2020-06-08

Prevalence of Genes Involved in Colistin Resistance in *Acinetobacter baumannii*: First Report from Iraq

Al-Kadmy, IMS

<http://hdl.handle.net/10026.1/15265>

10.1089/mdr.2019.0243

Microbial Drug Resistance

Mary Ann Liebert Inc

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Prevalence of genes involved in Colistin Resistance in <i>Acinetobacter baumannii</i>:	1
First report from Iraq	2
Running title: Colistin Resistance in <i>Acinetobacter baumannii</i>	3
	4
Israa M.S. Al-Kadmy ^{1&2} , Susan A. Ibrahim ² , Nadal Al-Saryi ² , Sarah Naji Aziz ² , Alexander	5
Besinis ¹ and Helal F. Hetta ^{3, 4} .	6
¹ Faculty of Science and Engineering, School of Engineering, University of Plymouth, Plymouth, PL4 8AA, UK	7
² Branch of Biotechnology, Department of Biology, College of Science, Mustansiriyah University, POX 10248,	8
Baghdad-Iraq	9
³ Department of Medical Microbiology and Immunology, faculty of medicine, Assiut University, Egypt.	10
⁴ Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA	11
	12
*Address correspondence to:	13
Israa M.S. Al-Kadmy	14
Faculty of Science and Engineering, School of Engineering, University of Plymouth,	15
Plymouth, PL4 8AA, UK	16
Email: israa.al-kadmy@plymouth.ac.uk	17
ORCID ID: 0000-0002-5109-7338	18
	19
	20
Author contribution:	21
All authors contributed equally to the Literature review, Study plan, Lab work, fund generation,	22
Manuscript writing and proof	23
	24
	25
	26
	27
	28
	29

Abstract 30

Background and aim: Colistin is increasingly being used as a ‘last-line’ therapy to 31
treat infections caused by multi-drug resistant *Acinetobacter baumannii* (*A. baumannii*) 32
isolates, when essentially no other options are available in these days. The aim of this 33
study was to detect genes associated with Colistin resistance in *A. baumannii*. 34

Methods: 121 isolates of *A. baumannii* were collected from clinical and environmental 35
samples during 2016 to 2018 in Baghdad. Isolates were diagnosed as *A. baumannii* by 36
using morphological tests, Vitek-2 system, 16SrRNA PCR amplification and 37
sequencing. Antibiotic susceptibility test was carried out using disc diffusion method. 38
Phenotypic detection of colistin resistance was performed by CHROMagarTM COL- 39
APSE medium and broth microdilution method for the determination of the minimal 40
inhibitory concentration (MIC). Molecular detection of genes responsible for colistin 41
resistance in *A. baumannii* was performed by PCR. 42

Results: 92 (76%) out of 121 *A. baumannii* isolates were colistin resistant. 26 (21.5%) 43
out of 121 isolates showed positive growth on CHROM agar *Acinetobacter* base for 44
MDR. PCR detected *mcr-1*, *mcr-2* and *mcr-3* genes in 89 (73.5%), 78 (64.5%) and 82 45
(67.8%) in the *A. baumannii* isolates respectively. 78 (64.5%) out of 121 isolates 46
harbored the integron *intI2* gene and 81 (66.9%) contained *intI3* gene. Moreover, 60 47
(49.6 %) out of 121 isolates were positive for the quorum sensing *Iasl* gene 48

Conclusion: The presence of a large percentage of colistin resistant *A. baumannii* 49
strains in Baghdad may be due to the presence of mobile genetic elements and it is 50
urgent to avoid unnecessary clinical use of colistin. 51

Keyword: Colistin, Resistance, *Acinetobacter*, CHROMagarTM COL-APSE, pEtN 52
gene, CMS, mobilized colistin resistance. 53

Introduction 54 55

Colistin is a polymyxin E, which possesses cyclic deca-peptide linked to a fatty acyl 56
chain by α -amide linkage. The only difference in structure between polymyxin E and 57
B is a single amino acid ¹. There are two forms of colistin that are commercially 58
available for use : colistin sulfate and sodium colistin methanesulfonate (CMS) ². In the 59

1970s, CMS was replaced by aminoglycosides because of the significant side effects of these antibiotics such as nephrotoxicity and neurotoxicity ³.

Colistin is an antibiotic that is significantly used against Gram-negative bacteria ⁴⁻⁵. Due to increased and sometimes-inappropriate use, a rise in colistin resistance was reported ⁶. The bacterial cell membrane can be disrupted by polymyxins, which interfere with phospholipids leading to damage to the osmotic barrier ⁷. Polymyxins are polypeptide molecule with positive charge that act as antimicrobial by disrupting the cell membrane and leading to death of the cell. This disruption occurs as a result of polymyxins binding with negatively charge in lipid A moiety of lipopolysaccharides (LPS) ⁸. Resistance to colistin might occur by alteration in binding site in lipid A or efflux pumps ⁹.

The modification of lipopolysaccharide (LPS) is most prevalent method of resistance, which involves an addition of phosphoethanolamine (PEtN) groups. This is thought to alter the physical properties of the outer membrane, which leads to polymyxin resistance ⁷. There are many well-known of PEtN transferases for example EptA from *E. coli*, *H. pylori* and *Vibrio cholerae*. Another example is *PmrC* from *A. baumannii*. These enzymes are chromosomally encoded and catalyze the transfer of PEtN from phosphatidylethanolamine (PE) onto the lipid A moiety of LPS⁸.

