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Colistin-Resistance Gene mcr-1 in Isolated *E. coli* Bacteria from Hospital Acquired Infection in Egypt

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ABSTRACT

Colistin(polymyxin E) is produced by certain strains of the bacteria called Paenibacillus polymyxa and related to the class of polymyxin of antibiotics. It is re-launched again as a last-line choice to treat highly antibiotic-resistant bacteria such as the strains of carbapenem-resistant.The resistance to colistin in *E.coli* has several molecular mechanisms that is associated with LPS alteration through various ways, so LPS modification is the main mechanism of colistin resistance.Mcr-1 is a novel, colistin-resistance, plasmid-mediated gene and showes high transfer rate in vitro between Escherichia coli Strains.The aim of this work is to evaluate the occurrence of mcr-1 gene among *E.coli* bacteria isolates. This study was conducted in microbiology unit, Clinical Pathology Department, Mansoura University, Mansoura, Egypt on 50 *E.coli* isolates *E.coli* culture from subjects , with hospital acquired infections, attending Mansoura University Hospitals from May 2017 to March 2019 . each sample were subjected to culture, identification by vitek II system, antibiotic susceptibility by disk diffusion and BMD methodes then detection of mcr1 gene was done by conventional pcr method. Plasmid –mediated colistin resistance gene (*mcr-1*) was found in 8 (16.0%) cases of 50 isolates of hospital acquired CoR EC.

Keywords: colistin, mcr1, *Ecoli*, BMD

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INTRODUCTION

Colistin(polymyxin E) is produced by certain strains of the bacteria called Paenibacillus polymyxa and related to the class of polymyxin of antibiotics. It is re-launched again as a last-line choice to treat highly antibiotic-resistant bacteria such as the strains of carbapenem-resistant. It is also utilized for prophylactic decontamination of the GIT in the (ICU) cases and decreasing the infections of respiratory system [1].

The resistance to colistin in *E.coli* has several molecular mechanisms that is associated with LPS alteration through various ways, so LPS modification is the main mechanism of colistin resistance. These routes include specific alteration of outer membrane porins and decreases in the overall negative charge of the LPS, over expression of efflux pump systems, over production of capsule polysaccharide and colistinase enzyme that can be produced by some strains [2].

Classical resistance to colistin is rare. However, its resistance is related to the mechanism of plasmid-mediated colistin resistance, including the gene of mcr-1, between the bacteria such as *E. coli* and K. pneumoniae [3] Mcr-1 is a novel, colistin-resistance, plasmid-mediated gene and showes high transfer rate in vitro between Escherichia coli Strains [4].

'Nosocomial' or 'healthcare associated infections' (HCAI) are infections that are obtained during the period of hospital care and they are not present at the time of admission. They happen > 2 days subsequent to admission which typically considered nosocomial. These infections can occur even subsequent to the release of the cases. Intestinal *E.coli* are the commonest cause of hospital acquired UTI [5]

The aim of this work is to evaluate the occurrence of mcr-1 gene among *E.coli* bacteria isolated from 95 subjects , with hospital acquired infections, attending Mansoura University Hospitals in Egypt .Out of them ,50 colistin-resistant strains with [MIC] >2 μg/mL had been studied.

SUBJECTS AND METHODS

This study was conducted in microbiology unit, Clinical Pathology Department, Mansoura University, Mansoura, Egypt on 50 E.coli isolates out of 95 +ve *E.coli* culture from subjects , with hospital acquired infections, attending Mansoura University Hospitals from May 2017 to March 2019 . They were 15 males and 35 females.They were admitted to the hospital for reasons other than *E.coli* infections and stayed for more than 3 days. Patients were selected according to definition of hospital acquired infection which is new symptoms appear within 48 hours of admission, three days after discharge, or 30 days after an operation [6].

