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CHROMagar COL-APSE medium as a screening method for fecal carriage of Colistin resistant *Enterobacteriaceae* among patients in Mansoura university hospitals, Egypt

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Abstract

Colistin resistance (Col-R) has been rising worldwide with high rate of morbidity and mortality. The emergence of mobile colistin resistance (*mcr*) harboring microorganisms become of a health concern. Hence, screening for fecal carriage of Col-R *Enterobacteriaceae* could aid in the prevention and control efforts of Col-R. This study aimed to screen for Col-R *Enterobacteriaceae* in the stool specimens of the hospitalized patients; explore for colistin minimal inhibitory concentrations (MIC) and the genetic determinants of these isolates, and to predict the risk factors among the studied patients' groups. Stool specimens from 290 hospitalized adult patients were screened for the presence of Col-R bacterial isolates using CHROMagar COL-APSE medium. Colistin MIC was estimated for Col-R *Enterobacteriaceae* using the broth microdilution (BMD) assay. Bacterial isolates were screened through the Polymerase chain reaction (PCR) for the existence of *mcr-1* and *mcr-2* genes. The fecal carriage of Col-R among the studied patients was 16.8 %. About 72 Col-R bacterial isolates were recovered. Col-R *Enterobacteriaceae* were predominant and were detected in 89.7 % of the bacterial isolates. Using the BMD, Col-R was confirmed and most of the isolates showed low resistance MIC titer (4 µg/ ml; 55.7 %). In addition, *mcr-1* gene was the most frequent Col-R gene detected (69.2 %), while *mcr-2* gene was less prevalent (11.5 %). The current study reported high prevalence of the Col-R and *mcr-1* gene harbored by the fecal flora; with the risk to be easily transmitted inside the hospitals and within the different communities. This highlights the need for active surveillance in addition to the infection control programs.

Keywords: Colistin resistance, Fecal Carriage, Mobile colistin resistance genes, *Enterobacteriaceae*, *mcr-1* gene

1. Introduction

Colistin is a polymyxin antimicrobial agent, which is important in curing infections triggered by the multiple drug-resistant (MDR) Gram negative rods (GNRs). Colistin interacts with the outer membrane of the Gram-negative bacterial cell wall; causing its damage as a bactericidal effect (Magiorakos *et al*., 2013). However, Ilsan *et al*., (2021) recently reported that Col-R has been rising worldwide mainly in Asia and in the Mediterranean region; with high morbidity and mortality rates.

Bacterial resistance to colistin is either acquired or intrinsically originated. Acquired Col-R is either encoded on the bacterial chromosome through un-transferable two-component systems PmrAB and PhoPQ; or more frequently be plasmid-borne through *mcr* genes, which encodes for Lipid A phosphoethanolamine transferase enzyme. The emergence of *mcr* harboring microorganisms become of health concern, which could be easily transferred between the different species (Nordmann *et al*., 2016; Grégoire *et al*., 2017).

Nine plasmid-mediated genes termed *mcr-1* through *mcr-9* had been reported in *Enterobacteriaceae*, and only *mcr-1* and *mcr-4* had been observed in *Pseudomonas* and *Acinetobacter* (Carroll *et al*., 2019). Moreover, Wang *et al*., (2020) reported another *mcr* variant; designated as *mcr-10* recorded in *Enterobacter* strain.

A study conducted by Richter *et al*., (2018) revealed that multiple risk factors have been associated with acquisition of Col-R infection and colonization, including old age; male patients, presence of co-morbidities, recent hospitalization, long hospital stay, previous carbapenem resistance, previous antibiotics treatment, exposure to chlorhexidine and the presence of devices. Furthermore, a previous study conducted by Kieffer *et al*., (2017) highlighted that bacteria carrying *mcr* genes have been detected in animals, due to the wide use of colistin in the veterinary medicine.

The intestinal microflora represents a major reserve of antimicrobial resistant microorganisms in human. Hence, screening for fecal carriage of Col-R in GNRs especially *Enterobacteriaceae* could aid in characterization of these isolates, and in the prevention and control efforts (Zurfluh *et al*., 2017).

Many phenotypic and molecular assays were described for Col-R detection including; CHROMagar COL-APSE medium, Super Polymyxin medium, BMD susceptibility test, Etest®, automated MIC-determining instruments, CT103XL microarray, eazyplex® SuperBug kit, sequencing, conventional, multiplex and Real-time PCR assays (Sekyere, 2019). CHROMagar COL-APSE medium is a chromogenic screening medium designed for detection of colistin resistant GNRs. Abdul Momin *et al*., (2017) revealed that growth of the Gram-positive bacteria and colistin sensitive GNRs is inhibited by this medium.

Effective, rapid and costly screening methods are needed to explore the individuals colonized with the Col-R isolates, which will play a central role in controlling the spread of drug resistance especially inside the hospitals, and in improving the patient's outcomes. The objectives of the current study were to screen for Col-R *Enterobacteriaceae* in the stool specimens of the hospitalized patients; explore for colistin MIC and genetic determinants of these isolates, and to predict the risk factors associated with Col-R among the studied patients' groups.

2. Patients and methods

2.1. Study design

During the time period extending from September, 2019-October, 2020, a descriptive cross sectional study was carried out on 290 patients admitted at different inpatient wards to Mansoura University Hospitals (MUHs). Informed consents were taken from the participating patients.

Stool specimens were collected from patients fulfilling the inclusion criteria, which were hospitalized adult patients ≥ 18 years with the absence of any gastrointestinal symptoms.

2.2. Risk factors associated with Col-R among the studied patients

Data collected from the patients' medical files included: demographic information; location before admission, admission days, underlying illness (i.e. neurologic disorder, diabetes, malignancy, cardiac disease, chronic lung disease, liver and renal impairment), intensive care unit (ICU) admission, presence of devices (such as; urinary catheter, tracheostomy and other ventilation methods), positive culture for carbapenem-resistannce GNRs, chlorhexidine use and receiving antibiotics in the previous 90 d including; carbapenem, colistin and anti-methicillin resistant *Staphylococcus aureus* (MRSA) drugs (i.e. vancomycin, daptomycin and linezolid), in reference to Richter *et al*., (2018).

