooden stick. This test was done to differentiate *Pseudomonas*, (oxidase positive, which give a blue- purple color within a few second), from other *Enterobacteriaceae* (oxidase negative, no color change).

3.9.5.2 Catalase test

This test was used to differentiate those bacteria that produce the enzyme catalase, such as *Staphylococci*, from non-catalase producing bacteria such as *Streptococci*. By using about 2–3 ml of the hydrogen peroxide solution in a test tube a several colonies of the test organism were immersed by using a wooden stick an immediate bubbling indicate positive result.

3.9.5.3 Indole test

The test organism was inoculated in a bijou bottle containing about 3 ml of sterile tryptone water (Appendix 2) under a septic condition, and sealed well. Then the tube wasincubated at 37˚C for up to 48h. Production of indolewas checked by additionof 0.5 ml of Kovac's reagent, shaked gently and examined for a red color in the surface layer.Within 10 minutes, most strains of *E. coli*, *P. vulgaris*, *P.rettgeri*, *M. morganii*, and*Providencia*species break down the amino acid tryptophan with the release of indole. Other *Enterobacteriaceae* are indole negative (no color change).

3.9.5.4 Urease test

The test organism was cultured in a medium which contains urea,(see Appendix 2), and the indicator phenol red, by using a sterile straight wire and under a septic condition, the media was inoculated and incubated overnight at 37^{o}C. This test was used to differentiate *Enterobacteriaceae*,*spp*; *Proteus*strains (strong Urease producers), *K. pneumoniae* (slow) are Urease positive (produce pink color) and *E. coli* is Urease negative.

3.9.5.5 Citrate test

The tested organisms were suspended in a normal saline and cultured in a Simmon's citrate agar (Appendix 2). By using a sterile straight wire, firstly the slope was streaked and then butt was stabbed and incubated over night at 37^{o}C. This test was used to assist in differentiation of *Enterobacteriaceae* spp. *K. pneumonia* and some strains of *Proteus spp*.gave positive result (bright blue). *E. coli* is citrate negative (no color changes).

3.9.5.6 Kligler Iron agar

Kligler Iron Agar medium (Appendix 2), was prepared according to the instructions of manufacturer. The KIAmedium was inoculated using sterilized straight wire, firstly, the butt was stabbed and then the slope streaked in a zig-zag pattern. After 24 hours of incubation at 37^{o}C, the KIA medium was observed for color changes, gas and H_2S production.

A yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose. This occurs with *E. coli* and *K. pneumoniae*. A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only, this occurred in most *of Proteus spp. and Pseudomonas spp*. Cracks and bubbles in the medium indicate gas production from glucose fermentation, this was observed in *K.pneumoniae,P.mirabilis* and most of *E.colispp*, *P.vulgaris.* A red-pink slope and butt indicate no fermentation of glucose or lactose. This occurred with most strains of *P.aeruginosa*. Blackening along the stab line or throughout the medium (hydrogensulphide (H_2S) production) was seen in *Proteus spp.*

3.9.6 Susceptibility test

All identified isolates were subjected to antibiotic Susceptibility testing discs by Kirby-Bauer diffusion methodaccording toPodschun and Ullmann, (1998).

Method:

1- Several colonies of tested organism were emulsified in small volume of sterile normal saline to make fine suspension. The turbidity of the suspension was matched against the turbidity of 0.5 McFarland standard (prepare by adding 0.6 ml of the barium chloride 1% v/v solution to 99.4 ml of the sulphuric acid 1% w/v solution). The suspension was used within 15 min after preparation.

2- A sterile cotton swab was dipped into the suspension and was rotated firmly several times against the upper side wall of the tube to removethe excessfluid.

3- The entire agar surface of Muller-Hinton agar (Appendix 2) plates were streaked three times; the plates were turn 60 degree between streaking to obtain even inoculums.

4- By using sterile forceps, under aseptic condition the antibiotic discs were applied onto the surface of the agar (ciprofloxacin, gentamycin, co trimexazole, cefixime, cefotaxime and cefuroxime. The discs were placed on the plates with at least 24 mm between them to avoid overlapping of inhibition zones and not less than 10 mm from the edge of Petridishes. The discs were pressed down with a sterile needle or forceps to make contact with the surface of media.

5- The plates were incubated innerted in the incubator at 37°C for overnight.

6- The plates were examined and the zones of inhibition were recorded (Podschun and Ullmann., 1998).

