Antibiotics Susceptibility Pattern of Clinical and Environmental Escherichia coli Isolates from Babylon Hospitals

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Abstract .A total of (400) clinical and environmental samples were collected during the period from Sep /2018 to Jan /2019 from different hospitals in Hilla city. The isolates were identified and based on microscopic features and standard biochemical tests. The results showed there were (150) isolates of *E.coli* from clinical and the environmental samples .The clinical isolates (67 isolates) were collected from patients suffering from different infections such as UTI (30 isolates) , gastroenteritis (diarrhea) (22 isolates) , and wound infections (15 isolates) . Also, the environmental isolates (83) were collected from the different site of hospitals environment include surgery room , hospital floors , the hospital cafeteria , the corridors of the hospital , and from workers dress and stool . All 150 *E.coli* isolates were primarily screened for colistin resistance, The following antibiotics were used for susceptibility testing to the resistance isolates for colistin (17 isolates) : Aminoglycosides (Amikacin , Gentamicin , kanamycin) , Sulfonamide (Trimethoprim) , Nitrofurans (Nitrofurantoin) , Fluoroquinolons (Ciprofloxacin , Norfloxacin) , Cefeme (Cephalothin , Ceftriaxone, Ceftazidime), Macrolides (Erythromycin) , carbapenems (Imipenem, meropenem) , beta-Lactams (Amoxicillin , Carbenicillin), Tetracyclin , Chloramphenicol . our result show that *E. coli* the most causative agent of UTI infection especially in women . there is a high resistance to cefems and beta- lactam antibiotic (100%) among these isolates which play as a cell wall inhibitors. And moderate resistance to ciprofloxacin (52.9%) , Chloramphenicol and Norfloxacin (29.14%) , and weak resistance to Gentamycin.

Introduction

one of the major problem to human health in this century is the antimicrobial resistance , the U.S centers for prevention and control disease and WHO are portraying worldwide emergency and a looming disaster of arrival to the pre-antibiotic era. The worldwide increase of production the carbapenemase by Enterobacteriaceae lead to increase use of colistin which certainly leads to emergence resistance . (Liu *et al*. ,2016)

The quick increasing of infections result from Gram-negative bacteria that resistant to antibiotic for example carbapenem-resistant Enterobacteriaceae ,has generated concerns globally . The progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. Also , the misuse and overuse of antibiotics in the treatment of

diarrhea could lead to a raise of antibiotic resistance . colistin is considered a replacement therapy for this infection. (Cao *et al*.,2018).

As of late Polymyxins have a critical job as final retreat antibiotic agents utilized for treating the diseases which are brought about by multidrug-resistance brought about by multidrug- resistance and seriously medicate safe Gram-negative pathogens. (Marco et al. , 2017)

Polymyxins considered a polypeptide antibiotic which consists of five different compounds (A-E) , they were found in 1947 . Just (B ,E) polymyxin which called colistin and has practice clinically . polymyxin have broad use in topical ophthalmic answer for a long time. (Falagas et al. , 2005)

In the outer membrane , the initial linkage of colistin with the membrane of bacteria occurs by electrostatic interactions between colistin which regarded as a cationic polypeptide and lipopolysaccharide (LPS) molecules as an anion , that leading to the disorder of the membrane . colistin is making to displace calcium (Ca+2) and magnesium (Mg+2) ,which stabilize the lipopolysaccharide molecules from its negative charged , that lead to a local disorder of the outer membrane ,which caused a high permeability of the cell envelope and infiltration of cell contents and thereby cell lysis . by dislodging divalent cations from the

contrarily charged phosphate gatherings of the Lipid An of

the lipopolysaccharide film, bringing about cell lysis. Colistin obstruction result from a chromosomal change in the qualities that encoding the PhoP/PhoQ , PmrA/PmrB two part that play as the administrative framework . These transformations lead to changes or once in a while completely loss of Lipid An atoms , mcr-1 quality is in charge of colistin obstruction which was indicated on an IncI2 plasmid , pHNSHP45 from E. coli isolates .The mcr-1 gene is providing resistance to colistin by encodes the enzyme (phoethanolamine transferase) which responsible for transport a phosphoethanolamine to Lipid A molecules . This plasmid appeared the ability to transfer by two means , transformation and conjugation that provide a sustain in *E. coli* for at least two weeks even in the absence of colistin . (Newton-Foot *et al.* , 2017).