2.3. Samples collection and processing

Stool samples were collected in clean, leak-proof screw capped containers, labeled and then sent immediately to the Medical Microbiology and Immunology department, Faculty of Medicine. Specimens were processed in the Microbiology Diagnostic and Infection Control Unit (MDICU) and the Genetic Unit. Once received, one loopful of each sample was enriched in 5 ml of selenite broth media for 24 h at 37°C (Cheesbrough, 2006).

2.4. CHROMagar COL-APSE medium for detection of Col-R isolates

According to Abdul Momin *et al*., (2017), about 10 µl of the enriched selenite broth was streaked onto CHROMagar COL-APSE medium (CHROMagar, Paris, France), and then incubated at 37°C for 18-24 h under aerobic conditions. After incubation, growth of the different colored bacterial colonies was interpreted using the manufacturer's criteria for selection of colistin-resistant GNRs. *Escherichia coli* (*E. coli*) has reddish colored colony, other Coliforms have metallic blue colonies, *Pseudomonas* has green colony, whereas *Acinetobacter* has cream colored opaque colony.

2.5. Biochemical identification of the bacterial isolates

Colistin-resistant GNRs were identified up to the species level through testing the colonial morphology; microscopic examination and biochemical assays including; Oxidase test, Kligler iron agar, Lysine iron agar, Motility, Ornithine, Indole test, Citrate utilization test (Oxoid, UK) (Mahon *et al*., 2014), and the Analytical Profile Index 20E API 20E (Bio-merieux SA, France). All intrinsically Col-R species were excluded from further testing such as *Proteus*; *Providencia*, *Hafnia*, *Morganella* and *Serratia* spp. (Jayol *et al*., 2017).

2.6. Susceptibility testing for Col-R *Enterobacteriaceae* isolates

Colistin non-intrinsically resistant *Enterobacteriaceae* isolates were subjected to BMD assay to determine colistin MIC. Results were estimated in accordance to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) break-points for *Enterobacteriaceae* (where; colistin-susceptible: MIC ≤ 2 mg/ l, and colistin-resistant: MIC >2 mg/ l), in reference to Nordmann *et al*., (2016); Clinical and Laboratory Standards Institute (CLSI. 2020).

A bacterial inoculum of 0.5 McFarland was prepared using 3-5 colonies from the 24 h old non selective culture plate; suspensions were made in tubes containing sterile saline and then mixed well. The 0.5 McFarland bacterial suspension was diluted to 1:75 by adding 10 µl to 740 µl of cation-adjusted Mueller-Hinton broth (CaMHB) with TES buffer (Sensititer TM FRCOL, Thermo Fisher Scientific, Germany), to reach pH 7.2-7.4. Approximately 25 µl from each diluted bacterial suspension was transferred to one of the microtiter wells in addition to 50 µl CaMHB and 25µl colistin sulfate (AGITECH; Sigma-Aldrich) to

yield bacterial concentration of 5 $\times 10^5$ cfu/ well. Each well should finally contain a total volume of 100 µl. Working stocks of colistin sulfate were freshly prepared and then diluted in sterile water for final concentration that ranges from 0.125 - 128 mg/ l. The microtiter plates were incubated within 15 min. at 35 ^oC for 24 h. Each plate was covered with a plastic cover to prevent drying. A colistin-susceptible control strain of *E. coli* (American Type Culture Collection (ATCC); 25922) provided by NAMRU-3, Naval Medical Research Unit Three, Cairo, Egypt, was used. Moreover, a medium control well containing 100 µl CaMHB was used. The MIC of colistin was calculated as the highest dilution of colistin that inhibited growth of the tested bacteria inside the wells (CLSI. 2020).

2.7. Molecular detection of Colistin resistant *mcr-1* and *mcr-2* genes using PCR

2.7.1. Extraction of DNA

Pure 24 h old colonies of Col-R *Enterobacteriaceae* strains were used for DNA extraction using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA products were kept frozen at -20ºC for PCR testing.

2.7.2. Amplification of *mcr-1* and *mcr-2* genes

The primers applied for PCR amplification of *mcr-1* and *mcr-2* genes are presented in Table (1). The amplification mixture was provided by Qiagen (Qiagen- Germantown-USA) with a total volume of 25 µl. The amplification cycles included 34 cycles of denaturation for 1 min. at 95°C; annealing at 52°C for 30 sec., extension for 1 min. at 72°C; followed by 1 cycle at 72 °C for 5 min., according to the previously described protocol of Liu *et al*., (2016). The amplification products were subjected to electrophoresis, and then visualized under an ultraviolet light.

2.8. Statistical analysis

Data were statistically analyzed using SPSS software version 22 (SPSS Inc., Chicago, IL, USA). The qualitative data was formulated as numbers and percentages. Comparison between qualitative data was carried out using Chi-square and *p* value, and then interpreted as significant if $p < 0.05$.

3. Results

3.1. Detection of Col-R isolates after culture on CHROMagar COL-APSE

About 290 stool samples were collected during this study; where 49 samples showed growth (Colistin resistant) on CHROMagar COL-APSE medium, 241 plates showed no growth (Colistin sensitive) and were thus excluded from further analysis. Therefore, the prevalence of fecal carriage of Col-R among the studied patients' groups was 16.8 % (49/290), as demonstrated in Fig. (1).

3.2. Risk factors associated with Col-R among the studied patients

Analysis of the patients' collected data revealed that Col-R isolation rate was higher in patient's ≥ 61 years (81.6 %), compared to the other age groups. For Col-R isolates, the distribution between male and female patients showed statistically insignificant difference. Several risk factors were significantly associated with Col-R such as; the presence of underlying illness, long hospitalization ≥ 10 d, ICU admission, advanced ventilation/ tracheostomy, and indwelling urinary catheters. In addition, treatment with carbapenem and colistin antibiotics within the previous 90 d was associated with an increased risk for Col-R. No significant association was recorded between Col-R and the following factors; previous hospitalization, previous carbapenem resistance, prior anti- MRSA treatment and chlorhexidine exposure (Table 2).