Plasmid-mediated colistin (COL) resistance due to the Mobilized colistin resistance *mcr-1 pEtN* gene has recently been identified in Asian countries. Bacteria carrying the *mcr-1* gene have been isolated from many clinical and environmental sources since it was first described. Moreover, these isolates are often pan-drug resistant (PDR was known as non-susceptibility to all agents in all antimicrobial categories) or extensively drug resistant (XDR was known as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories), bacterial isolates remain susceptible to only one or two categories), which significantly limits the therapeutic options for those organisms ¹⁰. Many studies have identified seven *mcr* gene families in addition to *mcr-1*: *mcr-2* ¹¹, *mcr-3* ¹², *mcr-4* ¹³, *mcr-5* ¹⁴, *mcr-6* ¹⁵, *mcr-7* ¹⁶ and *mcr-8* ¹⁷.

Acinetobacter baumannii (*A. baumannii*) is a multidrug resistant (MDR) bacteria that can spread to civilian hospitals by cross infection of injured military patients repatriated from war zones ¹⁸⁻¹⁹. Two different mechanisms of colistin resistance have been characterized in *A. baumannii* ²⁰⁻²¹. The first mechanism includes complete inactivation of the lipid A biosynthetic pathway and loss of outer membrane LPS. This pathway could be inactivated by deletions, point mutations or insertions in any of three

genes (*lpxA*, *lpxC*, and *lpxD*)²². Consequently, the interaction between LPS and colistin can be prevented leading to increase the MICs of colistin. Colistin resistance due to LPS inactivation has been identified in laboratory mutants and recent clinical isolates²³. The second mechanism of colistin resistance is mediated by the PmrAB two-component system²⁴⁻²⁵. It has been shown that mutations in *pmrB* increased cell sensitivity to colistin more than 100 fold²⁴.

Integrans are genetic elements that allow efficient capture and expression of exogenous genes that may lead to dissemination of antibiotic resistance, particularly among Gram-negative bacteria^{13, 16}. Integrans are reported to play a main role in the distribution of colistin resistance²⁴⁻²⁵.

Antibiotic resistant bacteria communicate through quorum sensing (QS). Quorum sensing system is widely spread in bacteria, which possesses an important role in controlling virulence factors. Therefore, it is considered as a “speaking” system in bacterium²⁶. QS is a way bacteria secret chemical signals called auto-inducers to communicates with each other and is often followed by alteration in expression in genes expression^{3, 10, 16}. The persistent modification of bacterial species or strains is a global issue. Both gram-positive and gram-negative bacteria uses inducer called acylated homoserine lactones (AHLs) as a chemical signal or auto chemo-inducer. Although the mechanisms of signaling are different from species to species^{3, 10, 16}. QS-controlled gene expression plays a major role in the antibiotic resistant in pathogens.

The aim of this study was to detect genes, which might be associated with colistin resistance in *A. baumannii*. As a result of the increasing distribution of serious infections with gram negative bacteria, colistin is increasingly being used as therapy to treat infections caused by MDR *A. baumannii* because there is a lack of other options.

Material and Methods

This work was done as a collaboration between Mustansiriyah University, Iraq, Assiut University, Egypt and University of Cincinnati medical center, USA.

In this study, 121 isolates of *A. baumannii* were collected from clinical (30 isolates from urine samples, 47 isolates from blood, 31 isolates from wound swabs, 4 isolates from cerebrospinal fluid and one isolate from endotracheal tube) and environmental (

8 isolates from soil) samples from different hospitals across Baghdad during 2016 - 2018.	126 127
Detection of <i>A. baumannii</i>:	128
The phenotypic characterization was performed using morphological tests, CHROMagar <i>Acinetobacter</i> , and Vitek-2 system (BioMérieux, France).	129 130
Conventional PCR was performed for the genotypic identification of <i>A. baumannii</i> species using specific primers for <i>16SrRNA</i> gene as previously described. ²⁷	131 132
The sequence of the primers and PCR cycling conditions are listed in Table (1).	133 134
Phenotypic detection of colistin resistance in <i>A. baumannii</i>:	135
We used CHROMagar™ COL-APSE (Paris, France) media for detection of colistin resistance and broth microdilution method for the determination of minimal inhibitory concentrations (MIC). Broth microdilution is recommended by CLSI for testing colistin susceptibility. Strains which showed colistin MIC values >2 µg/mL were interpreted as resistant according to CLSI, 2016 breakpoints ⁽²⁴⁾ , and using quality controlled standard strains (<i>Acinetobacter baumannii</i> ATCC BAA-747) obtained from American Type Culture Collection.	136 137 138 139 140 141 142 143
Phenotypic detection of MDR in <i>A. baumannii</i>	144
Then used CHROMagar <i>Acinetobacter</i> Base with supplement (S) and MDR Supplement for detection on MDR isolates (MDR: resistance to C3G, quinolones, carbapenem etc). We prepared CHROMagar™ COL-APSE plates using dehydrated CHROMagar™ base media (X207B) with the CHROMagar™ COL-APSE supplement (X207S) + CHROMagar™ Anti-swarmling supplement (X208). These mediums were not autoclaved in order preserve the CHROMogenic compounds included in the mixture and instead were sterilized by boiling at 100°C while swirling or stirring regularly, prior to the addition of the supplements.	145 146 147 148 149 150 151 152 153
The antibiotic susceptibility profile for <i>A. baumannii</i> isolates was determined using Kirby-Bauer disc diffusion test and interpreted as recommended by Clinical Laboratory Standards Institute ⁽²⁴⁾ . Susceptibility testing was performed by inoculating Mueller-Hinton agar plates (Thermo Fisher Scientific and Waltham, MA, USA) used the suspension equivalent in turbidity to 0.5 McFarland. Then, we incubated the plates overnight at 37°C before recording the results.	154 155 156 157 158 159

