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Original article

Detection of colistin resistant Gram negative bacilli in intensive care unit patients admitted to Ain Shams University Hospitals

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ABSTRACT

Background: Colistin is the last choice for serious infections caused by multidrug-resistant Gram negative bacteria and one of the prominent causes for spreading the resistance is Plasmid-borne Mobile Colistin Resistance (*mcr)*. Broth microdilution method (BMD) is the reference tool for colistin minimum inhibitory concentrations (MIC) determination, but it has many obstacles, so commercial BMD methods had been developed that are more user-friendly than the reference method and (Liofilchem ® ComASPTM) is one of them, which we used to determine colistin MIC in this study. **Objective:** To detect colistin resistant Gram negative bacilli (GNB) by ComASPTM colistin (formerly Sensi Test™Colistin) among Intensive Care Units (ICUs) patients admitted to Ain Shams university hospitals and to screen the presence of *mcr-1* gene by Polymerase Chain Reaction (PCR) in Colistin resistant isolates. **Method:** This Observational cross-sectional study was performed in the Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University between June 2019 to November 2019. One hundred isolates of Gram negative bacilli were obtained from patients admitted at different ICUs of Ain Shams University Hospitals. Full identification was done by conventional microbiological methods, Then MIC was measured for all isolated organisms by using commercial BMD ComASPTM colistin, PCR was done for colistin resistant isolates to detect *mcr-1* gene. **Results:** 60% of the GNB isolates were *K.pneumoniea*. Colistin resistance was 14% among 100 GNB, 35.7% of these colistin resistant were *K.pneumoniea* obtained from urine samples. Prevalence of *mcr-1* gene was 7.1%. **Conclusion:** Commercial BMD ComASPTM colistin is simple and uncomplicated method for detection colistin susceptibility.

Introduction

Today, antimicrobial resistance is one of the world's greatest health care problems, especially for gram-negative bacteria. Carbapenems were considered as effective and reliable antimicrobials for the treatment of β- lactamase (ESBL) extended-spectrum infections-Enterobacteriaceae. Currently, serious concerns were raised due to the global spread of carbapenem-resistant bacteria and doctors can "beam back" to the pre-antimicrobial era as there are only very few compounds available to treat infections with this multidrug-resistant microorganism [1,2].

This crisis has made colistin the last treatment option for infections caused by *Enterobacteriaceae* producing carbapenemase. That finding leads the World Health Organization (WHO) to classify colistin as an important drug for human's medicine [3].

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Colistin interacts with lipopolysaccharides on the outer membrane of Gram-negative bacteria and causes injury to the membrane leading to bacterial death. Multiple different mechanisms cause the loss or modification of the production of lipopolysaccharides in Gram negative bacteria resulting in resistance to colistin [4].

Colistin resistance results from two mechanisms: chromosomal defects or plasmid resistance. The chromosomal mutations occur in the PmrA / PmrB and PhoP / PhoQ encoding genes leading either to lipid A molecule modifications or even losses. These mutations are related to colistin usage [5]. Though, colistin resistance is present without prior exposure to Colistin, due to the presence of plasmid mediated *mcr-1* gene encoding the phosphoethanolamine transferase enzyme leading to the transfer of phosphoethanolamine to Lipid A; conferring colistin resistance [6].

The joint of Clinical Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing(CLSI-EUCAST) Polymyxin Breakpoints Working Group recently recommended that the ISO standard broth microdilution method (BMD) be the reference tool for Colistin MIC determination [7] ,but clinical microbiology laboratories rarely perform BMD reference as it requires freshly prepared or frozen antibiotic solutions.

So, it was mandatory to measure the presence of colistin resistance in our hospitals by more user-friendly tests. Few commercial BMD methods have recently become available as Liofilchem ® ComASPTM colistin (formerly SensiTestTM Colistin) which contains antibiotic in 7 twofold dilutions (0.25 to 16mg / L) and allowing simultaneous testing of four samples [8]. We used it to detect colistin resistant GNB among ICUs patients admitted to Ain Shams university hospitals.

Methodology

This Observational cross-sectional study was performed in the Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University and was approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University in the period between June 2019 to November 2019.