After the univariate regression analysis; the following variables were independent predictors for Col-R: previous carbapenem treatment (odds ratio (OR) = 6.5), advanced ventilation/ tracheostomy (OR= 4.1), ICU admission (OR= 3.6), Indwelling urinary catheters (OR= 2.5), presence of underlying illness

(OR= 2.4), long hospitalization (OR= 2.3), and previous colistin treatment (OR= 2.28). As presented in Table (3), after the multivariate regression analysis and adjustment of the confounding factors, only two variables were independent predictors for Col-R including; previous carbapenem treatment (OR= 6.1) and long hospitalization (OR= 2.9).

Table 1. Primers used for detection of Colistin resistant genes in the tested bacterial isolates

Gene	Primer sequence (5'-3')	Size (bp)	Reference
mcr-1	*CGG TCA GTC CGT TTG TTC* *CTT GGT CGG TCT GTA GGG*	309	Liu *et al*., (2016)
mcr-2	*ATG ACA TCA CAT CAC TCT TGG* *TTA CTG GAT AAA TGC CGC GC*	567	Liassine *et al*., (2106)

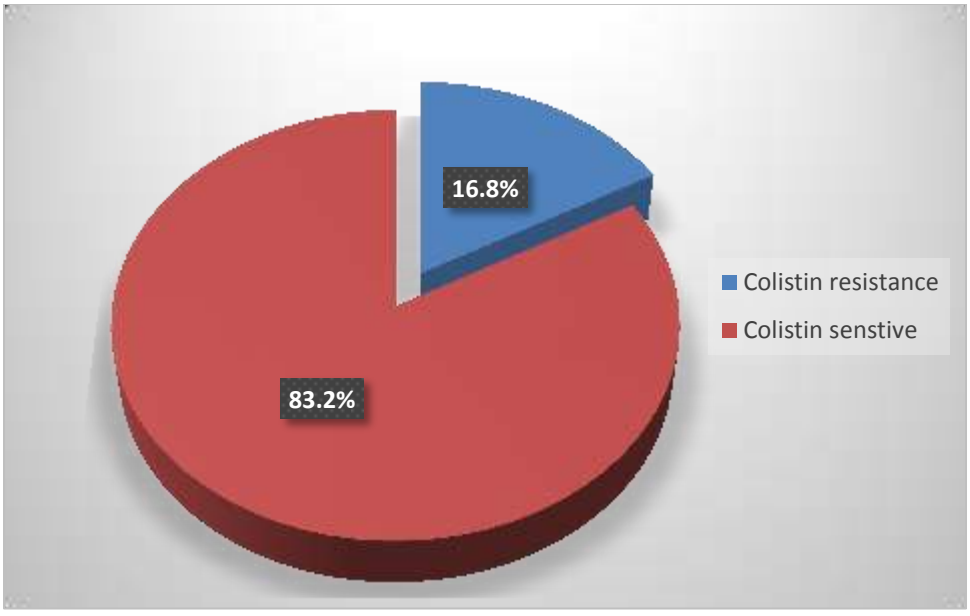

Fig. 1: Prevalence of fecal carriage of Colistin resistance among the studied patients' group

Table 2. Risk factors associated with Colistin resistance among the studied patients' groups

Risk factors	Colistin resistance (n= 49)		Colistin susceptible (n= 241)		*p* value	OR (95 % CI)
	No	%	No	%		
Age (years)						
18-40 (n= 33)	2	4.1	31	12.9	-	(r)
41-60 (n= 68)	8	16.3	60	24.9	0.37	2.1 (0.4-10)
≥ 61 (n= 189)	39	79.6	150	62.2	0.046*	4.03 (0.9-17)
Gender						
Male (n= 178)	31	63.2	147	60.9	0.766	1.1 (0.6-2.1)
Female (n=112)	18	36.7	94	39.1		(r)
Underlying illness	41	83.6	164	68.1	0.028*	2.4 (1.1-5.4)
Previous hospitalization	9	18.3	37	15.3	0.598	1.2 (0.5-2.8)
Long hospitalization	24	48.9	72	29.8	0.009*	2.3 (1.2-4.2)
ICU admission	18	36.7	33	13.6	≤ 0.001*	3.6 (1.8-7.3)
Previous carbapenem resistance	4	8.2	12	4.9	0.373	1.7 (0.5-5.5)
Prior colistin treatment	12	24.5	30	12.4	0.029*	2.28 (1.1-4.8)
Prior carbapenem treatment	35	71.4	67	27.8	≤0.001*	6.5 (3.3-12.8)
Prior anti- MRSA drugs	20	40.8	93	38.6	0.77	1.09 (0.6-2.1)
Chlorhexidine Exposure	7	14.3	25	10.4	0.42	1.4 (0.6-3.5)
Advanced ventilation/ tracheostomy	18	36.7	30	12.4	≤ 0.001*	4.1 (2.03-8.2)
Indwelling urinary catheters	29	59.1	88	36.5	0.003*	2.5 (1.3-4.7)

Where; (r): reference group, OR: odds ratio, CI: Confidence interval, *p value was significant if < 0.05

Table 3. Univariate and multivariate analysis for independent predictors of Colistin resistance among studied patient's groups

Risk factors	Univariate regression analysis		Multivariate regression analysis	
	***p* value**	**OR (95 % CI)**	***p* value**	**OR (95 % CI)**
Underlying illness	0.028	2.4 (1.1-5.4)	-	-
Long hospitalization	0.009	2.3 (1.2-4.2)	0.02	2.9 (1.2-7.4)
ICU admission	≤ 0.001	3.6 (1.8-7.3)	-	-
Prior colistin treatment	0.029	2.28 (1.1-4.8)	-	-
Prior carbapenem treatment	≤ 0.001	6.5 (3.3-12.8)	≤ 0.001	6.1 (2.2-16.9)
Advanced ventilation/ tracheostomy	≤ 0.001	4.1 (2.03-8.2)	-	-
Indwelling urinary catheters	0.003	2.5 (1.3-4.7)	-	-

Where; OR: odds ratio, CI: Confidence interval, *p* value was significant if < 0.05

3.3. Identification of the Col-R bacterial isolates

A total of 72 bacterial isolates were recovered from 49 positive stool specimen's, which were identified to the species level. Fourteen out of 72 isolates (19.4 %) were identified intrinsically as Col-R species, and were thus excluded from further testing's including; *Proteus* sp. (n = 6) (*P. mirabilis*, 5; *P. vulgaris*, 1), *Providencia* sp. (n = 4) (*P. rettgeri*, 3; *P. stuarti*, 1), *Morganella morganii* (n= 2) and *Serratia marcescens* (n= 2).