Every collected sample was inoculated into MacConkeys·s agar within 120 minutes of collection and incubated for 1 day at temperature of 37°C .Rose pink, round medium-sized colonies were observed.Biochemical identification for E.coli was done using vitek 2 automated system (biomurex).Testing of the susceptibility of the colistin antibiotic by Disc diffusion modified Kirby- Bauer-method reveal 55 colistin resistant isolates and when confirmed by Macrodilution broth method (BMD) for detection the minimal inhibitory concentration (MIC) of colistin there was 50 colistin resistant isolates and 5 isolates were sensitive to colistin [7]. Those 50 colistin resistant isolates by BMD were subjected to conventional PCR for detection of mcr1 resistant gene.

Detection of mcr1 by conventional PCR method:

Total DNA of all isolates were extracted with boiling method [8] **.**

PCR primers used for detecting *mcr1* gene:

The primers were adopted after [9]. Theprimers were supplied from (**Invitrogen**).DreamTaq Green PCR Master Mix (2x)**, Thermo fisher scientific,Egypt**) , commercial kits, ready to use.

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Target gene	primers sequences (5ˉ to 3ˉ)	Amplicon size(bp)
mcr1_ F	5 -ATGGCACGGTCTATGATA- 3ˉ	155
mcr1_ R	5 -CGGATAATCCACCTTAACA- 3ˉ	

* mcr1: for colistin resistant gene

Then the Eppendorf tubes were put in the thermal cycler device for amplification. The amplification was carried out according to the following condition:

An initial **denaturation** cycle for 15 min at 95°C,then the reaction mixes were subjected to 45 **amplification** cycles of (30 sec at 95°C, 30 sec at 55°C and 30 sec at72°C) and **final extension** of 7 min at 72°C.

The PCR products were analyzed by electrophoresis in 2% agarose gel to detect specific amplified product by comparing with standard molecular weight marker (DNA ladder 50 bps).

Data processing and statistical analysis

Statistical methods:

The collected data was revised, coded, tabulated by using excel (Microsoft office 2013) program and SPSS (Statistical Package For Social Science) program (SPSS, Inc, Chicago, IL) version 20.

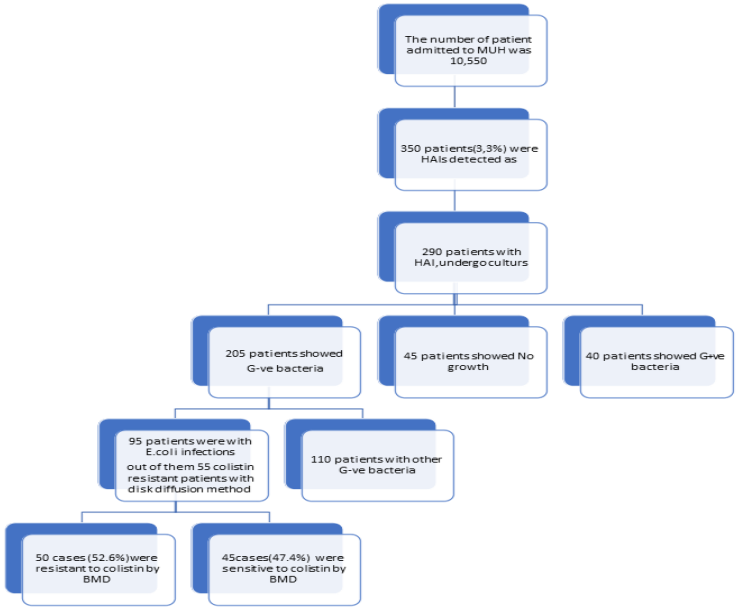
Descriptive statistics:

Qualitative data were presented as frequency and percentage
Quantitative data were presented as median and range, mean ± SD
Kolmogorov-Smirnov test was performed to assess normality of the data.

Analytical statistics:

Chi square test was used to compare groups.For comparison between two groups **Independent T test (for parametric data)** was used.**Where** : *p* is significant if < 0.05 at confidence interval 95%.
Result

Figure (I): Incidence of hospital acquired E.coli infection and colistin resistance in MUH from the period between May 2017 to Mar 2019:



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Among 95 studied isolates,there were 55 colistin resistant isolates by disk diffusion method and when confirmed by BMD only 50 isolates were colistin resistant and 5 isolates were colistin sensitive.

