Molecular study of colistin resistant clinical isolates of Enterobacteriaceae species

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Abstract

Background: There is recent concern about the development of colistin resistance that may disturb the antibiotics therapy used for extended beta lactamase producing Enterobacteriaceae species.

Aim: The aim of the present study was to investigate the presence of mcr-1 and mcr-2 genes in clinical isolates of Enterobacteriaceae spp. resistant to colistin.

Study design: Retrospective laboratory based study.

Material and Method The study was conducted on 50 Enterobacteriaceae species resistant to colistin collected from clinical samples from patients with health care associated infections according to CDC definitions. Minimum inhibitory concentrations (MICs) of colistin was performed by the use of the broth microdilution method according to CLSI. Isolates were reported resistant if MIC was >2 mg/L. Polymerase chain reaction (PCR) for mcr-1 and mcr-2 was performed.

Results: Colistin resistance genes was detected by PCR in 2 isolates. Mcr-1 gene was detected in 2 isolates (4%) and mcr-2 was not detected in any isolates. Mcr-1 was detected in one E.coli strain and in one K.pneumoniae strains. The presence of Mcr-1 was associated with high MIC >16 mg/L.

Conclusion: The present study highlights the emergence of colistin resistance among E.coli and K.pneumoniae to colistin resistant collected from clinical samples from patients with health care associated infections according to CDC definitions. Minimum inhibitory concentrations (MICs) of colistin was performed by the use of the broth microdilution method according to CLSI. Isolates were reported resistant if MIC was >2 mg/L. Polymerase chain reaction (PCR) for mcr-1 and mcr-2 was performed.

The aim of the present study was to investigate the presence of mcr-1 and mcr-2 genes in clinical isolates of Enterobacteriaceae spp. resistant to colistin from health-care associated infections in Mansoura University hospitals.

Material and method

The aim of the present study was to investigate the presence of mcr-1 and mcr-2 genes in clinical isolates of Enterobacteriaceae spp. resistant to colistin collected from clinical samples from patients with health care associated infections according to CDC. The study was approved by the ethical committee of our institute. Microbiological

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identifications of the isolates were performed by automated Microscan system (Beckman Coulter International, USA). Antibiotics susceptibility was performed by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19] to ceftazidime, cefotaxime, cefepime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, levofloxacin, sulfamethoxazole/trimethoprim and piperacillin/tazobactam. In addition, minimum inhibitory concentrations (MICs) of colistin was performed by the use of the broth microdilution method according to CLSI. Isolates were reported resistant if MIC was >2 mg/L [19].

PCR for mcr-1 and mcr-2

DNA extraction

Pure colonies of isolates were cultured on nutrient broth at 37°C for 24 hours. Later on, 100 micron of broth was centrifuged for 5 minutes and the deposit was resuspended in 100 micron distilled sterile water and heated in water bath at 95°C for 20 minutes. The supernatant was collected in sterile eppendorf and kept frozen at -20°C till amplification.

Amplification and detection of mcr-1 and mcr-2

The sequences of the used primers were summarized in Table 1, [8,20]. For amplification Qiagen amplification master mix was used (Qiagen). Total amplification volume was 25 micron with 3 μl of the bacterial crude lysate and 0.5 μM of each primer. The amplification procedure was performed with the following steps: 5 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 60°C (for mcr-1 gene) and 0.5 μM of each primer. The amplification was performed with the following steps 5 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 60°C (for mcr-1) or 1 min at 55°C (for mcr-2), 1 min at 72°C, and a final extension time of 7 min at 72°C [8,10,21].

Electrophoresis with gel 2% was performed for 20 minutes. The products was visualized by UV and compared with DNA ladder. The amplified products was confirmed by sequence analysis.

Results

The clinical isolates of Enterobacteriae spp. resistant to colistin was collected during 36 months from Mansoura University hospitals. The most common sources were urine (46%), blood (30%) and wounds (24%), Table 2.

The isolates were K. pneumoniae (44%), E. coli (42%), Enterobacter species (10%) and Acinetobacter baumannii (4%), Table 3.

The isolated strains had marked resistance to the third generation cephalosporines ceftazidime (60%) and cefotaxime (56%) and fourth generation cephalosporine, cefepime (78%). Resistance to carbapenem antibiotics imipenem and meropenem was 50% and 44% respectively. Less resistance was noticed for amikacin 42% and gentamicin 40%, Table 4.

Minimal inhibitory concentrations for colistin was found to be >16 mg/L in 30 isolates (60%), 8-16 mg/L in 8 isolates (16%) and 4-8 mg/L in 12 isolates (24%), Table 5, Figure 1.

Colistin resistance genes was detected by PCR in 2 isolates. Mcr-1 gene was detected in 2 isolates (4%) and mcr-2 was not detected in any isolates, Table 6.

Mcr-1 was detected in one E. coli strain and in one K. pneumoniae strains, Table 7.

The presence of Mcr-1 was associated with in high MIC >16 mg/L, Table 8, Figure 2.

