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

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Background and Aim: Colistin is increasingly being used as a ''last-line'' therapy to treat infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* isolates, when essentially no other options are available in these days. The aim of this study was to detect genes associated with colistin resistance in *A. baumannii*.
Methods: One hundred twenty-one isolates of *A. baumannii* were collected from clinical and environmental samples during 2016 to 2018 in Baghdad. Isolates were diagnosed as *A. baumannii* by using morphological tests, Vitek-2 system, 16SrRNA PCR amplification, and sequencing. Antibiotic susceptibility test was carried out using disk diffusion method. Phenotypic detection of colistin resistance was performed by CHROMagar™ COL-APSE medium and broth microdilution method for the determination of the minimal inhibitory concentration. Molecular detection of genes responsible for colistin resistance in *A. baumannii* was performed by PCR.
Results: Ninety-two (76%) of the 121 *A. baumannii* isolates were colistin resistant. Twenty-six (21.5%) of the 121 isolates showed positive growth on CHROMagar *Acinetobacter* base for MDR. PCR detected *mcr-1*, *mcr-2*, and *mcr-3* genes in 89 (73.5%), 78 (64.5%), and 82 (67.8%) *A. baumannii* isolates, respectively. Seventy-eight (64.5%) of the 121 isolates harbored the integron *intI2* gene and 81 (66.9%) contained *intI3* gene. Moreover, 60 (49.6%) of the 121 isolates were positive for the quorum sensing *lasI* gene.
Conclusion: The presence of a large percentage of colistin-resistant *A. baumannii* strains in Baghdad may be due to the presence of mobile genetic elements, and it is urgent to avoid unnecessary clinical use of colistin.

Keywords: colistin, resistance, *Acinetobacter*, CHROMagar COL-APSE, pEtN gene, CMS, mobilized colistin resistance

Introduction

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

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for example, EptA from *Escherichia coli*, *Helicobacter pylori*, and *Vibrio cholerae*. Another example is *PmrC* from *Acinetobacter baumannii*. These enzymes are chromosomally encoded and catalyze the transfer of PEtN from phosphatidylethanolamine (PE) onto the lipid A moiety of LPS.[8]

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, known as nonsusceptibility to all agents in all antimicrobial categories) or extensively drug resistant (XDR, known as nonsusceptibility 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]

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 minimal inhibitory concentrations (MICs) of colistin. Colistin resistance due to LPS inactivation has been identified in laboratory mutants and recent clinical isolates.[23] The second mechanism of colistin resistance is mediated by the PmrAB two-component system.[24,25] It has been shown that mutations in *pmrB* increased cell sensitivity to colistin more than 100-fold.[25]

Integrons 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] Integrons are reported to play a main role in the distribution of colistin resistance.[24,25]

Antibiotic resistant bacteria communicate through quorum sensing (QS). QS system is widely spread in bacteria, which possesses an important role in controlling virulence factors. Therefore, it is considered a ''speaking'' system in bacterium.[26] QS is a way bacteria secret chemical signals called autoinducers to communicate with each other and is often followed by alteration in gene expression.[3,10,16] The persistent modification of bacterial species or strains is a global issue. Both gram-positive and gram-negative bacteria use 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 resistance 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.

Materials and Methods

This work was performed 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 1 isolate from endotracheal tube) and environmental (8 isolates from soil) samples from different hospitals across Baghdad during 2016 to 2018.

Detection of A. baumannii

The phenotypic characterization was performed using morphological tests, CHROMagar™ *Acinetobacter*, and Vitek-2 system (BioMérieux, France).

Conventional PCR was performed for the genotypic identification of *A. baumannii* species using specific primers for *16SrRNA* gene, as previously described.[27]

The sequence of the primers and PCR cycling conditions are listed in Table 1.

Phenotypic detection of colistin resistance in A. baumannii

We used CHROMagar COL-APSE (Paris, France) media for the detection of colistin resistance and broth microdilution method for the determination of MICs. Broth microdilution is recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing colistin susceptibility. Strains that showed colistin MIC values >2 μg/mL were interpreted as resistant according to the CLSI, 2016 breakpoints,[24] and using quality-controlled standard strains (*A. baumannii ATCC BAA-747*) obtained from American Type Culture Collection.

Phenotypic detection of MDR in A. baumannii

We used CHROMagar *Acinetobacter* base with supplement (S) and MDR Supplement for the detection of 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-swarming supplement (X208). These mediums were not autoclaved to preserve the CHROMogenic compounds included in the mixture and instead were sterilized by boiling at 100°C while swirling or stirring regularly, before the addition of the supplements.