About 80.6 % of the isolates (58/ 72) were colistin resistant GNRs, which normally susceptible to colistin. Col-R *Enterobacteriaceae* were predominant and were detected in 89.7 % (52/58) of the GNRs isolates; such as *Klebsiella* sp. (n= 23; 44.2 %) (*K. pneumonia*, 21; *K. oxytoca*, 2), *E. coli* (n= 16; 30.8 %), *Citrobacter freundii* (n= 7; 13.5 %), and *Enterobacter* spp. (n= 6; 11.5 %) (*E. cloacae*, 5; *E. aerogenes*, 1). Six Col-R isolates (10.3 %) were related to GNRs other than the *Enterobacteriaceae*, such as *Acinetobacter baumanii* (n= 4); *Pseudomonas aeruginosa* (n= 1) and *Stenotrophomonas* sp. (n= 1), which were excluded from further testing's as demonstrated in Fig. (2).

3.4. Susceptibility testing for Col-R *Enterobacteriaceae* isolates

Using the BMD assay; determination of MIC of colistin susceptibility for Col-R *Enterobacteriaceae* isolates confirmed that Col-R in all the obtained isolates (52/ 52) by CHROMagar COL-APSE medium (100 % sensitivity) with MIC of colistin; lied between ≥ 4-128 mg/ l.

Most of the isolates showed low resistant MIC titer (4 µg/ ml; 55.7 %) followed by (8 µg/ ml; 26.9 %). The MIC results are demonstrated in Table (4).

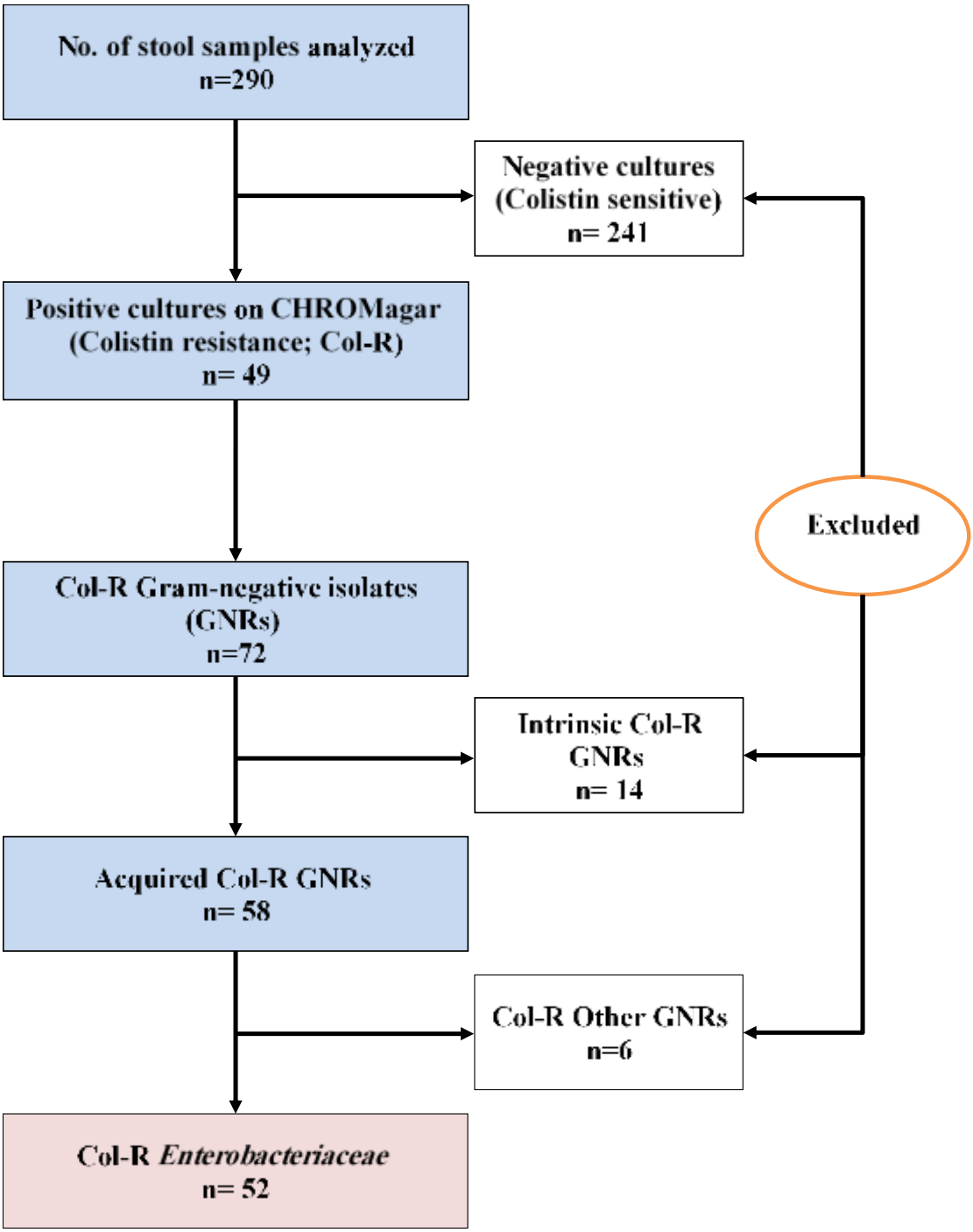

Fig. 2: Flow diagram showing the collection of stool samples; the isolation of colistin resistant strains from CHROMagar COL-APSE medium, the inclusion and exclusion of the bacterial strains in this study

Table 4. Colistin susceptibility MIC results of Colistin resistant *Enterobacteriaceae* isolates

Colistin MIC (µg/ ml)	**Colistin resistant *Enterobacteriaceae* isolates (n= 52)**	
	No	**%**
4	29	55.7
8	14	26.9
16	2	3.8
32	3	5.7
64	3	5.7
128	1	1.9