   The recorded zones were compared with those in the chart; results with specific organism reported as resistant (R), Moderate sensitive (M) or sensitive (S) (Black,. *et al* 1996)

3.10 Detection of *MCR-1*

3.10.1 DNA extraction:

Bacterial DNA was extracted by using G-spin TM Genomic DNA Extraction Kit.

3.10.2 Polymerase chain reaction:

3.10.2.1. Primer:

A universal primer pair, CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-R (5′-CTTGGTCGGTCTGTAGGG-3′), was used for screening as previously described (Liu *et al.*, 2016).

3.10.2.2 Preparation of primers

The primers were dissolved according to manufacture guide lines to prepare 10pmol/µl.

3.10.2.3. Preparation of10X TBE buffer

Amount of 108 grams of Tris base was added to 55g of boric acid and 40 ml of 0.5 EDTA, and then dissolved into 1 liter Deionized water pH 8.0.

3.10.2.4. Preparation of 1X TE buffer

10 ml of 10X were added to 90 ml Deionized water and heated until completely dissolved.

3.10.2.5. Preparation ofethidium bromide

Ten milligrams of ethidium bromide powder were dissolved into 500 µl DW and kept into brown bottle.

3.10.2.6. Preparation ofagarose gel

Amount of 2 g of agarose powder were dissolved in 100 ml 1X TE buffer. Then the mixture had been cooled to 55^{o}C in water bath. Then 2.5 µl of (20mg/ml) ethidium bromide was added, and mixed well and poured in a casting tray that had been taped up appropriately and equipped with suitable comb to form well in place. Any bubbles were removed and the gel allowed setting at room temp.

After solidification, the comb gently removed and the spacer from the opened slides was removed.

3.10.2.7. Master Mix:

Maxime PCR Premix Master mix (iNtRON BIOTECHNOLOGY, Korea) is a premixed ready to used solution containing all reagents required for

PCR (except water, template and primers) and additional compound needed for direct loading onto agarose gel tracking blue dye that allow the monitor progress during the electrophoresis.

3.10.2.8 Preparation of reaction mixture

The following reagents (25 µl reaction) were used for detection of *MCR-1*gene in the following volumes in 0.2 ml eppenddorf tube:

1. 17 µl Deionized sterile water.
2. 5 µl Maxime PCR PreMix (iNtRON BIOTECHNOLOGY).
3. 0.5 primer forward primer (Macrogen, Korea).
4. 0.5 primer reverse primer (Macrogen, Korea).
5. 2µl plasmid DNA (template DNA).

3.10.2.9 Protocol used for amplification of the *MCR-1*gene

The amplification was done by using TECHNE (TC- 312) thermal cycle (UK). The PCR mixture was subjected to initial denaturation step at 94°C for 5-min, followed by 30 cycles of denaturation at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds, followed by step of elongation at 72°C for 60 seconds and the final elongation at 72°C for 5 min.

3.10.2.10 Visualization of PCR product

The gel casting tray was flooded by 10x TBE buffer near the gel cover surface, then 7µl of PCR products of each samples was put into each well. Then to the firstwell of casting tray 7µl of DNA ladder (marker) was injected for each run. The gel electrophoresis apparatus (Primer, 125v, 500 mA, UK), was run at 100 v for 40 min. After that the gel was removed by gel holder and visualized by U.V transilluminator (Uvite–UK). The gel was photographed by using the Polaroid film.

Chapter four

Results

Chapter four

Results

4.1 Results

4.1. Enrolled patients

The distributions of enrolled patients were60% males and 40%females (figure 4.1),they were from different age groups. Males had been infected more than females. There was no association between MCR-1gene and gender of the patients (table 1).

Table (1) Relationship between *MCR-1*and gender

			mcr1			
			positive	Negative	Total	P- value
sex	male	Count	6	24	30	0.1
		% of Total	12.0%	48.0%	60.0%	
	female	Count	1	19	20	
		% of Total	2.0%	38.0%	40.0%	
Total		Count	7	43	50	
		% of Total	14.0%	86.0%	100.0%	