Patient selection and collection of samples

The samples (one hundred isolates of GNB) were obtained from patients admitted at different ICUs of Ain Shams University Hospitals. The age of the patients ranged from 22 years to 82 years. Prior to obtaining the samples, a written informed consent was obtained from each patient or from guardians of the patients after explaining the study and its goals to them.

Collection and identification of bacterial isolates

One hundred Gram-negative bacterial isolates (100) were collected from clinical samples from different infection site (blood, urine, sputum, wound and endotracheal tube) from different ICUs of Ain Shams University Hospitals in Cairo, Egypt. Out of 100 isolates of GNB, 56 urine samples, 25 respiratory specimens [12 sputum and 14 endotracheal aspirates (ETA)], 3 wound swabs specimens and 15 blood samples were collected under complete aseptic conditions. Samples were collected in sterile containers to be examined bacteriologically.

All bacterial isolates were identified using conventional methods depending on cultural and biochemical characteristics on MacConkey agar medium as described by Cheesbrough, 2006.

Antimicrobial susceptibility test (MIC) commercial BMD ComASPTM colistin.

All Gram-negative isolates were tested for colistin resistance by commercial BMD ComASPTM colistin.

A. Steps

A suspension equivalent in density to the 0.5 McFarland standard (BioMérieux, France) was prepared by diluting approximately 3-5 well-isolated colonies in sterile saline and then diluting 1:20 in saline to form solution A.

Solution B was provided by adding 400µl of solution A to the tube of (Mueller Hinton Broth) MH II Broth supplied in the package using a multichannel pipette (100 -1000µl). In each well in a row, 100 µl of solution B was added. The panel was coated with the lid and incubated for 20 hours in ambient air at 37.

B. Reading the results

At the end of the incubation period the growth was observed in the wells and the MIC was established, i.e. the lowest concentration of antibiotic that inhibits visible growth.

C. Results interpretation

The obtained MIC was interpreted according to interpretative criteria currently used by CLSI. According to CLSI, MIC of 2 µg / ml was considered susceptible and a MIC Of 4 µg /ml was considered colistin-resistant [9].

Molecular detection of colistin resistance

DNA extraction

Pure colonies from resistant isolates were cultured for 24 hours on a nutrient broth at 37 °C. Later, 100 microns of broth were centrifuged for 5 minutes, and the deposit was resuspended for 20 minutes in 100 microns of sterile distilled water and heated in water bath at 95°C. The supernatant was installed in sterile eppendorf and kept frozen at -20°C until amplification [10].

Amplification and detection of mcr-1 gene

The primers of *mcr-1* gene were summarized in **table (1**) [11] [12].

Qiagen amplification master mix (**Qiagen, Germany**) was used for the amplification. The total volume of amplification was 25 microns, with 3 µl of extracted bacterial DNA and 0.5 µM of each primer. The amplification procedure was carried out 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 *mcr1),* 1 min at 72 °C, and a final extension time of 7 min at 72 °C [6] [13].

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

Table 1. The primers sequence of *mcr-1* gene.

Gene		**Bp**
Mcr-1	F:5/- AGTCCGTTTGTTCTTGTGGC -/3 R:5/- AGATCCTTGGTCTCGGCTTG -/3	320 bp

Statistical analysis

Data collected were analyzed using the Social Sciences Statistical Package (SPSS) V.20. Data was presented using standard deviation, mean, median, minimum and maximum quantitative data, and categorical data using frequency (count) and relative frequency (percentage).

Results

This study encased 100 GNB isolates, from patients admitted at different ICUs of Ain Shams University Hospitals. These 100 isolates consisted of: *K. pneumoniae* (60%), *E.coli* (18%), *P.aeruginosa* (15%) and *Citrobacter* (7%).

Regarding antimicrobial susceptibility test (MIC) by commercial BMD (ComASPTM colistin), there were 14 resistant isolates (14%) out of 100 Gram negative isolates (the resistant isolate was identified by turbidity or as a button at the bottom of the well as in **figure (1)** and their distribution is shown in **figure (2)**.

For *K.pneumoniae*, 10 isolates (16.6%) were resistant, *P.aeruginosa*, 3 isolates (20%) were resistant, *E.coli*, only one isolate (5.5%) was resistant and *Citrobacter* all isolates were sensitive (7%). *Proteus* species were excluded from this result due to its intrinsic resistance to colistin.