The antibiotic susceptibility profile for *A. baumannii* isolates was determined using Kirby–Bauer disk diffusion test and interpreted as recommended by the CLSI.[24] Susceptibility testing was performed by inoculating Mueller–Hinton agar plates (Thermo Fisher Scientific, Waltham, MA) with bacterial suspension equivalent in turbidity to 0.5 McFarland. Then, we incubated the plates overnight at 37°C before recording the results.

The following commonly used antibiotics were tested: ampicillin, amoxicillin, aztreonam, cefepime, cefotaxime, cefoperazone, ceftazidime, imipenem, meropenem, clindamycin, colistin, gentamicin, amikacin, tetracycline, chloramphenicol, ciprofloxacin, amoxicillin/clavulanic acid, and trimethoprim/sulfamethoxazole.

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TABLE 1. THE POLYMERASE CHAIN REACTION PRIMERS USED AND THE AMPLICON SIZE OF DIFFERENT GENES INVOLVED IN THIS STUDY[27]

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

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Molecular detection of colistin resistance genes

The entire genomic DNA extraction was performed for all resistance isolates according to the modified microwave lysis method.[25]

Colistin resistance genes *mcr-1*, *mcr-2*, and *mcr-3* were detected by PCR for all isolates grown on CHROMagar COL-APSE.

The primer sequences and the amplicon size of different genes are listed in Table 1.

Briefly, the PCR mixture consisted of 12.5 μL of 2X GoTaq®Green Master Mix (KAPA, South Africa), 3 μL template DNA, 2 μL primers for each forward and reverse primers with final concentration (0.6 pmol/μL), and complete volume to 25 μL with nuclease-free water. The amplified PCR product was run in agarose gel electrophoresis and compared with 100 bp DNA ladder (KAPA, South Africa) and then visualized under UV transilluminator.

Detection of integrons on colistin-resistant A. baumannii

PCR was used to detect *intI2* and *intI3* genes, which represent the class 2 and class 3 integrons that are known to be associated with MDR *A. baumannii.* The primer sequences and the amplicon size of genes are listed in Table 1.

Detection of QS in colistin-resistant A. baumannii

The presences of *lasI* gene, as a part of the QS system, was investigated by PCR in *A. baumannii* isolates. The primer sequences and the amplicon size of different genes are listed in Table 1.

Results and Discussion

Phenotypic properties

One hundred twenty-one isolates of *A. baumannii* were collected from different samples during 2016 to 2018 in Baghdad. *16SrRNA* PCR amplification and sequencing were used to identify *A. baumannii* isolates. *A. baumannii* is a gram negative bacteria and can cause many infections due to its MDR.[18,20,25]

Twenty-six (21.5%) of the 121 isolates showed positive growth with red colonies on CHROMagar *Acinetobacter* base for MDR, suggesting that 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 the 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 1 environmental isolate) showed positive growth on the CHROMagar COL-APSE medium. In addition, the MIC for these isolate was measured using broth microdilution. The isolates showed MIC values ranging from 4 to 16 g/mL.

Antibiotic susceptibility pattern of *A. baumannii* isolated from clinical and environmental samples for different classes of antibiotics is shown in Table 2. The results showed that the isolates were 100% resistant to β-lactam and cefotaxime antibiotics, whereas they were less resistant (30%) to tetracycline. PCR was 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%) *A. baumannii* isolates, the *mcr-2* gene was detected in 78 (64.5%) *A. baumannii* isolates, and the *mcr-3* gene was detected in 82 (67.7%) *A. baumannii* isolates (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 medium for the growth of colistin-resistant bacterial pathogens, with a lower limit of detection of 10^1 colony forming unit (CFU).[26] Resistance to antibiotics is a global issue. The limitation of effective carbapenem treatment is leading to reduce treatment options for MDR bacteria.[28–34] The (mobilized colistin resistance) *mcr-1* gene has been reported in *E. coli* and *Klebsiella pneumoniae* from China, which encodes PEtN transferase.[10]

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Table 2. Antibiotic Resistance Pattern of *Acinetobacter baumannii* Isolates

Class of antibiotics	*Antibiotic tested*	*Resistant strains for 121* A. baumannii *isolates*
Penicillins	Ampicillin	100%
	Amoxicillin	100%
Monobactam	Aztreonam	90%
Third-generation cephalosporins	Cefotaxime	100%
	Cefoperazone	85.90%
	Ceftazidime	93%
Fourth-generation cephalosporin	Cefepime	96%
Carbapenems	Imipenem	44.70%
	Meropenem	36%
Polypeptides	Clindamycin	91.60%
	Colistin	76%
Aminoglycosides	Gentamicin	79%
	Amikacin	72%
Tetracycline	Tetracycline	30%
Amphenicols	Chloramphenicol	72%
Quinolone	Ciprofloxacin	79%
Combination	Amoxicillin/ clavulanic acid	96%
	Trimethoprim/ sulfamethoxazole	91.60%