	160
The following commonly used antibiotics were tested: ampicillin, amoxycillin,	161
aztreonam, cefepime, cefotaxime, cefoperazone, ceftazidime, imipenem, meropenem,	162
clindamycin, colistin, gentamicin, amikacin, tetracycline, chloramphenicol,	163
ciprofloxacin, amoxycillin/clavulanic acid and trimethoprim/sulphamethoxazole.	164
	165
Molecular detection of Colistin resistance genes:	166
The entire genomic DNA extraction was performed for all resistance isolates	167
according to modified Microwave lysis method ²⁵ .	168
Colistin resistance genes <i>mcr-1</i> , <i>mcr-2</i> and <i>mcr-3</i> were detected by PCR for all isolates	169
grown on CHROMagar TM COL-APSE.	170
The primer sequences and the amplicon size of different genes are listed in Table (1).	171
Briefly, the PCR reaction mixture consisted of 12.5 µl of 2X GoTaq®Green Master	172
Mix (KAPA, South Africa), 3 µl template DNA, 2 µl primers for each forward and	173
reverse primers with final concentration (0.6 pmol/ µl), and complete the volume to 25	174
µl with nuclease free water. The amplified PCR product was run in agarose gel	175
electrophoresis and compared with 100 bp DNA ladder (KAPA, South Africa) and then	176
visualized under UV trans-illuminator.	177
	178
Detection of integrons on colistin resistant <i>A. baumannii</i>:	179
PCR was used to detect <i>intI2</i> and <i>intI3</i> genes, which represent the class 2 and class 3	180
integrons that are known to be associated with MDR <i>A. baumannii</i> . The primer	181
sequences and the amplicon size of genes are listed in Table (1).	182
	183
Detection of quorum sensing in colistin resistant <i>A. baumannii</i>:	184
The presences of <i>Iasl</i> gene, as a part of the QS system, was investigated by PCR in <i>A.</i>	185
<i>baumannii</i> isolates. The primer sequences and the amplicon size of different genes are	186
listed in Table (1).	187
<u>Results and Discussion</u>	188
-Phenotypic properties	189
121 isolates of <i>A. baumannii</i> were collected from different samples during 2016 to	190
2018 in Baghdad. <i>16SrRNA</i> PCR amplification and sequencing were used to identify	191

A. baumannii isolates. *A. baumannii* is gram negative, can cause many infections due to its multi-drug resistance^(18, 20, 25).

26 (21.5%) out of 121 isolates showed positive growth with red colonies on CHROM agar *Acinetobacter* base for MDR suggesting the isolates were resistant to C3G, quinolones and carbapenem (CHROMagar™ *Acinetobacter*, 2018). The source of these 26 MDR isolates was environmental sample (n=1), from urinary tract infections (n=2), blood (n=12), wound swabs (n=6), cerebrospinal fluid (n=4) and endotracheal tube (n=1)

- Resistance of *Acinetobacter*:

The ability of the isolates to resist colistin was investigated by using CHROMagar™ COL-APSE (Paris, France) medium and confirmed by broth microdilution for determination of MIC. Isolates were detected by cream colonies on this medium (CHROMagar™ COL-APSE, 2018). The results showed that 92(76.03%) isolates (including one environmental isolate) showed positive growth on the CHROMagar™ COL-APSE medium. In addition, the MIC for these isolate was measured using broth microdilution for determination of MIC. The isolates showed MIC value ranged from 4 to 16 g/ml.

Antibiotic susceptibility pattern of *A. baumannii* isolated from clinical and environmental samples for different classes of antibiotics showed in Table (2). The result showed that the isolates were 100% resistant to β -lactum and Cefotaxime antibiotics, while they were less resistant (30%) to Tetracycline. PCR were performed to investigate the *mcr* related genes and their role in the resistance of *A. baumannii*

The *mcr*-1 gene was detected in 89 (73.6%) isolates; the *mcr*-2 gene detected in 78 (64.5%) and the *mcr*-3 gene was detected in 82 (67.7%) in the *A. baumannii* (Table 3).