The distribution of colistin resistant strains in different samples **(Figure 3)** is:

According to urine samples: 9 isolates (6.1%) out of 56 isolates are resistant, they include 5 isolates *K. pneumoniae* (55.56%), 3 isolates *P. aeruginosa* (33.3%) and one isolate for *E.coli* (11.1%).

For blood samples: 2 isolates (13.4%) out of 15 isolates are resistant, they include 2 isolates (100%) *K. pneumoniae.* For sputum samples: one isolate (7.4%) out of 12 isolates is resistant, which is *K. pneumoniae* (100%).

For wound exudate samples: one isolate (33.3%) out of 3 isolates is resistant, which is *K. pneumoniae* (100%). For tracheal aspirate: one isolate (7.14%) out of 14 isolates is resistant, which is *K. pneumoniae* (100%)

In the present study, history of Colistin intake was positive for only one patient (55 years old, male) admitted to ICU for pneumonia.

The prevalence of *mcr-1* gene was 7.1% and was referred to *K .pneumoniae* isolated from urine of 70 years male patient admitted to ICU for stroke and it is shown in **figure (4)**.

Figure 1. ComASPTM Colistin showing resistant isolate in the second row.

Figure 2. The distribution of sensitive and resistant strains of *Gram –ve* isolates.

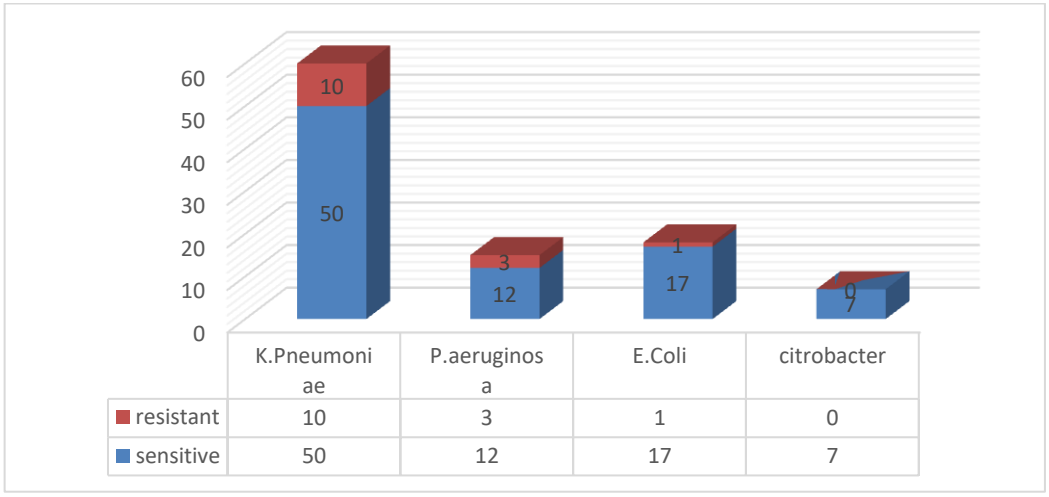

Figure 3. The distribution of colistin resistant strains in different samples.