Late information from the " U.S. National Healthcare Safety Network" exhibit that gram-negative microbes are in charge of over (30%) of emergency clinic gained diseases , and these microscopic organisms win in examples of ventilator-related pneumonia (47%) and UTI (45%) . In concentrated consideration units (ICUs) in the U.S , gram-negative microbes represent about (70%) of these sorts of contaminations , and practically identical data is represented from various pieces of the world . A scope of gram-negative living beings are in charge of medical clinic gained contaminations , the Enterobacteriaceae family being the more usually perceived gathering in general . Tragically , multidrug-safe life forms , including Pseudomonas aeruginosa , Acinetobacter baumannii , and carbapenemase-creating or expanded range β-lactamase (ESBL)– delivering Enterobacteriaceae , are progressively being represented far and wide. Contaminations brought about by gram-negative microscopic organisms have qualities that are of explicit concern . These life forms are especially capable at up-controlling or gaining qualities that code for instruments of anti-microbial medication obstruction , especially within the sight of anti-microbial determination weight . Additionally , they have accessible to them a plenty of opposition components , generally utilizing a solitary system to influence different anti-toxins or utilizing numerous instruments against a similar anti-microbial . Aggravating the issue of antimicrobial-medicate obstruction is the immediate risk of an inadequacy in the improvement and revelation of new anti-infection agents . Numerous elements have added to this decrease , including the expanding troubles of screening for new mixes , long time required for medication advancement and the high capital costs , the developing unpredictability of structuring and performing convincing clinical

preliminaries , and the worry about diminished medication life span because of the rise of opposition . Therefore , an ideal tempest has been made with thought to these diseases : rising medication obstruction without new medication advancement . (Peleg et al., 2010).

The present investigation was accomplished for recognition of antibiotics resistant pattern of clinical *E.coli* isolates in Hilla' s hospitals.

Materials & Method

This research included 400 samples, (150) of these samples are *E.coli* and others distributed among *Pseudomonas, Klebsiella*, Enterobacter and other Enterobacteriaceae , We collected these isolates from environmental and a clinical source from hospitals in Hilla city . In the present research , a total of clinical samples (67 isolates) were collected from patients suffering from different infections such as UTI (30 isolates) , gastroenteritis (diarrhea) (22 isolates) , and wound infections (15 isolates) . Also, the environmental sample (83) were collected from the different site of hospitals environment include surgery room , hospital floors , the hospital cafeteria , the corridors of the hospital , and from workers dress and stool , during a period extended from Sep 2018 to Jan 2019. The clinical and environmental isolates are collected from Hilla hospitals , by using disposable sterilized transport swabs . Each specimen was immediately inoculated into MacConkey , EMB agar and used chromagar (Chromogenic Testing Media for Colorful Microbial Detection) incubations at 37°C for 24 hours. Research center determination method can be relied upon the means suggested by (MaCfadden, 2000) for finding , from every positive culture take a solitary settlement and afterward distinguishing proof by relied upon morphological properties (shading creation , state shape, surface and edge), by using Gram`s stain for detection morphology properties of bacterial cells also help to different between Gram`s negative and gram positive (Winn *et al*., 2006) . after that used a biochemical tests to help for identification of *E.coli* such as Catalase test ,Oxidase test ,Vogues- Proskauer test , Indole test , Motility test , Citrate (Simmon`s) utilization test , Methyl red test according to (MacFaddin, 2000).