CHROMagar COL-APSE medium was able to support the growth of colistin resistant Gram-negative bacteria because it is a sensitive and specific media for the growth of colistin resistant bacterial pathogens with a lower limit of detection of 10¹ CFU²⁶. Resistance to antibiotic is a global issue. The limitation of effective treatment carbapenem treatment is leading to reduce treatment options for multidrug resistant bacteria²⁸⁻³⁴. The (mobilized colistin resistance) *mcr*-1 gene has been reported in *Escherichia coli* and *Klebsella pneumoniae* from China, which encodes phosphoethanolamine transferase¹⁰. It has the ability to be transferred between different

bacterial strains. This leads to antibiotic resistance because of alterations in the bacterial cell membrane lipid A ^{10, 23, 35}. Gene *mcr-2* has been reported in 76% of bacteria with *mcr-1* gene from Belgium ¹¹. In addition, *mcr-3* gene has been recently reported in *E. coli* of pig origin, which showed a 45.0% and 47.0% identity in nucleotide sequence to *mcr-1* and *mcr-2*, respectively ¹².

Results highlighted the rapid spreading of *mcr-1*, *mcr-2* and *mcr-3* genes globally. Recently, *mcr-1* has been isolated from *Enterobacteriaceae* (animals), products of animals, humans and environments in more than thirty different countries from five continents ³⁶. This rapid increase in the reporting of resistance mechanism in a short time is alarming.

- Role of integrons on MDR distribution

PCR was used to detect the class 2 and class 3 integrons in isolates of *Acinetobacter baumannii*, which associated with multi-drugs resistances. The results showed out of 121 isolates, 78 (64.5%) harbored *intI2* gene and 81 (66.9%) contained *intI3* gene (Table 3). These results confirmed the role of integrons in MDS distribution in *A. baumannii*, which is similar to the role of integrons in the distribution of MDR in *Salmonella* spp. in Rajaei, et al. ³⁷.

Integron genes play a key role in the horizontal transfer of antibiotic multi-resistance accompanying with genetic element. Resistance genes are either on the host plasmid or bacterial chromosome ^{13, 38-39}. The *intI* gene encodes for an integrase, which belongs to the tyrosine-recombinase family ⁴⁰. The activity of integrase includes recombination of separate DNA molecules as gene cassettes. Integrons are divided into two subsets: the mobile integrons that are responsible for spreading the anti-drug resistance genes and super integrons. According to sequencing, there are five classes of integrons ⁴¹⁻⁴².

Integrons have the ability to capture the antibiotic resistance cassettes genes that lead to distribution of MDR and decrease the infection treatment options ⁴³. Resistance cassettes have been reported in both gram negative bacteria and gram positive bacteria ⁴⁴⁻⁴⁶.

- Quorum sensing detection in *Acinetobacter*

This system was first discovered in 1994 by Dr. Peter Greenberg in *Vibrio fischeri*. The QS system is a chemical mediated cell-to-cell communication that can regulate gene expression and the activity of the group in communities ⁴⁷. There are

many activities depending on the QS system such as production, secretion, and
detection of small signaling molecules named Autoinducers (AIs) ⁴⁸.

The presences of *Iasl* gene in *Acinetobacter* isolates were investigated by PCR.
The results indicated that 60 (49.6 %) out of 121 isolates were positive for *Iasl* gene
(Table 3). Bacteria use QS to regulate genes expression, facilitate pathogenic invasion
and spread virulence factors ⁴⁹. The QS controls local bacteria population and cell
density, which make the bacteria behave as a collaborative community such as
multicellular organism⁵⁰. Bacterial QS regulates bioluminescence, competence,
antibiotic production and secretion of virulence factors ⁵¹. This affects the formation of
biofilm ⁵²⁻⁵³, drug sensitivity ⁵⁴ and bacterial virulence ⁵⁵.
The *lasI* gene is as a part of QS system, the product of this gene being N-(3-oxo-
dodecanoyl) -L- homoserine lactone (3-oxo-C12-AHL), which interacts with *LasR* and
activates target promoters ⁵⁶. Only the multimeric form of this protein is active and can
bind to target DNA and regulate the transcription of multiple genes at high cell densities
⁵⁷.

Conclusion

MDR *A. baumannii* is considered to be a serious threat. The current study showed that
there is a high prevalence of colistin resistance in *A. baumannii* strains isolated from
Iraq. This is associated with the ability of this pathogen to acquire new genetic material
leading to increase the resistance. In addition, integrons showed a major role in
extending the bacterial ability to grow in different challenge conditions because it
allows *A. baumannii* to capture additional genetic material from other species. This
leads to the distribution and increased the resistance of *A. baumannii*. This resistance
can transform in over the world by natural transformation. The presence of a large
percentage of colistin resistant *A. baumannii* strains in Baghdad makes it urgent to avoid
unnecessary clinical use of colistin.

Acknowledgement

The authors would like to thank Mustansiriyah University
(<https://uomustansiriyah.edu.iq/>) / Baghdad, Iraq for its support to complete this work. In

addition, we would like to thank Mr. Gwladys Chansigaud and R&D Department CHROMagar	290
(www.CHROMagar.com) in Paris to support us all CHROM agar media.	291
	292
Disclosure Statement: No competing financial interests exist.	293
	294