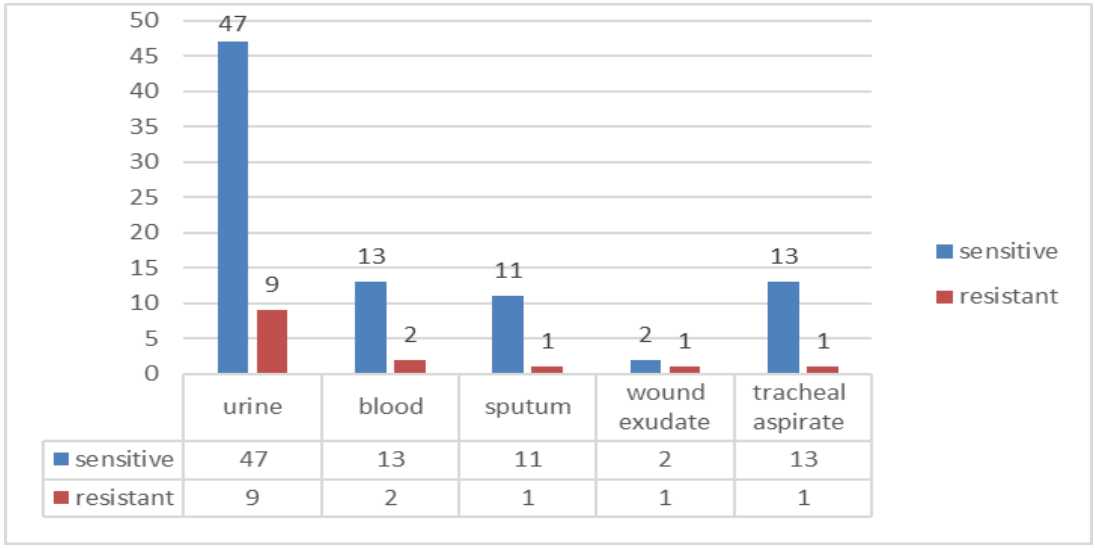

Figure 4. Gel electrophoresis of *mcr-1* gene (320 bp) encoding for colistin resistance (strain no 12 is the resistant strain), Pc: positive control and NC**:**(negative control).

Discussion

Increased use of inappropriate antimicrobials has resulted in the emergence of MDR bacteria, which are extremely hard to treat [14]. Colistin recently returns to use as an effective antibiotic, particularly for treating severe health care associated infections with multiple antibiotic resistances [15].

Colistin resistance detection currently relies on MIC determinations using BMD, and routine Colistin resistance detection using traditional methods such as PCR-based tests

In this study, the isolates were *K.pneumoniae* (60%), *E.coli* (18%), *P.aeurogenosa* (15%), and *citrobacter* (7%). Similar studies in Egypt [16] found that *K.pneumoniae* (43.4%), *E.coli* (29.1%), *P.aeruginosa* (13.5%), *A.baumannii* (5.3%), *Enterobacter spp.* (2%) , *Citrobacter* (0.8%) , *Proteus* (4.9%) , *Serratia* (0.4%) and *Morganella morgagni* (0.4%), and in India **ML and Raja** [17] found that the most prevalent organisms isolated were *K.pneumoniae* (37.4%) followed by *E.Coli* (24.5%) and *Pseudomonas* species (13.6%). In contrast, **Moosavian and Emam** [18] reported that the percentage of *E. coli* was (74.7%) and *K. pneumoniae* was (25.3%).

Such disparity in outcomes can be explained by variance in sample form and number of cases, differences in patient overall health, or discrepancy between countries. The important differing aspects is compliance with measures to control infections.

The prevalence rate of colistin resistance by commercial BMD ComASPTM colistin in our study was (14%), while (86%) of Gram –ve isolates were sensitive to colistin. Similarly, in Egypt, **Emara et al.** [16] disclosed that only 10 (16.4%) isolates were resistant to colistin and in Iran **Moosavian and Emam** [18] reported colistin resistant isolates were 13.6% (64 out of 470 isolates) but by disk diffusion method. On the contrary, **Kandee** [19] reported that colistin resistance against *A. baumannii* (2.8%) and for *P. aeruginosa* (7.9%) by agar dilution method in Egypt. In Hungary [20] the rate of colistin resistance was 0.6%, 1.3% and 2.6% in *Enterobacteriaceae*, *Pseudomonas spp.* and *Acinetobacter spp.*, respectively.

The most resistant isolate in this study was *P.aeruginosa* (20%), followed by *K.pneumoniae* (16.6%), while (5.5%) of *E.coli* was resistance, and all isolates of *Citrobacter* were sensitive (7%).In contrast, another study in Egypt by **Emara et al.** [16] reported that the most common isolated organisms were *K. pneumoniae* (80%), followed by *E. coli* (10%) and *P. aeruginosa* (10%) but in Iran ,previous study[18] reported that *E.coli* colistin resistant strains were 59.4% and *K. pneumoniae* colistin resistant strains were 40.6%.