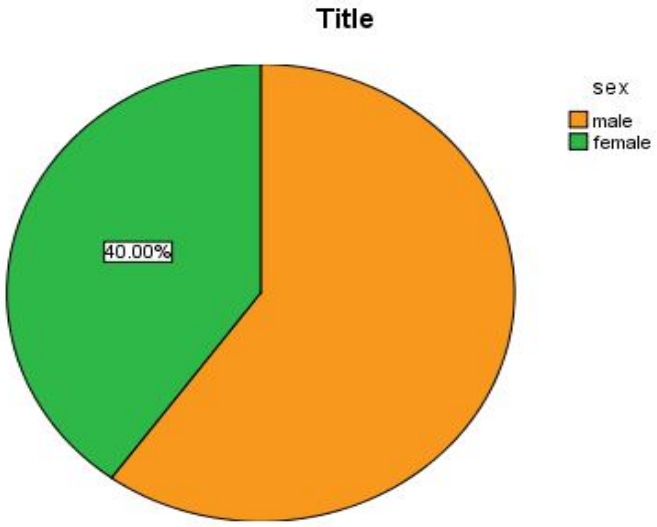

Figure (4.1): Distribution of male and female patients

The highest age group thatsuffered from *Enterobacteriaceae* infections was (61-90) 42% as shown in (figure 4.2) and the lowest one was (20 – 40). Statically there was no significant association between age of patients and the presence of *MCR-1* gene.

Table (2): Relationship between the presence*MCR-1*and age group

			mcr1			
			positive	Negative	Total	P-value
age2	20-40	Count	1	15	16	0.2
		% of Total	2.0%	30.0%	32.0%	
	41-60	Count	1	12	13	
		% of Total	2.0%	24.0%	26.0%	
	61-90	Count	5	16	21	
		% of Total	10.0%	32.0%	42.0%	
Total		Count	7	43	50	

Table (2): Relationship between the presence*MCR-1*and age group

			mcr1			
			positive	Negative	Total	P-value
age2	20-40	Count	1	15	16	0.2
		% of Total	2.0%	30.0%	32.0%	
	41-60	Count	1	12	13	
		% of Total	2.0%	24.0%	26.0%	
	61-90	Count	5	16	21	
		% of Total	10.0%	32.0%	42.0%	
Total		Count	7	43	50	
		% of Total	14.0%	86.0%	100.0%	

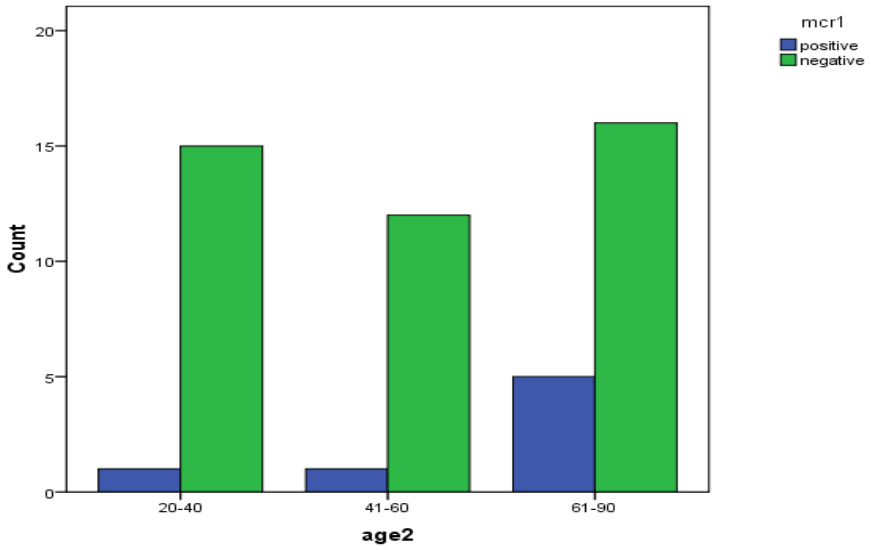

Figure 4.2. Distribution of patients according to the age group

4.2. Distribution of collected specimens

The specimens were collected from different sites; urine (60%), swabs (32%) and others sites like tissues and aspiration fluid (8%) as shown in (Table 3). Statically there was no association between the presence of *MCR-1* gene and the site that from which sample was isolated (p-value = 0.3).|

Table (3): Relationship between *MCR-1* and sites of isolation

			mcr1			
	site		positive	Total		P-value
	urine	Count	6	24	30	0.3
		% of Total	12.0%	48.0%	60.0%	
	swab	Count	1	15	16	
		% of Total	2.0%	30.0%	32.0%	
	others	Count	0	4	4	
		% of Total	.0%	8.0%	8.0%	
Total		Count	7	43	50	
		% of Total	14.0%	86.0%	100.0%	