References:	295
1. Storm, D. R.; Rosenthal, K. S.; Swanson, P. E., Polymyxin and related peptide antibiotics. <i>Annual review of biochemistry</i> 1977 , <i>46</i> (1), 723-763.	296 297
2. Bergen, P. J.; Li, J.; Rayner, C. R.; Nation, R. L., Colistin methanesulfonate is an inactive prodrug of colistin against <i>Pseudomonas aeruginosa</i> . <i>Antimicrobial agents and chemotherapy</i> 2006 , <i>50</i> (6), 1953-1958.	298 299 300
3. Li, J.; Nation, R. L.; Turnidge, J. D.; Milne, R. W.; Coulthard, K.; Rayner, C. R.; Paterson, D. L., Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. <i>The Lancet infectious diseases</i> 2006 , <i>6</i> (9), 589-601.	301 302 303
4. Nation, R. L.; Li, J., Colistin in the 21st century. <i>Current opinion in infectious diseases</i> 2009 , <i>22</i> (6), 535.	304 305
5. El-Mokhtar, M. A.; Mandour, S. A.; Shahat, A. A., Colistin resistance among multidrug-resistant <i>E. coli</i> isolated from Upper Egypt. <i>Egyptian Journal of Medical Microbiology</i> 2019 , <i>28</i> (2), 11-17.	306 307 308
6. Ko, K. S.; Suh, J. Y.; Kwon, K. T.; Jung, S.-I.; Park, K.-H.; Kang, C. I.; Chung, D. R.; Peck, K. R.; Song, J.-H., High rates of resistance to colistin and polymyxin B in subgroups of <i>Acinetobacter baumannii</i> isolates from Korea. <i>Journal of Antimicrobial Chemotherapy</i> 2007 , <i>60</i> (5), 1163-1167.	309 310 311 312
7. Hawley, J. S.; Murray, C. K.; Jorgensen, J. H., Development of colistin-dependent <i>Acinetobacter baumannii</i> - <i>Acinetobacter calcoaceticus</i> complex. <i>Antimicrobial agents and chemotherapy</i> 2007 , <i>51</i> (12), 4529-4530.	313 314 315
8. Yahav, D.; Farbman, L.; Leibovici, L.; Paul, M., Colistin: new lessons on an old antibiotic. <i>Clinical microbiology and infection</i> 2012 , <i>18</i> (1), 18-29.	316 317
9. Tzeng, Y.-L.; Ambrose, K. D.; Zughaier, S.; Zhou, X.; Miller, Y. K.; Shafer, W. M.; Stephens, D. S., Cationic antimicrobial peptide resistance in <i>Neisseria meningitidis</i> . <i>Journal of bacteriology</i> 2005 , <i>187</i> (15), 5387-5396.	318 319 320
10. Liu, Y.-Y.; Wang, Y.; Walsh, T. R.; Yi, L.-X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X., Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. <i>The Lancet infectious diseases</i> 2016 , <i>16</i> (2), 161-168.	321 322 323 324
11. Xavier, B. B.; Lammens, C.; Ruhel, R.; Kumar-Singh, S.; Butaye, P.; Goossens, H.; Malhotra-Kumar, S., Identification of a novel plasmid-mediated colistin-resistance gene, <i>mcr-2</i> , in <i>Escherichia coli</i> , Belgium, June 2016. <i>EuroSurveillance Monthly</i> 2016 , <i>21</i> (27), 30280.	325 326 327
12. Yin, W.; Li, H.; Shen, Y.; Liu, Z.; Wang, S.; Shen, Z.; Zhang, R.; Walsh, T. R.; Shen, J.; Wang, Y., Novel plasmid-mediated colistin resistance gene <i>mcr-3</i> in <i>Escherichia coli</i> . <i>MBio</i> 2017 , <i>8</i> (3), e00543-17.	328 329 330
13. Carattoli, A., Importance of integrons in the diffusion of resistance. <i>Veterinary research</i> 2001 , <i>32</i> (3-4), 243-259.	331 332
14. Borowiak, M.; Fischer, J.; Hammerl, J. A.; Hendriksen, R. S.; Szabo, I.; Malorny, B., Identification of a novel transposon-associated phosphoethanolamine transferase	333 334