### Table 1. Genes and primers sequences with amplified bp

| Gene     | Sequence            | bp   |
|----------|---------------------|------|
| mcr-1    | F: 5′-CGGTTCAGAGCCGTTTCTGTA GG−3′<br>R: 5′-GTGTTGCGGTCGAGATGA GCCG−3′ | 509 bp |
| mcr-2    | F: 5′-TTGTTGCGGTCGAGATGA GCCG−3′<br>R: 5′-CTTTGAGATTGGGTTATGA GCCG−3′ | 567 bp |

### Table 2. Source of colistin resistant strains

| Source          | No. | %   |
|-----------------|-----|-----|
| Blood           | 15  | 30.0|
| Urine           | 23  | 46.0|
| Wound           | 12  | 24.0|
| Total           | 50  | 100.0|

### Table 3. Isolated Enterobacteriae species

| Enterobacteriae species | No. | %   |
|-------------------------|-----|-----|
| A. baumannii            | 2   | 4.0 |
| E. coli                 | 21  | 42.0|
| Enterobacter             | 5   | 10.0|
| K. pneumoniae           | 22  | 44.0|
| Total                   | 50  | 100.0|

### Table 4. Antibiotics resistance of the isolated Enterobacteriae spp

| Antibiotics             | No. | %   |
|-------------------------|-----|-----|
| Amikacin                | 21  | 42.0|
| Cefepime                | 39  | 78.0|
| Cefotaxime              | 28  | 56.0|
| Cefotaxime              | 30  | 60.0|
| Imipenem                | 25  | 50.0|
| Meropenem               | 22  | 44.0|
| Levofloxacin            | 33  | 66.0|
| Gentamicin              | 20  | 40.0|
| Ciprofloxacin           | 32  | 64.0|
| Sulfamethoxazole/trimethoprim | 22  | 44.0|
| Piperacillin/tazobactam | 33  | 66.0|

### Table 5. Distribution of levels of minimal inhibitory concentration of colistin among isolated strains

| MIC Mg/L | No. | %   |
|----------|-----|-----|
| >16      | 30  | 60  |
| Aug-16   | 8   | 16  |
| 04-Aug   | 12  | 24  |
| Total    | 50  | 100 |

### Table 6. Frequency of Mcr-1 and MCR-2 detection by PCR

| Gene     | No. | %   |
|----------|-----|-----|
| Mcr-1    | 2   | 4   |
| Mcr-2    | 0   | 0   |
| Total    | 50  | 100 |

### Table 7. Distribution of Mcr-1 among Enterobacteriae species

| Enterobacteriae species | Mcr-1 No. (%) | N0. (%) |
|-------------------------|---------------|---------|
| E. coli                 | 1 (4.7%)      | 21 (100%)|
| K. pneumoniae           | 1 (4.5%)      | 21 (100%)|

### Table 8. Distribution of Mcr-1 according to MICs of colistin.

| MIC of colistin | Mcr-1 | N0. (%) |
|----------------|-------|---------|
| >16            | 2     | 3.3%    |
|                |       | 30 100% |
Several reports had documented the association of carbapenem resistance and resistance to the third generation of cephalosporines with colistin resistance among different species of Enterobacteriaceae [27-30]. Therefore there is a need for the study of the prevalence of colistin resistance among isolates with extended spectrum beta lactamase resistance [31,32]. Less resistance was noticed for amikacin 42% and gentamicin (40%). This on contrary to previous results reporting poor sensitivity to aminoglycosides [23,33]. This difference in susceptibility may reflect the difference of antibiotics policy among different health care settings in different geographical regions.

The use of PCR to detect mcr-1 gene in the present study has revealed the presence of mcr-1 in one E.coli strain and in one K.pneumoniae strain. There are several reports about the dissemination of strains of K.pneumoniae and E.coli with detected mcr-1 gene including Egypt and Arabian Peninsula [34,35]. These findings highlights that the presence of such gene among clinical isolates. Thus the presence of mcr-1 should be monitored and studies should be carried on large number of isolates. Spread of the mcr-1 gene in the community and successively in the hospital would pose a threat to patients developing an infection with mcr-1 containing multidrug resistant Enterobacteriaceae isolates as this will limit the therapeutics options [36].

In the present study none of the isolates had mcr-2. Recently, it was reported that a novel gene carried on plasmid-, mcr-2, also confers resistance to colistin [8], although it seems unusual that the mcr-2 gene is detected only in Belgium [37]. This posed a hypothesis that might be due to a mechanism for mcr-2 dissemination different from that of the paradigm mcr-1 gene.

The presence of Mcr-1 was associated with in high MIC >16mg/L. Generally most isolates with colistin resistant strains had MICs in the range of 4 or 8 mg/L. Even in one study the strains which harbor mcr-1 were susceptible to colistin with a MIC of ≤ 0.25mg/L. The discrepancy in the results of MICs may be attributed to the difference in the number of the tested isolates.

Conclusion

The present study highlights the emergence of colistin resistance among E.coli and K.pneumoniae in tertiary health care setting. The gene that was responsible for this resistance was mcr-1 while mcr-2 was not detected. There is a need for future studies with large number of clinical isolates to determine the prevalence of colistin resistance and the responsible molecular mechanism for such resistance.

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