In this study, Prevalence of *mcr-1* gene resistant isolates was (7.1%) and referred to *K.pneumoniae* isolated from urine of 70 years male patient admitted to ICU for stroke. This finding go in accordance with the results of a study carried out in Egypt by **Zaki et al.** [21] who found that *mcr-1* gene

was detected in 2 isolates (4%) (One *E.coli* strain and in one *K.pneumoniae* strains) and study carried out in the Arabian Peninsula by **Sonnevend et al.** [22] found 4 (5.3%) *E coli* strains carrying the *mcr-1* gene, 2 from Bahrain, one from Saudi Arabia and one from the UAE were detected in this collection, respectively. Two *E. coli* were isolated from blood in 2012 and in 2013, a urine and a wound isolate were recovered in 2015. On the contrary, in Iran, **Moosavian and Emam** [18] found that 1.7% (n=8 out of 470) of *E. coli* and *K. pneumoniae* strains carried *mcr-1* gene. And studies carried out in China [23] found that 16 *E coli* isolates (1%) of 1322 samples from inpatients with infection have *mcr-1* gene, in Hungary **Juhász et al.** [20] reported only one strain, *E. coli* isolated from the blood sample of a hemato-oncology patient in 2011 was positive for *mcr-1*. Meanwhile [18], *mcr-1* gene was not detected in any of the tested colistin-resistant isolates. All this low prevalence may be related to a ban on the use of colistin in agriculture and good practice of Colistin intake.

At the other hand, the prevalence of [24] *mcr-1* genes in Assiut and Minia University Hospital was (20.8%) and (23.1%). This discrepancy can be explained by the fact that all isolates were multidrug resistant *E. coli* collected from urine samples. This coincides with our study, where urine samples were the most common source of resistant isolates.

The distribution of colistin resistant strains in different samples in this study as following: Urine samples: (9%) resistant, including 5 isolates *K.pneumoniae*, 3 isolates *P.aerogenosa* and one isolate for *E.coli*, for blood samples: (2%) resistant, they include 2 isolates *K.pneumoniae*, for sputum samples: (1%) resistant, which is *K.pneumoniae*, for wound exudate samples: (1%) resistant, which is *K.pneumoniae*, for tracheal aspirate:(1%) resistant, which is *K.pneumoniae.*

In contrary, resistant isolates in urine were (37.5%), blood resistant isolates were (25%), in sputum samples (20.8%) were resistant and in wound isolates (16.7%) were resistant [25].

In the present study, history of colistin intake was positive in 1 case (10%) for 55 years male patient admitted to ICU for pneumonia, similar result was reported in Egypt [18] where the history of colistin intake was (20%). In Brazil, [26] 252 colistin-resistant Gram-negative bacteria have emerged independently (without colistin therapy) from this city. On the other hand, in Pennsylvania [27] (95%) of cases had received colistin before colistin-resistant isolates were identified.

Conclusion

Colistin resistance Gram–ve isolates is increasing even without history of colistin intake.in this study, we used Commercial BMD ComASPTM colistin and it was easy to perform and simple method for detection colistin susceptibility.

Recommendations

Strict application of infection control and antibiotic policies to control spread of antibiotic resistance

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Authorship

Each author listed in the manuscript had approved the submission of this version of the manuscript and takes full responsibility for it.

Ethical approval

The study was approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University, Egypt.

References

1-**Przybysz SM, Correa-Martinez C, Kock R, Becker K, Schaumburg F.** SuperPolymyxin Medium for the Screening of Colistin-Resistant Gram-Negative Bacteria in Stool Samples. Front Microbiol 2018; 9: 2809.

2-**Gregoire N, Aranzana-Climent V, Magreault S, Marchand S, Couet W.** Clinical Pharmacokinetics and Pharmacodynamics of Colistin. Clin Pharmacokinet 2017; 56(12): 1441-1460.

3- **World Health Organization**. Critically important antimicrobials for human medicine: ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use, 2017. Licence: CC BY-NC-SA 3.0 IGO.

4-**Mulvey MR, Mataseje LF, Robertson J, Nash JH, Boerlin P, Toye B, et al.** Dissemination of the mcr-1 colistin resistance

gene. The Lancet Infectious Diseases 2016; 16(3):289-90.

5-**Olaitan AO, Morand S, Rolain JM.** Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Frontiers in microbiology 2014; 5: 643.

6-**Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al.** Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. Eurosurveillance 2016;21(27):30280.

7-**Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, et al.** European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. Clinical Microbiology and Infection 2006; 12(6):501-3.

8-**Galani I, Adamou P, Karaiskos I, Giamarellou H, Souli M.** Evaluation of ComASP Colistin (formerly SensiTest Colistin), a commercial broth microdilution-based method to evaluate the colistin minimum inhibitory concentration for carbapenem-resistant *Klebsiella pneumoniae* isolates. J Glob Antimicrob Resist 2018;15:123-126.