gene, mcr-5, conferring colistin resistance in d-tartrate fermenting <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Paratyphi B. <i>Journal of Antimicrobial Chemotherapy</i> 2017 , 72 (12), 3317-3324.	335 336 337
15. Partridge, S. R.; Di Pilato, V.; Doi, Y.; Feldgarden, M.; Haft, D. H.; Klimke, W.; Kumar-Singh, S.; Liu, J.-H.; Malhotra-Kumar, S.; Prasad, A., Proposal for assignment of allele numbers for mobile colistin resistance (mcr) genes. <i>Journal of Antimicrobial Chemotherapy</i> 2018 , 73 (10), 2625-2630.	338 339 340 341
16. Wang, X.; Wang, Y.; Zhou, Y.; Li, J.; Yin, W.; Wang, S.; Zhang, S.; Shen, J.; Shen, Z.; Wang, Y., Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing <i>Klebsiella pneumoniae</i> . <i>Emerging microbes & infections</i> 2018 , 7 (1), 122.	342 343 344
17. Litrup, E.; Kiil, K.; Hammerum, A. M.; Roer, L.; Nielsen, E. M.; Torpdahl, M., Plasmid-borne colistin resistance gene mcr-3 in <i>Salmonella</i> isolates from human infections, Denmark, 2009–17. <i>Eurosurveillance</i> 2017 , 22 (31).	345 346 347
18. Peleg, A. Y.; Seifert, H.; Paterson, D. L., <i>Acinetobacter baumannii</i> : emergence of a successful pathogen. <i>Clinical microbiology reviews</i> 2008 , 21 (3), 538-582.	348 349
19. Kareem, S. M.; Al-Kadmy, I. M.; Al-Kaabi, M. H.; Aziz, S.N.; Ahmad, M., <i>Acinetobacter baumannii</i> virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB). <i>Microbial pathogenesis</i> 2017 , 110, 568-572.	350 351 352
20. Cai, Y.; Chai, D.; Wang, R.; Liang, B.; Bai, N., Colistin resistance of <i>Acinetobacter baumannii</i> : clinical reports, mechanisms and antimicrobial strategies. <i>Journal of antimicrobial chemotherapy</i> 2012 , 67 (7), 1607-1615.	353 354 355
21. AL-Kadmy, I. M.; Ali, A. N. M.; Salman, I. M. A.; Khazaal, S. S., Molecular characterization of <i>Acinetobacter baumannii</i> isolated from Iraqi hospital environment. <i>New microbes and new infections</i> 2018 , 21, 51-57.	356 357 358
22. Henry, R.; Vithanage, N.; Harrison, P.; Seemann, T.; Coutts, S.; Moffatt, J. H.; Nation, R. L.; Li, J.; Harper, M.; Adler, B., Colistin-resistant, lipopolysaccharide-deficient <i>Acinetobacter baumannii</i> responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids, and poly- β -1, 6-N-acetylglucosamine. <i>Antimicrobial agents and chemotherapy</i> 2012 , 56 (1), 59-69.	359 360 361 362 363
23. Moffatt, J. H.; Harper, M.; Harrison, P.; Hale, J. D.; Vinogradov, E.; Seemann, T.; Henry, R.; Crane, B.; Michael, F. S.; Cox, A. D., Colistin resistance in <i>Acinetobacter baumannii</i> is mediated by complete loss of lipopolysaccharide production. <i>Antimicrobial agents and chemotherapy</i> 2010 , 54 (12), 4971-4977.	364 365 366 367
24. CLSI. (2016). Performance standard for antimicrobial susceptibility testing; Twenty-First informational supplement. M100-S26.vol.36 No.(1).	368 369
25. Adams, M. D.; Nickel, G. C.; Bajaksouzian, S.; Lavender, H.; Murthy, A. R.; Jacobs, M. R.; Bonomo, R. A., Resistance to colistin in <i>Acinetobacter baumannii</i> associated with mutations in the PmrAB two-component system. <i>Antimicrobial agents and chemotherapy</i> 2009 , 53 (9), 3628-3634.	370 371 372 373
26. Yin, W.-F.; Purmal, K.; Chin, S.; Chan, X.-Y.; Koh, C.-L.; Sam, C.-K.; Chan, K.-G., N-acetyl homoserine lactone production by <i>Klebsiella pneumoniae</i> isolated from human tongue surface. <i>Sensors</i> 2012 , 12 (3), 3472-3483.	374 375 376

27. Hasen Esaa, R.; N N Naji, E.; Sami, H., <i>Comparison of three diagnostic methods for Acinetobacter baumannii Isolated from Baghdad Hospitals</i> . 2016.	377 378
28. Balaji, V.; Jeremiah, S.; Baliga, P., Polymyxins: antimicrobial susceptibility concerns and therapeutic options. <i>Indian journal of medical microbiology</i> 2011 , 29 (3), 230.	379 380
29. Farhan, S. M.; Ibrahim, R. A.; Hetta, H. F.; Mahran, K. M.; Abdelbaky, R. M., PREVALENCE OF OXA-23 IN MULTIDRUG RESISTANCE ACINETOBACTER BAUMANNII ISOLATED FROM DIFFERENT INFECTIONS AT MINIA UNIVERSITY HOSPITAL. 2018 .	381 382 383
30. El-Mokhtar, M. A.; Hetta, H. F., Ambulance vehicles as a source of multidrug-resistant infections: a multicenter study in Assiut City, Egypt. <i>Infection and drug resistance</i> 2018 , 11, 587.	384 385 386
31. Ahmed, S.; Ahmed, S.; Mohamed, W.; Feky, M.; Daef, E.; Badary, M.; Hetta, H., Nosocomial vancomycin and methicillin resistant staphylococcal infections in intensive care units in Assiut University Hospitals. <i>Egyptian Journal of Medical Microbiology</i> 2011 , 20 (2).	387 388 389
32. Farhan, S. M.; Ibrahim, R. A.; Mahran, K. M.; Hetta, H. F.; El-Baky, R. M. A., Antimicrobial resistance pattern and molecular genetic distribution of metallo- β -lactamases producing <i>Pseudomonas aeruginosa</i> isolated from hospitals in Minia, Egypt. <i>Infection and Drug Resistance</i> 2019 , 12, 2125.	390 391 392 393
33. El-Baky, R. M. A.; Sandle, T.; John, J.; Abuo-Rahma, G. E.-D. A.; Hetta, H. F., A novel mechanism of action of ketoconazole: inhibition of the NorA efflux pump system and biofilm formation in multidrug-resistant <i>Staphylococcus aureus</i> . <i>Infection and drug resistance</i> 2019 , 12, 1703.	394 395 396 397
34. Al-Saryi, N.; Ibrahim, S. A.; AL-Kadmy, I. M.; Hetta, H. F., Whole genome sequencing of <i>Streptococcus pneumoniae</i> serotype 33C causing fatal sepsis in a hospitalized patient with nephrotic syndrome. <i>Gene Reports</i> 2019 , 100434.	398 399 400
35. Hinchliffe, P.; Yang, Q. E.; Portal, E.; Young, T.; Li, H.; Tooke, C. L.; Carvalho, M. J.; Paterson, N. G.; Brem, J.; Niumsup, P. R., Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1. <i>Scientific reports</i> 2017 , 7, 39392.	401 402 403 404
36. Wang, Y.; Tian, G.-B.; Zhang, R.; Shen, Y.; Tyrrell, J. M.; Huang, X.; Zhou, H.; Lei, L.; Li, H.-Y.; Doi, Y., Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. <i>The Lancet Infectious Diseases</i> 2017 , 17 (4), 390-399.	405 406 407 408
37. Rajaei, B.; Siadat, S. D.; Razavi, M. R.; Aghasadeghi, M. R.; Rad, N. S.; Badmasti, F.; Jafroodi, S. K.; Rajaei, T.; Moshiri, A.; Javadian, S., Expanding drug resistance through integron acquisition in <i>Salmonella</i> spp. isolates obtained in Iran. <i>African Journal of Microbiology Research</i> 2011 , 5 (16), 2249-2253.	409 410 411 412
38. Tamang, M. D.; Oh, J. Y.; Seol, S. Y.; Kang, H. Y.; Lee, J. C.; Lee, Y. C.; Cho, D. T.; Kim, J., Emergence of multidrug-resistant <i>Salmonella enterica</i> serovar Typhi associated with a class 1 integron carrying the <i>dfrA7</i> gene cassette in Nepal. <i>International journal of antimicrobial agents</i> 2007 , 30 (4), 330-335.	413 414 415 416