Table (1): Incidence of hospital acquired infection(HAI) by E.coli in MUH in the duration between May 2017 to Mar 2019:

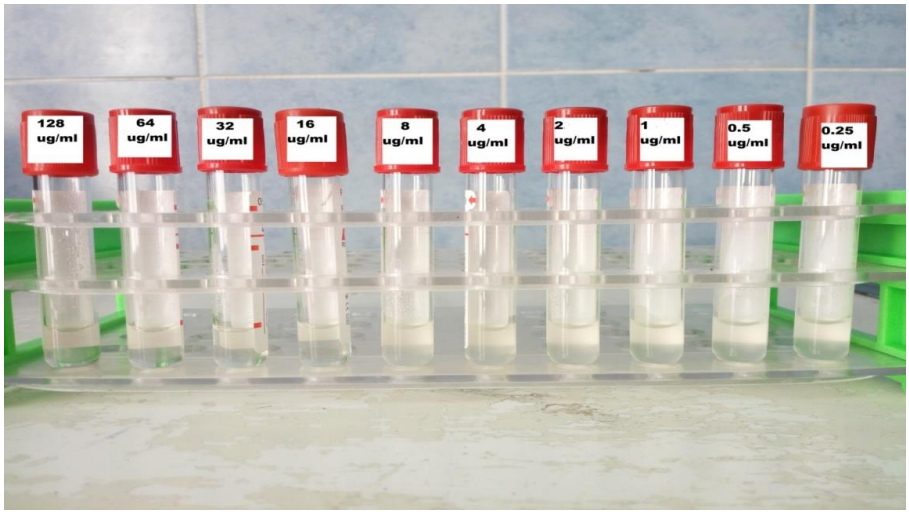
No of admissions	Incidence of HAIs	Time
10,550	95	May 2017 to Mar.2019
100%	0.9%	—

The incidence of HAI cases by E.coli in MUH from 10,550 patients admitted to hospital was 0.9% (provided from the statistical unit in hospital) .

Table (2): Comparison between Disc diffusion modified Kirby- Bauer- method and Macrodilution broth method among studied groups (n=55):

Parameter	Resistent to colistin		Sensitive to colistin	
	No	%	No	%
Disc diffusion method	55	100%	0	0.00%
Macrodilution broth method	50	90.9%	5	9.1%

This comparison between two methods showed that about 9.1% of the cases had different susceptibility to colistin, this may highlight the disadvantages of Disc diffusion method in detection of colistin susceptibility.



Picture (1): Macrodilution Method: - Serial dilution of colistin antibiotic, after incubation with half McFarland E.coli suspension, showed that 16 ug/ml was the minimal conc. which inhibit the bacterial growth.

Table (3) : Broth Macrodilution (BMD) method for colistin.

Parameter			Cases (N=55)	
MIC	Median		16.0	
	Min	Max	0.25ug/ml	128.0ug/ml
Sensitive (MIC≤2ug/ml)			5	9.1%
Resistant (MIC≥4ug/ml)			50	90.9%

- 5(9.1%) cases were sensitive to colistin and the rest 50 (90.9%) cases were resistant.

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Table (4): MIC concentrations for E.coli isolates resistant to colistin.

MIC conc (ug/ml)	No	%
4	**7**	**14%**
8	**14**	**28%**
16(median)	**12**	**24%**
32	**5**	**10%**
64	**6**	**12%**
128	**6**	**12%**
Total	**50**	**100%**

The most detected MIC among cases was 8 ug/ml (28%) followed by 16 ug/ml (24%).

Table (5):MIC results in relation to presence of mcr1 gene among studied isolates (n=50)

MIC(ug/ml)	(Group A) Mcr 1 +ve	(Group B) Mcr1 –ve	P
4	**0**	**7**	**0.522**
8	**4**	**10**	
16	**2**	**10**	
32	**1**	**4**	
64	**1**	**5**	
128	**0**	**6**	
Total	**8**	**42**	

This table shows that mcr1 gene was present more frequent in 8ug/ml MIC, followed by16ug/ml MIC for E.coli.

Table (6): Detection of mcr1 gene by conventional PCR in 50 colistin resistant cases by macrodilution broth.