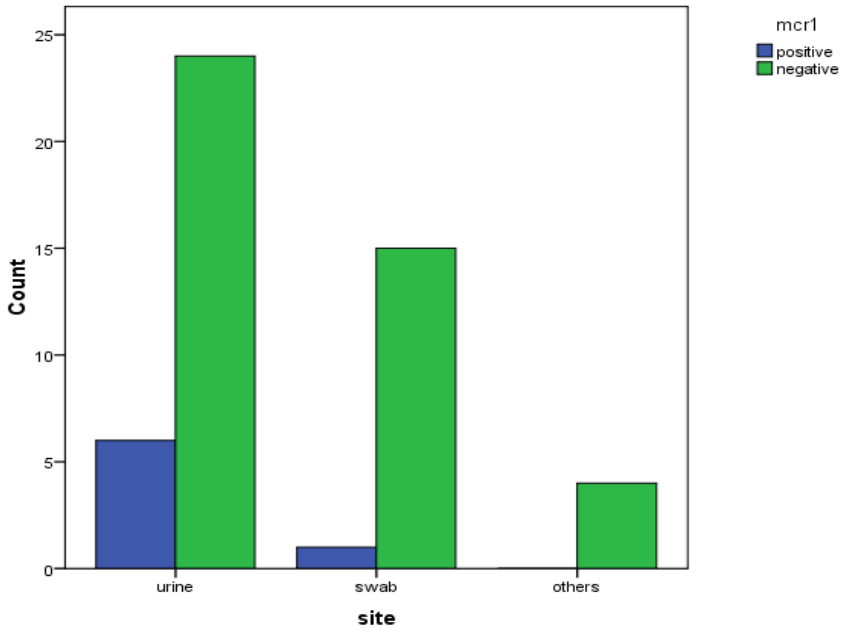

Figure: 4.3. Distribution of sample isolation site

4.3. Distribution of isolated organisms

The isolated organisms were as follows; *E. coli* (38%), *K. pneumoniae* (4%) and *Proteusspp*. (8%). There was significant statistical association between the isolated organisms and the presence of *MCR-1* gene ($p < 0.05$).

Table (4): Relationship between MCR-1 and isolated organisms

	Pathogen		*MCR-1* positive	*MCR-1* Negative	Total	p-value
	E.coli	Count	6	32	38	
		% of Total	12.0%	64.0%	76.0%	
	Proteuss pp.	Count	0	8	8	
		% of Total	.0%	16.0%	16.0%	0.04
	Klebsiell apneumo niae	Count	1	3	4	
		% of Total	2.0%	6.0%	8%	
Total		Count	7	43	50	
		% of Total	14.0%	86.0%	100.0 %	

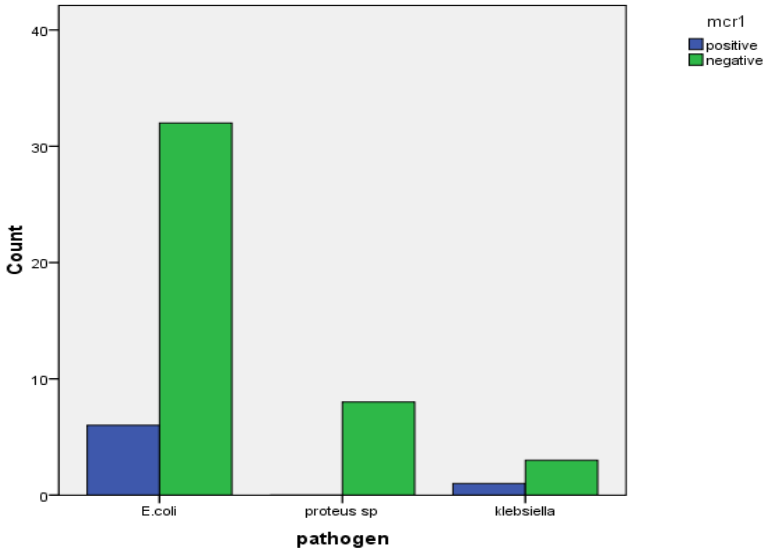

Figure 4.4: Distribution of the isolated organisms

4.4. Antimicrobial susceptibility testing

4.4.1 Susceptibility of ciprofloxacin

Sixty six percent ofall isolated organisms were resistant to ciprofloxacin and 34% were sensitive. There was significant association (P-value <0.05) between the presence of -*MCR-1* gene and resistance to ciprofloxacin.