39. Yang, B.; Zheng, J.; Brown, E. W.; Zhao, S.; Meng, J., Characterisation of antimicrobial resistance-associated integrons and mismatch repair gene mutations in Salmonella serotypes. <i>International journal of antimicrobial agents</i> 2009 , 33 (2), 120-124.	417 418 419
40. Hall, R. M.; Collis, C. M., Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. <i>Molecular microbiology</i> 1995 , 15 (4), 593-600.	420 421
41. Hall, R. M. In <i>Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria</i> , Ciba Foundation Symposium 207-Antibiotic Resistance: Origins, Evolution, Selection and Spread: Antibiotic Resistance: Origins, Evolution, Selection and Spread: Ciba Foundation Symposium 207, Wiley Online Library: 2007; pp 192-205.	422 423 424 425
42. Mazel, D., Integrons: agents of bacterial evolution. <i>Nature Reviews Microbiology</i> 2006 , 4 (8), 608.	426 427
43. Stokes, H. t.; Hall, R. M., A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. <i>Molecular microbiology</i> 1989 , 3 (12), 1669-1683.	428 429 430
44. Nešvera, J.; Hochmannová, J.; Pátek, M., An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium <i>Corynebacterium glutamicum</i> . <i>FEMS microbiology letters</i> 1998 , 169 (2), 391-395.	431 432 433
45. Nandi, S.; Maurer, J. J.; Hofacre, C.; Summers, A. O., Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. <i>Proceedings of the National Academy of Sciences</i> 2004 , 101 (18), 7118-7122.	434 435 436
46. Tauch, A.; Götter, S.; Pühler, A.; Kalinowski, J.; Thierbach, G., The 27.8-kb R-plasmid pTET3 from <i>Corynebacterium glutamicum</i> encodes the aminoglycoside adenylyltransferase gene cassette aadA9 and the regulated tetracycline efflux system Tet 33 flanked by active copies of the widespread insertion sequence IS6100. <i>Plasmid</i> 2002 , 48 (2), 117-129.	437 438 439 440
47. Yang, L.; Tolker-Nielsen, T.; Molin, S. <i>Pseudomonas aeruginosa</i> quorum-sensing-A factor in biofilm development, and an antipathogenic drug target. Technical University of Denmark (DTU), 2009.	441 442 443
48. Kalia, V. C.; Purohit, H. J., Quenching the quorum sensing system: potential antibacterial drug targets. <i>Critical reviews in microbiology</i> 2011 , 37 (2), 121-140.	444 445
49. Bose, S.; Ghosh, A. K., Understanding of quorum-sensing: a possible solution for drug resistance in bacteria. <i>Int J Curr Microbiol App Sci</i> 2016 , 5 (2), 540-546.	446 447
50. LaSarre, B.; Federle, M. J., Exploiting quorum sensing to confuse bacterial pathogens. <i>Microbiology and molecular biology reviews</i> 2013 , 77 (1), 73-111.	448 449
51. Labbate, M.; Queck, S. Y.; Koh, K. S.; Rice, S. A.; Givskov, M.; Kjelleberg, S., Quorum sensing-controlled biofilm development in <i>Serratia liquefaciens</i> MG1. <i>Journal of bacteriology</i> 2004 , 186 (3), 692-698.	450 451 452
52. Cady, N. C.; McKean, K. A.; Behnke, J.; Kubec, R.; Mosier, A. P.; Kasper, S. H.; Burz, D. S.; Musah, R. A., Inhibition of biofilm formation, quorum sensing and infection in <i>Pseudomonas aeruginosa</i> by natural products-inspired organosulfur compounds. <i>PLoS One</i> 2012 , 7 (6), e38492.	453 454 455 456