Parameter		Group B (resistant to colistin) (n=50)	
Mcr 1 gene by PCR	Negative	42	84.0%
	Positive	8	16.0%

Only 8 cases were positive for mcr1 gene by conventional PCR and the rest 42 cases were negative.

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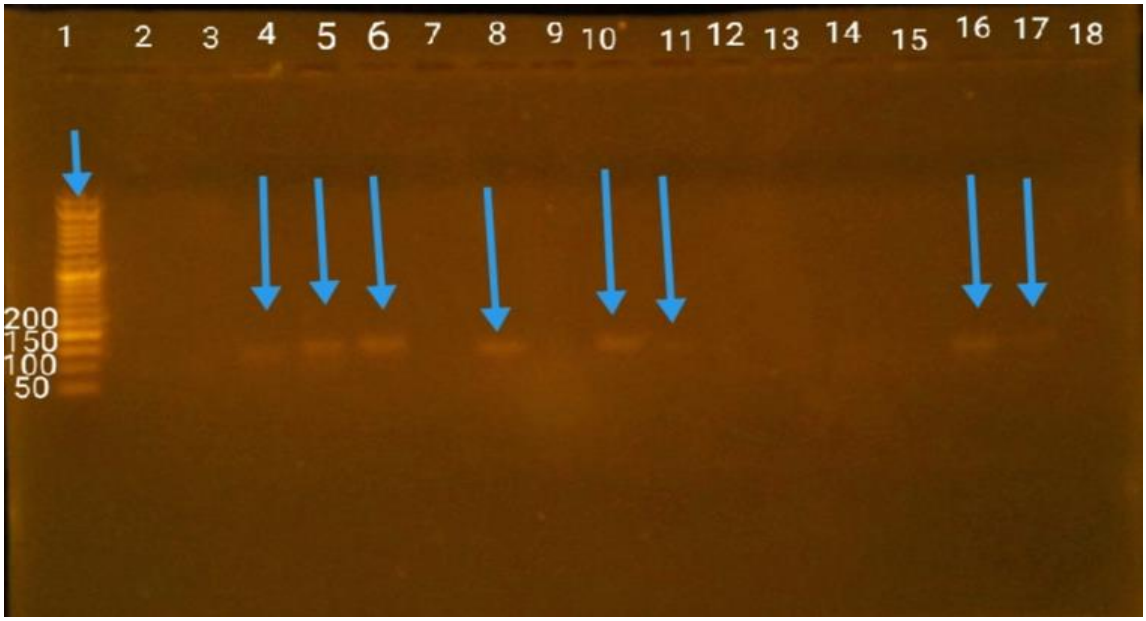
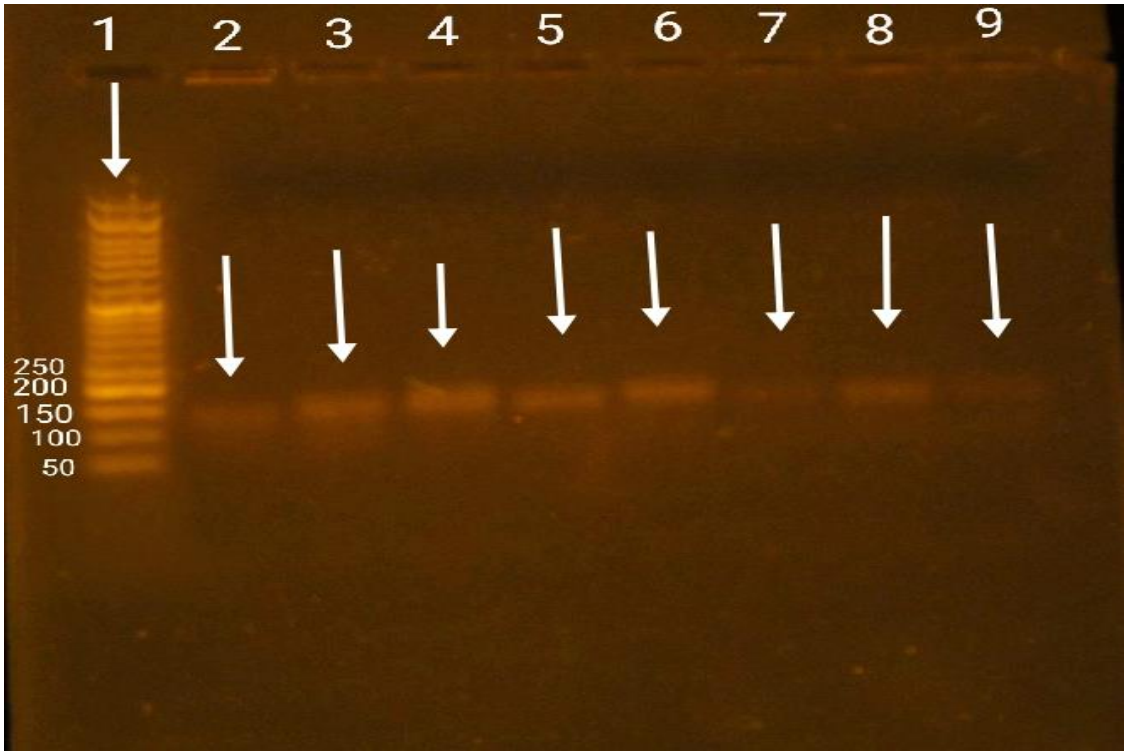