Table (5): Relationship between resistance to ciprofloxacin and the presence of *MCR-1* gene

			MCR-1			
			Negative	positive	Total	p- value
cipro	resist	Count	33	0	33	0.00
		% of Total	66.0%	.0%	66.0%	
	Sensitive	Count	10	7	17	
		% of Total	18.0%	16.0%	34.0%	
Total		Count	43	7	50	
		% of Total	86.0%	14.0%	100.0%	

4.4.2 Susceptibility of Gentamicin

There were 34 (68%) isolates resistant to gentamicin and 16(32%) sensitive. There was significantly association between *MCR-1*gene and gentamicin.

Table (6): Relationship between resistance to gentamicin and the presence of*MCR-I* gene

gentamicin			*MCR-1*		Total	P- value
			Negative	positive		
	resist	Count	34	0	34	0.00
		% of Total	68.0%	.0%	68.0%	
	Sensitive	Count	9	7	16	
		% of Total	16.0%	16.0%	32.0%	
Total		Count	43	7	50	
		% of Total	86.0%	14.0%	100.0 %	

4.4.3 Susceptibility of Cefuroxime

There were 42(96%) isolates resistant to cefuroxime and 2(4%) were sensitive. There was significant association between resistance to cefuroxime and the presence of *MCR-1* gene (p- value >0.05).

Table (7): Relationship between the presence of *MCR-1* gene and resistant to cefuroxime

Cefuroxime			*MCR-1* gene			
			Negative	positive	Total	P- value
	Resist	Count	43	5	48	0.01
		% of Total	86.0%	10.0%	96.0%	
	Sensitive	Count	0	2	2	
		% of Total	.0%	4.0%	4.0%	
Total		Count	43	7	50	
		% of Total	86.0%	14.0%	100.0%	

4.5. PCR

A total of 50isolateswere subjected to PCR for detection of *mcr-1* gene (that showed a band typical in size309bp). Seven (14%) isolates were positive for *mcr-1* gene. While, 43 (86%) gave negative result (figure 4.5).