3.5. Molecular detection of Colistin resistant *mcr−1* and *mcr−2* genes

By PCR, *mcr-1* gene was the most frequent Col-R gene detected in 36/52 of the isolates (69.2 %), while *mcr-2* gene was less prevalent and recorded in 6/52 of the isolates (11.5 %), as clear in Table (5). Furthermore, *mcr-1*and *mcr-2* genes co-occurred together in 2 isolates (3.8 %). About 12 isolates (23.1 %) showed the absence of *mcr-1* and *mcr-2* genes. Gel electrophoresis of PCR amplification products of the *mcr-1* gene is demonstrated in Fig. (3). Isolates 1 (*E. coli*), 2 (*K. pneumoniae*), 3 (*K. pneumoniae*) and 4 (*E. coli*) recorded a single band at 309 bp of the *mcr-1* gene, while isolate 5 (*Enterobacter cloacae*) did not present any bands for this gene. As shown in Table (6), significant association was detected between *mcr-1* positive *Enterobacteriaceae* isolates and the colistin low resistant MIC titer 4 µg/ ml (27/36; 75 %).

4. Discussion

The World Health Organization (WHO) has approved colistin as a "highest priority critically important antibacterial agent for human" (WHO. 2017). Furthermore, Wang *et al*., (2020) recently added that worldwide spread of *mcr* -linked Col-R in *Enterobacteriaceae* represents a health catastrophe; with the need for powerful screening algorithms to identify these clinical bacterial isolates. Currently, 290 stool samples were collected from adult patients admitted at MUHs, who play central roles in transmitting the antibiotic-resistant microorganisms to the community. The prevalence of fecal carriage of Col-R among the studied patients' groups was 16.8 % (49/290) after culture on CHROMagar COL-APSE medium. Results of this study are close to the findings observed in two studies carried out in Germany and Singapore; which recorded that 19.5 % (279/1,430) and 11.4 % (23/201) Col-R isolates were detected in stool samples through screening several culture media, respectively (Przybysz *et al*., 2018; La *et al*., 2019). Other studies carried out in Switzerland and United States by Liassine *et al*., (2106); Richter *et al*., (2018) showed lower prevalence rates of Col-R, recording 0.26 % and 0.4 %; respectively. These variations might be attributed to the differences in geographical location; type of specimen, application of effective infection control program, and the application of antibiotic policy that restricts colistin use in treating the community infections. These variables could support or inhibit the selection of Col-R strains in these regions (Castanheira *et al*., 2016).

Table 5: Detection of *mcr-1* and *mcr-2* genes in Colistin resistant *Enterobacteriaceae* isolates using PCR

Gene	**Colistin resistant *Enterobacteriaceae* isolates** (n= 52)	
	No	**%**
***mcr-1* positive**	36	69.2
mcr-2* positive	6	11.5
***mcr-1* and *mcr-2* (negative)**	12	23.1

*Co-existence of *mcr-1*and *mcr-2* genes in 2 isolates

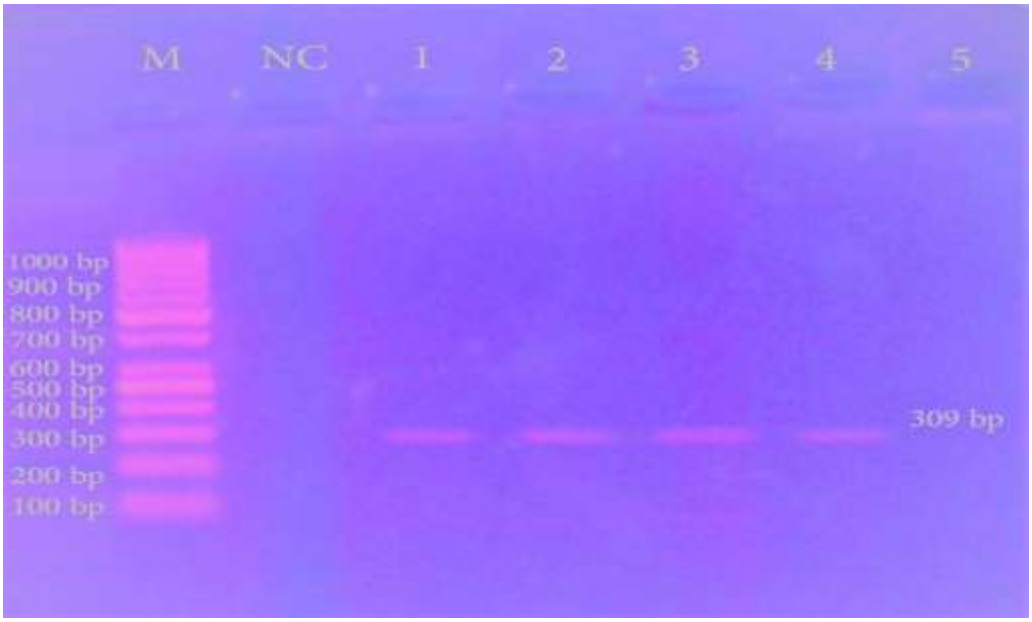

Fig. 3: Gel electrophoresis showing PCR amplification of *mcr-1* gene in colistin resistant *Enterobacteriaceae* isolates. Lanes; 1: (*E. coli*), 2: (*K. pneumoniae*), 3: (*K. pneumoniae*) and 4: (*E. coli*) showed a single band of *mcr-1* gene at 309 bp, while lane 5 (*Enterobacter cloacae*) did not show any bands for this gene. The M lane is 100 bp DNA marker, whereas the NC lane represents the negative control

Table 6: Colistin susceptibility MIC results of *mcr-1* positive *Enterobacteriaceae* isolates

Colistin MIC	***mcr-1* positive *Enterobacteriaceae* (n= 36)**	
(µg/ ml)	**No**	**%**
4	27	75
8	6	16.7
16	2	5.5
64	1	2.8

Furthermore, CHROMagar COL-APSE medium is selective and can discriminate between Col-R *Enterobacteriaceae* as well as the other Col-R GNRs. Preparation of such medium does not need a skilled individual and takes only 18-24 h of incubation till growth appearance (Abdul Momin *et al*., 2017).