Determination of Antibacterial Susceptibility Test

The in vitro susceptibility of *E. coli* isolates to 16 antimicrobial agents were determined via disk diffusion method according to clinical and laboratory standards institute instructions (CLSI, 2016) .Activation of isolates was performed using nutrient broth for 18 hrs at 37°C and the growth was adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/mL) ,

The kibry-Baure susceptibility test was examined by using a pure culture of selected identified bacterial isolate , then colistin antibiotic discs were added to each plate , and incubation for 18 hours , by measuring the inhibition zone can classify bacterial sensitivity (Cockerill *et al*., 2010). The following antibiotics were used for susceptibility testing to the resistance isolates for colistin : Aminoglycosides (Amikacin , Gentamicin , kanamycin) , Sulfonamide (Trimethoprim) , Nitrofurans (Nitrofurantoin), Fluoroquinolons (Ciprofloxacin , Norfloxacin) , Cefeme (Cephalothin, Ceftriaxone, Ceftazidime), Macrolides (Erythromycin) , carbapenems (Imipenem, meropenem) , beta-Lactams (Amoxicillin , Carbenicillin), Tetracyclin , Chloramphenicol .

Results and Discussion

A total of (45) midstream urine samples were collected from 7 male and 38 female whose visit urology consultant clinic at Hilla Hospitals during a period from October 2018 to January 2019. these samples were obtained from patients whose suffering from UTI . After centrifugation at 6000 rpm for 3minutes, these samples were subjected to microscopic examination to investigate RBC ,WBCs , crystals and the precipitate plated onto eosin –methylene- blue (EMB) and Mac-Conkey -agar plates followed by incubation for (24 hrs) at (37°C) for confirmation between lactose and non-lactose fermenting strains. The results showed that 30 (66.67%) of midstream urine samples gave growth of

Gram negative bacteria on MacConkey agar and show Green Metallic Sheen of *Escherichia coli* on EMB while 15(33.3%) show Other G-ve bacteria.

These results in accordance with several studies such as (Sharma *et al.*, 2016) who found that *E. coli* recovered from (67.66 %) of the (UTI) patients . Ghanbari *et al.*, (2017) found the most prevalence isolated were *E. coli* (58.28 %) . Paulo, (2016) suggested that most prevalent and active pathogen was *E. coli* (60.4%) among patients with(UTIs) . Several studies stated that the isolation percentage of *E. coli* among (UTI) ranged from (40-80%) of collected midstream urine samples (Abdi and Rashji, 2014) .

Table (1): type of bacterial isolates

Bacterial isolates	**No.**	**Percentage %**
E. coli	150	42.86%
Klebsiella	100	28.57%
Pseudomonas	20	5.71%
Enterobacter	35	10%
others	45	12.86%
Percentage %	350	100%

Table (2): Number and percentage of *E.coli* isolates among different clinical samples.

Clinical sample	**No. of samples**	**No. of *E.coli* isolates**
UTI	45	30
gastroenteritis (diarrhea)	30	22
wound infections	120	15
total	195	67

Table (3): Number and percentage of *E.coli* isolates among different environmental samples.

Environmental sample	**No. of sampl**	**No. (%) of *E.coli* isolat**
surgery room, hospital floor and cafeteria	105	33
workers dress	50	20
workers stool	50	30
Total	205	83

Indeed, that both the sexual orientations are defenseless to the contamination , because of their life systems and conceptive physiology ladies are for the most part powerless against the disease . the contamination which more often than not happens Through the urethra which considered the Entry of an irresistible pathogen in the urinary tract . As the shorter length of urethra in ladies which makes them powerless against such diseases ,this is one reason for bigger contamination among ladies than men (Foxman and Brown, 2003)