Picture (2): Agarose gel stained with ethedium bromide of conventional PC :Showing
LAN (1): 50 bp DNA ladder
LANs (2,3,7,9,12, 14,15 and 18): show no bands (Negative results)
LANs(4,5,6,8,10,11,16 and 17) :show bands at 155 bp which corresponds to mcr1 gene



Picture (3): Agarose gel stained with ethedium bromide of conventional PCR Showing:

LAN (1): 50 bp DNA ladder

LANs(2,3,4,5,6,7,8,9):show bands at 155 bp which corresponds to *mcr1 gen*e

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DISCUSSION

Nowadays, colistin has been reused for critical infections especially after spread of multi antibiotic-resistant organisms. Colistin resistant *E.coli* species has an ability to accumulate resistance genes through horizontal gene transfer and this cross-transfer occurs between human and animal products due to using of colistin in veterinary medicine [10].

The mechanism of colistin resistant in *E.coli* is mostly due to mutational change within chromosomal genes encoding biosynthesis of LPS and the first plasmid –mediated colistin resistance gene (*mcr-1*) was reported in China [11].

In MUH during this study the prevalence of HAIs by G-ve bacteria was reported to be 58.6% (205 isolates).*E.coli* was represented by 27.1%(95 isolates)from all HAIs and colistin resistant *E.coli by BMD* was 52.6% (50 isolates) out of the 95 isolates.The prevalence of HAIs by *E.coli* (27.1%) was nearly the same as the study performed in National Trauma Centerin **Nepal 2019** which was about 28.3% and **Hormozi SF 2018** in which colistin resistant *E.coli* represent about 22.1%
[12,13]
This high prevalence may be due to poor practices in maintaining sterility or cleanliness in health care facilities. Oral-fecal transmission is a main reason of *E.coli* infections so unwashed hands in a hospital setting can cause a higher incidence of *E.coli* infection, particularly in immunosuppressed or elderly cases . [14]

Wangchinda W and his colleagues,2018 found that cases suffering from Co R EC or Co R KP colonization in the hospital may transmit these infections to other cases, healthcare staff, in the hospital and into the hospital environment. Cases that had plasmid-mediated gene for antibiotic-resistance, such as mcr-1, may transmit this plasmid containing the gene to other bacteria in their bodies, other cases, or the hospital environment, that may lead to further appearance and spread ofcolistin-resistant pathogens. Cases suffering from constant colonization of Co R EC or CoR KP may transmit them to others in home and community on going back home from hospital. They can lead to community-acquired infections because people that are receiving colistin are at danger of having CoR EC or CoR infection.