In the present study, Col-R isolation rate was higher in patient's ≥ 61 years (81.6 %). Several risk factors were observed to be significantly associated with Col-R, such as presence of underlying illness; long hospitalization ≥ 10 d, ICU admission, advanced ventilation/ tracheostomy and indwelling urinary catheters. Moreover, prior treatment with carbapenem and colistin within 90 d was associated with an increased risk. These results are in accordance with those of the earlier studies conducted by Shorr *et al*., (2008); Kontopidou *et al*., (2011); Giacobbe *et al*., (2015); Jayol *et al*., (2016). These findings demonstrate the importance of availability of all data in the medical records, which could help clinicians for better management of their patients.

In this study, no significant association was detected between Col-R and the following factors; previous hospitalization, previous carbapenem resistance, prior anti- MRSA drugs and chlorhexidine exposure. Results of current analysis are against those of the previous studies of Shorr *et al*., (2008); Giacobbe *et al*., (2015); Richter *et al*., (2018). Variations of results might be related to the difference in the studied population with either infection or colonization and application of antibiotic policy, which necessitate carrying out the culture and sensitivity testing's, in addition to the use of chlorhexidine antibiotic in treatment of some patients.

According to Caselli *et al*., (2018), surface contamination in hospitals may trigger selection and spread of Col-R among GNRs. This may be enhanced by selective pressure of some antiseptics like chlorhexidine. Thus, screening for Col-R should include environmental as well as clinical samples.

In this study, the seven independent predictors for Col-R that were significant on univariate regression analysis were tested against each other's in groups. After multivariate regression analysis and adjustment of the confounding factors, only two variables were independent predictors for Col-R including; previous carbapenem treatment (OR= 6.1) and long hospitalization (OR= 2.9). Current results are in accordance with those achieved by Richter *et al*., (2018), where the multivariate models recorded that recent exposure to carbapenems may serve as a marker for Col-R risk. Although this may be improbable to occur at the theoretical level; however, carbapenem treatment is a proxy for infection with MDR GNRs including Col-R harboring strains.

Currently, about 72 Col-R bacterial isolates were recovered. 80.6 % of the isolates (58/72) were colistin resistant GNRs normally susceptible to colistin. Col-R *Enterobacteriaceae* were predominant and recorded in 89.7 % (52/58) of the GNRs isolates. In agreement to our findings, two studies conducted by Przybysz *et al*., (2018); La *et al*., (2019) in Germany and Singapore recorded that 77.3 % and 73.9 % of the bacterial isolates were Col-R *Enterobacteriaceae* normally susceptible to colistin, respectively.

Among the Col-R *Enterobacteriaceae* isolates; *Klebsiella* (n= 23; 44.2 %) and *E. coli* (n= 16; 30.8 %) were predominant in this study, in agreement with the previous studies conducted by Richter *et al*., (2018); La *et al*., (2019). Another study carried out in Lebanon by Al-Bayssari *et al*., (2021) demonstrated that all *E. coli* recovered from patients either at hospital admission or after one day were Col-R. This can be interpreted as that recovery of *E. coli* and *K. pneumoniae* from community and hospital acquired infections is a result of interaction between the environmental and the hospital factors, which play central roles in exchange of the *mcr* genes, as suggested by Liassine *et al*., (2106).

Colistin MIC determined by BMD assay for Col-R *Enterobacteriaceae* isolates; confirmed the presence of Col-R in all the recovered isolates by the CHROMagar COL-APSE (52/52) (100 % sensitivity). The majority of isolates showed low resistance MIC titer recording; 4 µg/ ml; 55.7 %, followed by 8 µg/ ml; 26.9 %. This is in accordance with the previous study carried out by Zurfluh *et al*., (2017) in Switzerland, where the majority of Col-R isolates had MIC of 4 µg/ ml (6/18; 33.3 %) and 8 µg/ ml (11/18; 61.1 %). Another study conducted in Egypt by Zafer et al., (2019) showed similar results. As recommended by CLSI; BMD is the reference test for colistin susceptibility, but it has a work load and is time consuming. Moreover, Col-R is under-estimation by disk diffusion assay (CLSI. 2020). This supports the importance of CHROMagar COL-APSE medium as a sensitive and rapid test for screening of Col-R, compared to BMD assay.

In the present study, results of the PCR technique showed that *mcr-1* gene was the most frequent Col-R gene detected in 36/52 isolates (69.2 %), while *mcr-2* gene was less prevalent and recorded in 6/52 isolates (11.5 %). Furthermore, *mcr-1* and *mcr-2* genes co-existed together in 2 isolates (3.8 %). About 12 bacterial isolates (23.1 %) recorded the absence of *mcr-1* and *mcr-2* genes, and were mostly related to other Col-R genes. Our results are in line with the recent study held in Lebanon by Al-Bayssari *et al*., (2021), which recorded that all *E. coli* isolates are MDR and carry the *mcr-1* gene. In addition, *mcr-1* gene was detected in 70.6 % (12/17) of the isolates in Singaphore (La *et al*., 2019).

The risk of *mcr-1* gene transfer from food, the environmental or hospital agents needs medical attention. Therefore, implementation of effective infection control program that limits the spread of *mcr* containing MDR isolates is mandatory. Screening for *mcr-1* gene carrying strains among the hospitalized patients is important; mainly for ICU and hematologic patients (Terveer *et al*., 2017). Furthermore, a previous study conducted by Long *et al*., (2019) reported that two or more *mcr* genes could exist simultaneously in the same isolate; which could be explained by the genetic relatedness present between the different *mcr* genes, and this will cause an increased risk of gene transmission between the species.

Furthermore, significant association was observed between *mcr-1* positive *Enterobacteriaceae* isolates and the colistin low resistant MIC titer 4 µg/ ml (27/36; 75 %). In Italy, a similar study carried out by Caselli *et al*. (2018) reported that all the *mcr-1* carrying isolates had MIC for colistin of 4 µg/ ml. These observations must be taken into consideration while selecting a screening culture method that does not skip the *mcr* carrying isolates, and those with low resistant MIC titer.

Limitations of the current study mainly were; the BMD susceptibility assay was not used to check for Col-R in all the collected stool specimens, and search was not carried out for all the known *mcr* genes.