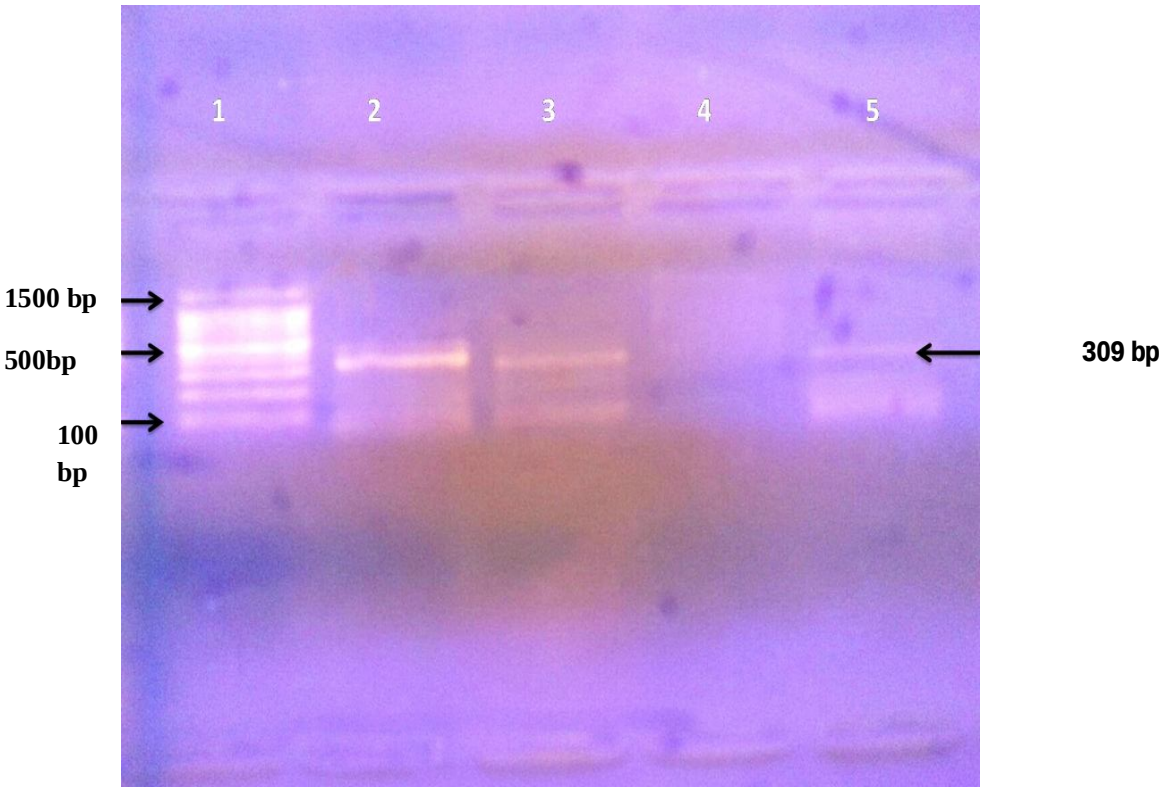

Figure 4.5: PCR amplification of *mcr-1*gene on 2% agarose gel electrophoresis. Lane 1 DNA ladder: MW 100-1500bp. Lane 2, 3and 5 showing typical bands size of 309bp corresponding to the molecular size of *mcr-1* gene. Lane 4 is negative control.

Chapter five

Discussion

Chapter five

5.1 Discussion

Colistin is an antibiotic that is used as a 'last-line' therapy to treat infections caused by MDR Gram-negative bacteria, when essentially no other options are available (Nation and Li,2009). The spread of the plasmid-mediated colistin resistance gene, *mcr-1*,into *Enterobacteriaceae* clinical isolates poses a significant threat to global health. Here we report thepresence of *mcr-1* gene in seven (14%) isolates of *Enterobacteriaceae*, isolated from Alzaytona and ShargElneel, in Khartoum, Sudan. This result indicates the spread of *mcr-1* in different Sudanese hospitals. In addition, this finding is higher than previous reports of *mcr-1*gene that have been reported in Egyptand Spain (Elnahriry*et al*., 2016; Prim *et al*., 2015).

We found *mcr-1* gene is most common in *E.coli*(6/32). Also we reported the presence of mcr-1 gene in *K*. pneumoniae (1/4), but *Proteus spp*. were negative for mcr-1 gene. These results are in line with global reports of*mcr-1*that was most common in *E. coli* but also was detected in *K*. pneumoniae(Skov and Monnet, 2016;Gu*et al*., 2016). Most of the isolated organisms were highly resistant to Cefuroxime (48/50), cefotaxime (46/50), co-trimoxazol (28/50), ciprofloxacin (33/50) and gentamicin (34/50). This could be attributed to irrational use of antibiotics in Sudan (Elsiddi*et al*., 2010; Awad*et al*., 2005), which increases the selection pressure for resistance on bacteria (Awad*et al*., 2005).

The bacterial infection in this study is common in elderly patient (61-90 years) and this due to decrease efficiency of immune system (Greenwood, *et al*., 2002). There was no association between *MCR-1* gene and age of the patients (p- value >0.05).

The prevalence of bacterial infection especially urinary tract infections in this study is increased in males (60%) more than female (40%). There was no association between gender of the patients and *MCR-1* gene (p-value >0.05).

5.2 Conclusion

1- This study confirms the presence of *MCR-1* gene in clinical isolates of *Enterobacteriaceae* in Khartoum State for the first time.

2- The most common bacteria that harbored*MCR-1* gene was*E. coli*.

5.3 Recommendations:

- Thegovernment must take some steps to stop spread of resistance bacteria, especially which is positive to *mcr-1* gene.
- Antibiotics should only be used whenneeded, the right drug, right dose, right route and in the right time.
- Narrow spectrum antibiotics should be used rather than broad spectrum.
- Culturingand sensitivity testing of pathogenic bacteria should be taken before treatment begins.
- Health care provider should be try to minimize the spread of resistant bacteria by using proper sanitation techniques includes hand washing or disinfecting between each patient.
- Other studies should be done, with large sample size and covered other region in Sudan.

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Appendices

APPENDIX 1

Color plate

Theromocycler

Appendix 2

Preparation of reagents and culture media

1. Blood agar base

Blood agar base is recommended as base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms.

Compositions

Ingredients **Gms/L**

Beef heart, infusion (beef extract)…… 5000

Tryptose ……..10

Sodium chloride ……..5

Final pH …….....…7.3

Directions

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. Cool to 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile petridishes.

2. Crystal violet Grams stain

To make 1 liter

Crystal violet…….…… 20 g

Ammonium oxalate ……9 g

Ethanol or methanol , absolute ……..……95ml

Distilled water …….……to 1 litter

3. Weight the crystal violet on a piece of clean paper. Transferred to a brown bottle pre marked to hold one litter
4. Add the absolute ethanol or methanol and mix until the dye is completely dissolved.
5. Weight the ammonium oxalate and dissolve in about 200 ml of distilled water. Add the stain, make up to one litter with distilled water and mixed well.**Proteus**
6. Label the bottle and store it at room temperature. The stain is stable for several months.
7.