In our study MICs values of colistin for isolated *E.coli*, were obtained by BMD method andthe most detected values among cases was 8 µg /ml (28%) followed by 16 µg /ml (24%).Also there were 5 cases (0.9%) (5/55)that were resistant to colistin by Disc diffusion method but were sensitive by BMD method(MIC≤ 2 µg/ml) and this is may be due to binding of colistin to plastic leading to poor diffusion in agar and false resistant zone [15]**.**

Median MIC values for colistin resistant (CR) strains were 16 µg/ml and this result was not in accordance to previous study in **Greece** since**Turlej-RogackaA et al.,2018** found that 64 µg/ml were the median MIC values for CR strains.

In the current study 8 (16.0%) cases of 50 isolates of CoR EC were found to harbor *mcr-1* gene and *E. coli* harboring mcr-1 gene showed colistin MIC ranged from 8 to 64 µg/ml. One was isolated from sputum sample and the other 7 from urine samples.On contrary to this data a higher data was reported in**Thailand2018**,in which the mcr-1 gene was found in (29.7%)E. coli isolates. The MIC of colistin in mcr-1-positive isolates ranged from 4–32 mg/L.There was no mcr-1 gene in the isolates with MIC 2 mg/L. among the 13 colistin-resistant E. coli isolates, 11 had the mcr-1 gene [16] .

In **Wangchinda W. et al.,2018** study,the gene was found in 13.0% of isolates of CoRECorCoR KP while it was found in 57.7% of CoR EC isolates.The MIC of colistin of CoR EC isolates having the generanged from 4 to 16 mg/L.The sites of infections were UTIs, pneumonia and bacteremia. **In our study** the gene was found in 7 UTI samples and one sputum samples [17].

But in **Elnahriry SS et al.,2016** study**,** the gene was found in only one E. coli isolate that was from a patient suffering from bacteremia who was hospitalized in ICU of a Cairo hospital without history of traveling abroad. The colistin MIC for this isolate was 16 mg/L [18].

In Phillippines 2018, three identified isolates from 123 drug resistant isolates showed MIC of ≥4 µg/ml to colistin by Becton Dickinson Phoenix M50 system.Conventional PCR to detect mcr-1gene was

carried out on the 3 isolates. Two E. coli isolates were positive for carriage of mcr-1 gene and both showed MIC of colistin of 8 µg/ml through broth microdilution testing. One was collected from foot wound and the other from blood sample. The cases had no previous colistin therapy documented in their medical history and both cases did not travel abroad within 0.5 year before admission [19].

A similar MIC cut-off value for colistin for Enterobacteriaceae (MIC >2 mg/L) was reported in **Eiamphungporn W et al.,2018** study. Despite colistin resistance is characterized as result of non-transferable chromosomal mutations, on the other hand the transferable plasmid-mediated gene mcr-1 was recently found in E. coli inside China [20].

Also in **Eiamphungporn W et al.,2018** study, this gene was less frequent in K. pneumonia isolates than *E.coli* isolates from humans in Thailand. Past studies documented a moderately decreased prevalence of this gene in Enterobacteriaceae, up to 1% [21] **.** The presence of this gene in the clinical isolates is uncommon in Europe [19] .Remarkably, its prevalence was lower in Enterobacteriaceae isolates from humans than in isolates from food and animals [20].

But in **Papa-Ezdra R et al.,2019** study three colistin resistant *E. coli* isolates carriedthe *mcr-1* gene and they were isolated from blood, urine and rectal swab samples.

CONCLUSION

In conclusion, plasmid –mediated colistin resistance gene (*mcr-1*) was found in 8 (16.0%) cases of 50 isolates of hospital acquired CoR EC [21].

The misuse of colistin in agriculture and the poultry industry may be the main cause of the high incidence of mcr-1 in bacteria from animals and animal products. This issue should be addressed by all appropriate authorities by prohibition the careless use of colistin in agriculture.

Recommendation

Colistin should not be used as empirical antimicrobial therapy. However, even appropriate use of colistin is still a key driver of the predictable emergence of CoR EC.Increased awareness and realization of antibiotic supervision programs are needed across healthcare and agricultural sectors to control and slow the spread of antibiotic resistance.It is necessary to have infection control team (ICT) that have a broad experience covering knowledge of infection control.

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