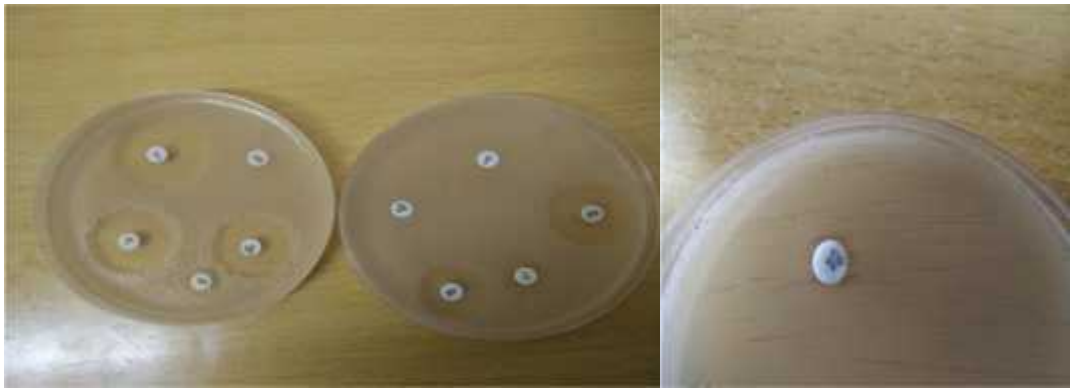

Figure (1): Antibiotic sensitivity test of *E.coli* isolates by Kirby –Bauer method on Mollar Hinton Agar

Antibiotic susceptibility testing was carried out following the method outlined by the (CLSI, 2016) . 16 antibiotics were tested for antibacterial activity by Kirby-Bauer Disc diffusion method against 17 isolates that are show resistance for colistin . The results revealed that , the resistance were (100%) for Erythromycin , Cephalothin , Ceftazidime, Imipenem and Carbenicillin , 16(94.12%) for Amoxicillin and Ceftriaxone, 5(29.14%) for Chloramphenicol and Norfloxacin , 1 (5.8%) for Amikacin , 3(17.65%) for kanamycin, 9(52.9%) for ciprofloxacin, 15(88.24%) for tetracyclin , 12 (70.59%) for Nitrofurantoin, and resistance to trimethoprim were 10 (58.82 %).

Table (4): Distribution of antibiotics resistance among *E. coli* isolates.

Antibiotics Name	Resistance %	Mode of Action
Colistin(µg)	100	Cell membraine inhibitor
Cephalothin(30µg) , Ceftazidime (30µg)Imipenem (10µg)and Carbenicillin(100µg)	100	cell wall synthesis inhibitor
Trimethoprim(5µg)	58.82	folate synthesis inhibitor
Erythromycin(15µg)	100	protein synthesis inhibitor
kanamycin(30µg)	17.65	protein synthesis inhibitor
Amikacin(30µg)	5.8	protein synthesis inhibitor
Norfloxacin(10µg)	29.14	DNA synthesis inhibitor
Ciprofloxacin(5µg)	52.9	DNA synthesis inhibitor
Gentamycin(10µg)	0	protein synthesis inhibitor
Nitrofurantion(µg)	70.59	DNA synthesis inhibitor
Amoxicillin(30µg) , Ceftriaxone(30µg)	94.12	cell wall synthesis inhibitor
Tetracycline(30µg)	88.24	DNA synthesis inhibitor
Chloramphenicol(30µg)	18.3	protein synthesis inhibitor

The high level of resistance to different classes of antibiotics in several study indicated the easy availability , unregulated use , animal feed loaded with antibiotics , and the emergence of infectious diseases are among significant purposes behind current circumstance. Antibiotics are amongst the most critical achievements of the 12th century , used to kill or inhibit the growth of microorganisms . Antibiotic resistance in *E. coli* isolated from(UTIs) is is expanding step by step , making it a major general medical issue. So it is very vital to decide the antibiotic resistance designs in *E. coli* isolates for appropriate and exact prescriptions . (Bockstael and Aerschot, 2009).

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