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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Received: 3 October, 2021; Accepted: 31 October, 2021; Published online: 15 November, 2021

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 the 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

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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 untransferable 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-resistant 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, Haifnia, 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 FRKOL, 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 ×10⁵ 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 °C 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 -20°C 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 | 309       | Liu et al., (2016) |
|      | CTT GGT CGG TCT GTA GGG |           |                    |
| mcr-2| ATG ACA TCA CAT CAC TCT TGG | 567       | Liassine et al., (2016) |
|      | TTA CTG GAT AAA TGC CGC GC |           |                    |

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           |    |         |      |         | 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 specimens, 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., (2016); 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 \textit{mcr}-1 and \textit{mcr}-2 genes in Colistin resistant \textit{Enterobacteriaceae} isolates using PCR

| Gene                          | No | %     |
|-------------------------------|----|-------|
| \textit{mcr}-1 positive       | 36 | 69.2  |
| \textit{mcr}-2 positive*      | 6  | 11.5  |
| \textit{mcr}-1 and \textit{mcr}-2 (negative) | 12 | 23.1  |

*Co-existence of \textit{mcr}-1 and \textit{mcr}-2 genes in 2 isolates

**Fig. 3**: Gel electrophoresis showing PCR amplification of \textit{mcr}-1 gene in colistin resistant \textit{Enterobacteriaceae} isolates. Lanes; 1: \textit{(E. coli)}, 2: \textit{(K. pneumoniae)}, 3: \textit{(K. pneumoniae)} and 4: \textit{(E. coli)} showed a single band of \textit{mcr}-1 gene at 309 bp, while lane 5 \textit{(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 (μg/ml) | mcr-1 positive Enterobacteriaceae (n= 36) | 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).

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 carbapenem 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.

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 carbapenem 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 (52/58) (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 Singapore (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 \textit{mcr} genes are less common, and the European Center for Disease Prevention and Control suggested screening mainly for the \textit{mcr-1} gene (\textit{European Centre for Disease Prevention and Control}, 2016; \textit{Sun et al.}, 2018).

\textbf{Conclusion}

This study highlighted the high prevalence of Col-R and \textit{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.

\textbf{Authors’ contributions statement}

All the authors have contributed equally in this study.

\textbf{Acknowledgements}

The authors would like to acknowledge all the supporting staff and the patients participated in this study.

\textbf{Conflict of interests}

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

\textbf{Funding}

This study did not receive a fund from any agency.

\textbf{Ethical approval}

The current research protocol was ethically approved by the Mansoura University Ethical Committee, Egypt (no. R.21.01.1150.R1). The patient's consents and statement of protection of the patient's privacy are provided.

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