53. Jakobsen, T. H.; Bragason, S. K.; Phipps, R. K.; Christensen, L. D.; van Gennip, M.; Alhede, M.; Skindersoe, M.; Larsen, T. O.; Høiby, N.; Bjarnsholt, T., Food as a source for quorum sensing inhibitors: iberin from horseradish revealed as a quorum sensing inhibitor of <i>Pseudomonas aeruginosa</i> . <i>Appl. Environ. Microbiol.</i> 2012 , 78 (7), 2410-2421.	457 458 459 460
54. Brackman, G.; Cos, P.; Maes, L.; Nelis, H. J.; Coenye, T., Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. <i>Antimicrobial agents and chemotherapy</i> 2011 , 55 (6), 2655-2661.	461 462 463
55. Koh, K. H.; Tham, F.-Y., Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. <i>Journal of Microbiology, Immunology and Infection</i> 2011 , 44 (2), 144-148.	464 465 466
56. Smith, R. S.; Iglewski, B. H., <i>Pseudomonas aeruginosa</i> quorum sensing as a potential antimicrobial target. <i>Journal of Clinical Investigation</i> 2003 , 112 (10), 1460.	467 468
57. Siehnel, R.; Traxler, B.; An, D. D.; Parsek, M. R.; Schaefer, A. L.; Singh, P. K., A unique regulator controls the activation threshold of quorum-regulated genes in <i>Pseudomonas aeruginosa</i> . <i>Proceedings of the National Academy of Sciences</i> 2010 , 107 (17), 7916-7921.	469 470 471 472 473

Table 1: The PCR primers used and the amplicon size of different genes involved in this study

27

474

475

Gene	Sequence	TM (°C)	Products size (bp)
<i>16srRNA</i>	5'-TTTAAGCGAGGAGGAGG-3' 5'-ATTCTACCATCCTCTCCC-3'	58	240
<i>mcr-1</i>	5'-CACTTATGGCACGGTCTATGA-3' 5'-CCCAAACCAATGATACGCAT-3'	59	956
<i>mcr-2</i>	5'-TGGTACAGCCCCCTTATT-3' 5'-GCTTGAGATTGGGTTATGA-3'	57	1,617
<i>mcr-3</i>	5-TTGGCACTGTATTTTGCATTT-3 5-TTAACGAAATTGGCTGGAACA-3	50	542
<i>intI2</i>	5'-CAC GGA TAT GCGACA AAA AGG-3' 5'-TGTA GCA AAC GAGTGA CGA AAT G-3'	60	788
<i>intI3</i>	5'-AGT GGG TGG CGAATG AGT G-3' 5'-TGT TCT TGT ATCGGC AGG TG-3'	60	600
<i>lasI</i>	5'- TCGACGAGATGGAAATCGATG-3' 5'- GCTCGATGCCGATCTTCAG-3'	59	402

476

477

Table (2): Antibiotic resistance pattern of *A. baumannii* isolates.

478

Class of antibiotics	Antibiotic tested	Resistant strains for 121 of <i>A. baumannii</i> isolates
penicillins	Ampicillin	% 100
	Amoxycillin	% 100
Monobactam	Aztereonam	%90
3rd generation cephalosporin	Cefotaxime	% 100
	Cefoperazone	%85.9
	Ceftazidine	%93
4 th generation cephalosporin	Cefepime	%96
Carbapenemes	Imipenem	%44.7
	Meropenem	%36
Polypeptide	Clindamycin	%91.6
	Colistin	%76
Aminoglycosides	Gentamicin	%79
	Amikacin	%72
Tetracyclines	Tetracycline	%30
Amphenicols	Chloramphenicol	%72
quinolones	Ciprofloxacin	%79
Combination	Amoxycillin/clavulanicacid	%96
	Trimethoprim/sulphametoxazole	%91.6

479

480

481

482

483

Table (3): Frequency of genes involved in colistin resistance in *Acinetobacter baumannii* isolates. 484

PCR test	Positive result (%)	Negative result
<i>mcr-1</i>	89 (73.6%)	32 (26.4%)
<i>mcr-2</i>	78 (64.5%)	43 (35.5%)
<i>mcr-3</i>	82 (67.7%)	39 (32.2%)
<i>mcr-1</i> + <i>mcr-2</i>	74(61.1%)	47(38.8%)
<i>mcr-1</i> + <i>mcr-3</i>	77(63.6%)	44(36.3%)
<i>mcr-2</i> + <i>mcr-3</i>	69(57.02%)	52(42.9%)
<i>mcr-1</i> + <i>mcr-2</i> + <i>mcr-3</i>	66(54.5%)	55(45.4%)
<i>intI2</i>	78 (64.5%)	43 (35.5%)
<i>intI3</i>	81 (66.9%)	40 (33.1%)
<i>lasI</i>	60 (49.6%)	61 (50.4%)

485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503