However, the other *mcr* genes are less common, and the European Center for Disease Prevention and Control suggested screening mainly for the *mcr-1* gene (European Centre for Disease Prevention and Control. 2016; Sun *et al*., 2018).

Conclusion

This study highlighted the high prevalence of Col-R and *mcr-1* harbored by the fecal flora; with the risk to be easily transmitted inside the hospitals and to the community in the surrounding locality. This necessitates the use of active surveillance besides the infection control programs.

The CHROMagar COL-APSE medium is able to screen for the Col-R microorganisms regardless of the mechanism of resistance. The PCR is fast and sensitive but is specific to the tested resistant genes, which does not indicate that the corresponding isolate is resistant. The phenotypic and molecular assays could be complementary for diagnosis of the Col-R infections.

Authors' contributions statement

All the authors have contributed equally in this study.

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Conflict of interests

The authors declare non-existence of any conflict of interests.

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Ethical approval

The current research protocol was ethically approved by the Mansoura University Ethical Committee, Egypt (no. R.21.01.1150.R1).

5. References

Abdul Momin, M.H.F.; Bean, D.C.; Hendriksen, R.S.; Haenni, M.; Phee, L.M. and Wareham, D.W. (2017). CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram negative pathogens. Journal of Medical Microbiology. 66(11): 1554-1561. https://doi.org/10.1099/jmm.0.000602

Al-Bayssari, C.; Dagher, T.N.; El Hamoui, S.; Fenianos, F.; Makdissy, N.; Rolain, J.M. and Nasreddine, N. (2021). Carbapenem and colistin-resistant bacteria in North Lebanon: Coexistence of *mcr-1* and *NDM-4* genes in *Escherichia coli*. The Journal of Infection in Developing Countries. 15(7): 934-942. https://doi.org/10.3855/jidc.14176

Carroll, L.M.; Gaballa, A.; Guldimann, C.; Sullivan, G.; Henderson, L.O. and Wiedmann, M. (2019). Identification of Novel Mobilized Colistin Resistance Gene *mcr-9* in a Multidrug-Resistant, Colistin-Susceptible *Salmonella enterica* Serotype *Typhimurium* Isolate. mBio Journal. 10(3): e00853-19. https://doi.org/10.1128/mBio.00853-19

Caselli, E.D.; Accolti, M.; Soffritti, I.; Piffanelli, M. and Mazzacane, S. (2018). Spread of *mcr-1* driven colistin resistance on hospital surfaces, Italy. Emerging Infectious Disease. 24(9): 1752-1753. https://doi.org/10.3201/eid2409.171386

Castanheira, M.; Griffin, M.A.; Deshpande, L.M.; Mendes, R.E.; Jones, R.N. and Flamm, R. (2016). Detection of *mcr-1* among *Escherichia coli* clinical isolates collected worldwide as part of the SENTRY antimicrobial surveillance program during 2014-2015. Antimicrobial Agents Chemotherapy. 60(9): 5623-5624. https://doi.org/10.1128/AAC.01267-16

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries-Part 2. 2nd Edition. 2: 26-130. Cambridge University Press, New York. https://doi.org/10.1017/CBO9780511543470

Clinical and Laboratory Standards Institute (CLSI). (2020). Performance Standards for Antimicrobial Susceptibility Testing; 30th Edition. CLSI document M100-A30 Edition. Wayne, PA.

European Centre for Disease Prevention and Control. (2016). Plasmid-Mediated Colistin Resistance in *Enterobacteriaceae*. Stockholm. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-plasmid-mediated-colistin-resistance enterobacteriaceae-15

Giacobbe, D.R.; Del Bono, V.; Trecarichi, E.M.; De Rosa, F.G.; Giannella, M.; Bassetti, M.; Bartoloni, A.; Losito, A.R.; Corcione, S.; Bartoletti, M.; Mantengoli, E.; Saffioti, C.; Pagani, N.; Tedeschi, S.; Spanu, T.; Rossolini, G.M.; Marchese, A.; Ambretti, S.; Cauda, R.; Viale, P.; Viscoli, C.; Tumbarello, M. and Isgri, S. (2015). Risk factors for bloodstream infections due to colistin-resistant KPC-producing *Klebsiella pneumoniae*: Results from a multicenter case-control-control study. Clinical Microbiological Infections. 21(12): 1106.e1-8. https://doi.org/10.1016/j.cmi.2015.08.001

Grégoire, N.; Aranzana-Climent, V.; Magréault, S.; Marchand, S. and Couet, W. (2017). Clinical pharmacokinetics and pharmacodynamics of colistin. Clinical Pharmacokinetics Journal. 56 (12): 1441-1460. https://doi.org/10.1007/s40262-017-0561-1

Ilsan, N.A.; Lee, Y.J.; Kuo, S.C.; Lee, I.H. and Huang, T.W. (2021). Antimicrobial Resistance Mechanisms and Virulence of Colistin- and Carbapenem-Resistant *Acinetobacter baumannii* Isolated from a Teaching Hospital in Taiwan. Microorganisms. 9(6): 1295-1312. https://doi.org/10.3390/microorganisms9061295

Jayol, A.; Saly, M.; Nordmann, P.; Menard, A.; Poirel, L. and Dubois, V. (2017). *Hafnia*, an enterobacterial genus naturally resistant to colistin revealed by three susceptibility testing methods. Journal of Antimicrobial Chemotherapy. 72: 2507-2511. https://doi.org/10.1093/jac/dkx154

Jayol, A.; Poirel, L.; Dortet, L. and Nordmann, P. (2016). National survey of colistin resistance among carbapenemase-producing *Enterobacteriaceae* and outbreak caused by colistin-resistant OXA-48-producing *Klebsiella pneumoniae*, France, 2014. Eurosurveillance Journal. 21(37): 30339. https://doi.org/10.2807/1560-7917.ES.2016.21.37.30339

Kieffer, N.; Aires-de-Sousa, M.; Nordmann, P. and Poirel, L. (2017). High Rate of MCR-1-Producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal. Emerging Infectious Disease. 23: 2023-2029. https://doi.org/10.3201/eid2312.170883

Kontopidou, F.; Plachouras, D.; Papadomichelakis, E.; Koukos, G.; Galani, I.; Poulakou, G.; Dimopoulos, G.; Antoniadou, A.; Armaganidis, A. and Giamarellou, H. (2011). Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Clinical Microbiology Infections. 17: E9-E11. https://doi.org/10.1111/j.1469-0691.2011.03649.x