3. Kliger Iron Agar (KIA)

KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

Compositions

Ingredients **Gms/L**

Peptic digest of animal tissue…….…… 15

Yeast extract……..…….3

Beef extract……..……..…3

Peptose peptone ……...……...…5

Dextrose ……...……. 1

Lactose ……..……10

Ferrous sulphate …….……... 0.20

Sodium chloride …….…..…..5

Sodium thiosulphate…….…….. .0.3

Phenol red…….…… …0.042

Agar…….…….. 15

Final pH(at 25°C)……..……7.4

Directions

Suspend 57.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour. set as slope with butt.

4. Lugol's iodine solution

To make one litter

Potassium iodine solution ……20 g

Iodine …….. 10 g

Distilled water …….……to 1 litter

1. Weight the potassium iodine , and transfer to brown bottle pre marked to hold 1 litter .
2. Add about quarter of the volume of water, and mix until the potassium iodine solution is completely dissolved .
3. Weight the iodine, and add to potassium iodide solution. Mix until the iodine is dissolved .

4. Make up to 1 litter distilled water, mix well. Label the bottle and marked toxic. Store at dark place

5. Mac Conkey Agar medium

Mac Conkey Agar medium is a differential medium to distinguish between bacteria by neutral red indicator which changes colour when acid is produced following fermentation of lactose sugar.

Composition

Ingredients **Gms/L**

Peptic digest of animal tissue…… 17

Protease peptone……..……. 3

Lactose……... 10

Bile salts ……1.5

Sodium chloride ……..……..5

Neutral red……..…….03

Agar…….. 15

Final pH(at 25°C)……..……7.2

Directions

Suspend 51.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour.

6. McFarland Standard Turbidity tube 0.5 :

Ingredients

Conc. Sulphuric acid ……..……1 ml

Dehydrated barium chloride ……..……..0.5g

Distilled water ……..……..……99 ml

Prepare 1% V/V of sulphuric acid solution by adding 1 ml of concentrated sulphuric acid to 99 ml of DW and mix. Prepare 1% w/v solution of barium chloride by dissolve 0.5g of dehydrated barium chloride in 50 ml of distilled water. Add 0.6 ml of sulphuric acid then mix well.

7. Muller Hinton agar

Muller Hinton agar is used for testing susceptibility of common and rabidly growing bacteria using antimicrobial disc, it manufactured to contain low level of thymine, thymidine, calcium and magnesium.

Compositions

Ingredients **Gms/L**

Casein acid hydrolysate …… 17

Beef heart infusion ……. …… 2

Starch soluble …… 1.5

Agar……17

Final pH(at 25°C)…….... 7.3

Directions

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour.

8. Nutrient agar

Nutrient agar is used for cultivation of less fastidious organisms, can be enriched with blood or other biological fluids.

Compositions

Ingredients **Gms/L**

Peptone …… 10

Beef extract ……. ……..... 10

Sodium chloride ……5

Yeast xtract……1.5

Agar……15

Final pH(at 25°C)……... 7.3

Directions

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour.

9. Oxidase Reagent

Prepare fresh before use.

To make 10 ml:

Tetramethyle-*p*-phenylenediaminedihydrochloride …….0.1 g

Distilled water ……10ml

Dissolve the chemical in water . The reagent is not stable .

10. Peptone water

Used for culturing organisms to proceed indole test in the presence of Kovac's or Ehrlich's reagent that reacts with the indole to produce a red coloured compound.

Compositions

Ingredients **Gms/L**

Peptic digest of animal tissue……..…… 10

Sodium chloride …….……..5

Final pH(at 25°C)…….……7.2

Directions

Suspend 15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour.

11. Simmons citrate Agar

This test is used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Compositions

Ingredients **Gms/L**

Magnesium sulphate …….…… 0.20

Ammonium dihydrogenphosphate ……..……..……. 1

Dipotassium phosphate ……..……... 1

Sodium citrate ……..2

Sodium chloride ……..…….5

Bromothymol blue ……..…….0.08

Agar…….. 15

Final pH(at 25°C)…….…...……6.8

Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour. set as slope .