9-**Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al.** Heteroresistance to colistin in multidrug-resistant Acinetobacter baumannii. Antimicrobial agents and chemotherapy 2006; 50(9):2946-50.

10-**Handbook QD.** Qiagen DNeasy DNA extraction protocol for bacterial cultures. Adapted from QIAgen DNeasy handbook, July 2006.

11- **Cavaco LM, Mordhorst H, Hendriksen R.** PCR for plasmid-mediated Colistin resistance genes: mcr-1 and mcr-2 (Multiplex). Denmark: Protocol optimized at National Food Institute 2016 Oct 30.

12-**Osei Sekyere J.** Mcr colistin resistance gene: a systematic review of current diagnostics and detection methods. Microbiologyopen 2019; 8(4): e00682.

13-**Quinones B, Swimley MS, Narm KE, Patel RN, Cooley MB, Mandrell RE**. O-antigen and virulence profiling of shiga toxin-producing *Escherichia coli* by a rapid and cost-effective DNA microarray colorimetric method. Front Cell Infect Microbiol 2012;2:61.

14-**Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, et al**. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. Journal of clinical microbiology 2007; 45(10):3352-9.

15-**Sınırtaş M, Akalın H, Gedikoğlu S.** Investigation of colistin sensitivity via three different methods in Acinetobacter baumannii isolates with multiple antibiotic resistance. International Journal of Infectious Diseases 2009;13(5):e217-e220.

16-**Emara MM, Abd-Elmonsef MM, Elnasr LM, Elfeky AA**. Study of mcr-1 Gene-Mediated Colistin-Resistance in Gram-Negative Isolates in Egypt. Egypt J Med Microbiol 2019;28(3):9-16.

17-**ML KY, Raja A.** Bacteriological profile and antibiogram of the gram negative clinical isolates from a tertiary care centre. Blood 2014; 20:3-50.

18-**Moosavian M, Emam N.** The first report of emerging mobilized colistin-resistance (mcr) genes and ERIC-PCR typing in *Escherichia coli* and *Klebsiella pneumoniae* clinical

isolates in southwest Iran. Infection and drug resistance 2019;12:1001.

19-**Kandee A.** Detection of colistin susceptibility in multi- drug resistant pseudomonas aeruginosa and acinetobacter baumannii by four different methods, Egypt. J. Med. Microbiol 2016; 25 (2): 17-23

20-**Juhász E, Iván M, Pintér E, Pongrácz J, Kristóf K**. Colistin resistance among blood culture isolates at a tertiary care centre in Hungary. Journal of global antimicrobial resistance 2017;11:167-70.

21-**Zaki ME, ElKheir NA, Mofreh M**. Molecular study of colistin resistant clinical isolates of Enterobacteriaceae species. J Clin Mol Med 2018;1(1):1-4.

22-**Sonnevend Á, Ghazawi A, Alqahtani M, Shibl A, Jamal W, Hashmey, R, et al** . Plasmid-mediated colistin resistance in Escherichia coli from the Arabian Peninsula. International Journal of Infectious Diseases 2016;50:85-90.

23-**Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al.** Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet infectious diseases 2016;16(2):161-8.

24-**El-Mokhtar MA, Mandour SA, Shahat AA.** Colistin resistance among multidrug-resistant E. coli isolated from Upper Egypt. Egypt. JMed Microbiol 2019;28:11-7.

25-**Rabie RA, Abdallah AL.** Plasmid mediated colistin resistant genes mcr-1 and mcr-2 among Escherichia coli and Klebsiella Pneumoniae isolates at Zagazig University Hospitals, Egypt. Internal medicine 2020 ;14:0-05.

26-**Rossi F, Girardello R, Cury AP, Di Gioia TS, Almeida JN Jr, Duarte AJ.** Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years. Braz J Infect Dis 2017;21(1):98-101.

27-**Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al** . Colistin-resistant Acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis 2015 ;60(9):1295-303.

Abo El Naga E, Mahmoud Y, Ali AK, Ibrahim W. Detection of colistin resistant Gram-negative bacilli in intensive care unit patients admitted to Ain Shams University Hospitals. Microbes Infect Dis 2021; 2(1): 92-99.