La, M.V.; Leeb, B.; Hongc, B.Z.M.; Yahc, J.Y.; Kood, S.H.; Jianga, B.; Lily Nga, S.Y. and Tana, T.Y. (2019). Prevalence and antibiotic susceptibility of colistin-resistance gene (*mcr-1*) positive *Enterobacteriaceae* in stool specimens of patients attending a tertiary care hospital in Singapore. International Journal of Infectious Diseases. 85: 124-126. https://doi.org/10.1016/j.ijid.2019.05.029

Liassine, N.; Assouvie, L.; Descombes, M.C.; Tendon, V.D.; Kieffer, N.; Poirel, L. and Nordmann, P. (2016). Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract *Enterobacteriaceae* in Switzerland. International Journal of Infectious Disease. 51: 4-5. https://doi.org/10.1016/j.ijid.2016.08.008

Liu, Y.Y.; Wang, Y.; Walsh, T.R.; Yi, L.X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X.; Yu, L.F.; Gu, D.; Ren, H.; Chen, X.; Lv, L.; He, D.; Zhou, H.; Liang, Z.; Liu, J.H. and Shen, J. (2016). Emergence of plasmid-mediated colistin

resistance mechanism *MCR-1* in animals and human beings in China: A microbiological and molecular biological study. Lancet Infectious Diseases 16(2): 161-168. https://doi.org/10.1016/S1473-3099(15)00424-7

Long, H.; Feng, Y.; Ma, K.; Liu, L.; McNally, A. and Zong, Z. (2019). The co-transfer of plasmid-borne colistin-resistant genes *mcr-1* and *mcr-3*, the carbapenemase gene *blaNDM-5* and the 16S methylase gene *rmtB* from *Escherichia coli*. Scientific Reports. 9: 696. https://doi.org/10.1038/s41598-018-37125-1

Magiorakos, A.P.; Suetens, C.; Monnet, D.L.; Gagliotti, C. and Heuer, O.E. (2013). EARS-net coordination group and EARS-Net participants. The rise of carbapenem resistance in Europe: just the tip of the iceberg?. Antimicrobial Resistance and Infection Control. 2 (1): 6-8. https://doi.org/10.1186/2047-2994-2 -6

Mahon, C.R.; Lehman, D.C. and Manuselis, G. (2014). Textbook of Diagnostic Microbiology. eBook ISBN: 9780323292627, 5th Edition. 1,096 pages. Published by Saunders. Elsevier Health Sciences.

Nordmann, P.; Jayol. A. and Poirel, L. (2016). A universal culture medium for screening polymyxin-resistant gram-negative isolates. Journal of Clinical Microbiolgy. 54: 1395-1399. https://doi.org/10.1128/JCM.00446-16

Przybysz, S.; Correa-Martinez, M.C.; Köck, R.; Becker, K. and Schaumburg, F. (2018). Super Polymyxin TM Medium for the Screening of Colistin-Resistant Gram-Negative Bacteria in Stool Samples. Frontiers in Microbiology. 9: 2809-2816. https://doi.org/10.3389/fmicb.2018.02809

Richter, S.E.; Miller, L.; Uslan, D.Z.; Bell, D.; Watson, K.; Humphries, R. and McKinnell, J.A. (2018). Risk Factors for Colistin Resistance among Gram-Negative Rods and *Klebsiella pneumoniae* Isolates. Journal of Clinical Microbiology. 56(9): e00149-18. https://doi.org/10.1128/JCM.00149-18

Sekyere, J.O. (2019). *Mcr* colistin resistance gene: a systematic review of current diagnostics and detection methods. Microbiology Open. 8(4): e682. https://doi.org/10.1002/mbo3.682

Shorr, A.F.; Zilberberg, M.D.; Micek, S.T. and Kollef, M.H. (2008). Prediction of infection due to antibiotic-resistant bacteria by select risk factors for health care-associated pneumonia. Archives of Internal Medicine. 168: 2205-2210. https://doi.org/10.1001/archinte.168.20.2205

Sun, J.; Zhang, H.; Liu, Y.H. and Feng, Y. (2018). Towards understanding *MCR*-like colistin resistance. Trends in Microbiology. 26: 794-808. https://doi.org/10.1016/j.tim.2018.02.006

Terveer, E.M.; Nijhuis, R.H.T.; Crobach, M.J.T.; Knetsch, C.W.; Veldkamp, K.E.; Gooskens, J.; Kuijper, E.D.J. and Claas, E.C.J. (2017). Prevalence of colistin resistance gene *(mcr-1)* containing *Enterobacteriaceae* in feces of patients attending a tertiary care hospital and detection of a *mcr-1* containing, colistin susceptible *E. coli.* Plos One. 12(6): e0178598. https://doi.org/10.1371/journal.pone.0178598

Wang, C.; Feng, Y.; Liu, L.; Wei, L.; Kang, M. and Zong, Z. (2020). Identification of novel mobile colistin resistance gene *mcr-10*. Emerging Microbes and Infections. 9(1): 508-516. https://doi.org/10.1080/22221751.2020.1732231

World Health Organization (WHO). (2017). Critically Important Antimicrobials for Human Medicine: Ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use. 5th Revision. https://apps.who.int/iris/handle/10665/255027

Zafer, M.M.; El−Mahallawy, H.A.; Abdulhak, A.; Amin, M.A.; Al−Agamy, M.H. and Radwan, H.H. (2019). Emergence of colistin resistance in multidrug‐resistant *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from cancer patients. Annals

of Clinical Microbiology and Antimicrobials*;* 18(1): 40-48. https://doi.org/10.1186/s12941-019-0339-4

Zurfluh, K.; Stephan, R.; Widmer, A.; Poirel, L.; Nordmann, P.; Nüesch, H.J.; Hächler, H. and Nüesch-Inderbinen, M. (2017). Screening for fecal carriage of *MCR* producing *Enterobacteriaceae* in healthy humans and primary care patients. Antimicrobial Resistance and Infection Control. 6(28): 1-4. https://doi.org/10.1186/s13756-017-0186-z