12. Urea Agar Base (Christensen)

Testing for Urease enzyme activity is important in differentiating enterobacteria. Especially for *proteusspp*

Compositions

Ingredients **Gms/L**

Peptic digest of animal tissue……..…..…… 1

Dextrose …….……. 1

Disodium phosphate ……... 1.20

Monopotassiumphosphate ……..…0.80

Sodium chloride ……..5

Phenol red……..…….… 0.012

Agar……15

Final pH(at 25℃)……..…6.8

Directions

Suspend 24 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 10 ibs pressure (115°C) for 20 min. Cool to 50°C and a aseptically add 50 ml of sterile 40% of urea solution (FD048) and mix.

APPENDIX 2

Questionnaire

Name: ……

Sex: ……

Age: ……

Isolatedorganisms: ……

Sensitive to: ……

Resistant to: ……

Results of antimicrobial sensitivitytesting:

No	Sex	Isolate	Cipro	Genta	Co	Cefot	Cefta	cefuro	*mcr-1*
1	Male	*E. coli*	*S*	S	R	R	R	R	+
2	Female	*E. coli*	*S*	R	R	R	R	R	-
3	Female	*E. coli*	*S*	R	R	R	R	R	-
4	Male	*E. coli*	*R*	R	S	R	R	R	-
5	Male	*E. coli*	*R*	R	S	S	S	S	-
6	Male	*E. coli*	*S*	S	S	R	R	R	+
7	Male	*E. coli*	*S*	S	R	R	R	R	+
8	Male	*E. coli*	*R*	S	S	R	R	R	-
9	Female	*E. coli*	*R*	R	R	R	R	R	-
10	Male	*E. coli*	*S*	R	R	R	R	R	-
11	Male	*E. coli*	*R*	R	R	R	R	R	-
12	Male	*E. coli*	*R*	R	S	R	R	R	-
13	Female	*E. coli*	*R*	S	S	R	R	R	-

14	Female	*E. coli*	*R*	R	S	R	R	R	-
15	female	*E. coli*	*R*	R	S	R	R	R	-
16	Male	*E. coli*	*R*	R	R	R	R	R	-
17	Male	*E. coli*	*R*	R	R	R	R	R	-
18	Male	*E. coli*	*R*	R	R	S	S	R	-
19	Female	*E. coli*	*R*	R	R	R	R	R	-
20	Female	*E. coli*	*S*	S	R	R	S	R	+
21	Female	*E. coli*	*R*	R	R	R	R	R	-
22	Male	*E. coli*	*R*	S	R	R	R	R	-
23	Male	*E. coli*	*R*	R	S	R	R	R	-
24	Female	*E. coli*	*R*	R	S	R	R	R	-
25	Male	*E. coli*	*S*	R	S	R	R	R	-
26	Male	*E. coli*	*S*	R	R	R	R	R	-
27	Female	*E. coli*	*S*	S	R	R	R	R	+
28	Male	*E. coli*	*R*	R	S	R	R	R	-
29	Male	*E. coli*	*S*	S	R	R	R	R	+
30	Female	*E. coli*	*R*	S	S	R	R	R	-
31	Male	*E. coli*	*R*	S	S	R	R	R	-
32	Female	*E. coli*	*S*	S	R	R	R	R	-
33	Male	*E. coli*	*R*	R	S	S	S	R	-
34	Female	*E. coli*	*R*	R	R	R	R	R	-
35	Female	*E. coli*	*S*	R	S	R	S	R	-
36	Male	*E. coli*	*R*	R	R	R	R	R	-
37	Male	*E. coli*	*R*	S	S	R	R	R	-
38	Male	*E. coli*	*R*	R	S	R	R	R	-
39	Female	*K. pneumonia*	S	S	R	R	R	R	+
40	Male	*K. pneumonia*	R	R	R	R	R	R	-

41	Female	*Proteus*	R	R	S	R	R	R	-
42	Male	*Proteus*	R	R	R	R	R	R	-
43	Male	*Proteus*	S	S	S	R	R	R	-
44	Male	*Proteus*	R	R	R	R	R	R	-
45	Female	*Proteus*	S	S	S	R	S	R	-
46	Male	*Proteus*	S	S	S	R	R	R	-
47	Male	*Proteus*	R	R	R	R	R	S	-
48	Female	*Proteus*	S	S	R	S	R	R	-
49	Female	*K. pneumonia*	S	R	R	R	R	R	-
50	Female	*K. pneumonia*	R	R	R	R	R	R	-