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] The *mcr-2* gene 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]

The results highlight the rapid spreading of *mcr-1*, *mcr-2*, and *mcr-3* genes globally. Recently, *mcr-1* gene has been isolated from Enterobacteriaceae (animals), products of animals, humans, and environments in more than 30 different countries from 5 continents.[36] This rapid increase in the reporting of resistance mechanism in a short time is alarming.

Table 3. Frequency of Genes Involved in Colistin Resistance in *Acinetobacter baumannii* Isolates

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

Role of integrons on MDR distribution

PCR was used to detect the class 2 and class 3 integrons in the *A. baumannii* isolates, which are associated with MDR. The results showed that of the 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 MDR distribution in *A. baumannii*, which is similar to the role of integrons in the distribution of MDR in *Salmonella* spp. in the study by Rajaei *et al.*[37]

Integron genes play a key role in the horizontal transfer of antibiotic multiresistance companying with genetic element. Resistance genes are either on the host plasmid or on the 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 antidrug 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 the distribution of MDR and decrease the infection treatment options.[43] Resistance cassettes have been reported in both gram-negative and gram-positive bacteria.[44–46]

QS detection in Acinetobacter

The QS system was first discovered in 1994 by Dr. Peter Greenberg in *Vibrio fischeri.* This 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.[48]

The presence of *lasI* gene in *Acinetobacter* isolates was investigated by PCR.

The results indicated that 60 (49.6%) of the 121 isolates were positive for *lasI* gene (Table 3). Bacteria use QS to regulate gene 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 organism.[50] 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 a part of the 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 resistance of *A. baumannii.* This resistance can transform all 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.

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Authors' Contribution

All authors contributed equally to the literature review, study plan, laboratory work, fund generation, and writing and proofing of the article.

Disclosure Statement

No competing financial interests exist.

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References

1. Storm, D.R., K.S. Rosenthal, and P.E. Swanson. 1977. Polymyxin and related peptide antibiotics. Annu. Rev. Biochem. 46:723–763.
2. Bergen, P.J., J. Li, C.R. Rayner, and R.L. Nation. 2006. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 50:1953–1958.
3. Li, J., R.L. Nation, J.D. Turnidge, R.W. Milne, K. Coulthard, C.R. Rayner, and D.L. Paterson. 2006. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect. Dis. 6:589–601.
4. Nation, R.L., and J. Li. 2009. Colistin in the 21st century. Curr. Opin. Infect. Dis. 22:535.
5. El-Mokhtar, M.A., S.A. Mandour, and A.A. Shahat. 2019. Colistin resistance among multidrug-resistant *E. coli* isolated from Upper Egypt. Egypt. J. Med. Microbiol. 28: 11–17.
6. Ko, K.S., J.Y. Suh, K.T. Kwon, S.-I. Jung, K.-H. Park, C.I. Kang, D.R. Chung, K.R. Peck, and J.-H. Song. 2007. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J. Antimicrob. Chemother. 60:1163–1167.
7. Hawley, J.S., C.K. Murray, and J.H. Jorgensen. 2007. Development of colistin-dependent *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex. Antimicrob. Agents Chemother. 51:4529–4530.
8. Yahav, D., L. Farbman, L. Leibovici, and M. Paul. 2012. Colistin: new lessons on an old antibiotic. Clin. Microbiol. Infect. 18:18–29.
9. Tzeng, Y.-L., K.D. Ambrose, S. Zughaier, X. Zhou, Y.K. Miller, W.M. Shafer, and D.S. Stephens. 2005. Cationic antimicrobial peptide resistance in *Neisseria meningitidis*. J. Bacteriol. 187:5387–5396.
10. Liu, Y.-Y., Y. Wang, T.R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, and X. Huang. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16:161–168.
11. Xavier, B.B., C. Lammens, R. Ruhal, S. Kumar-Singh, P. Butaye, H. Goossens, and S. Malhotra-Kumar. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. Euro. Surveill. 21:30280.
12. Yin, W., H. Li, Y. Shen, Z. Liu, S. Wang, Z. Shen, R. Zhang, T.R. Walsh, J. Shen, and Y. Wang. 2017. Novel plasmid-mediated colistin resistance gene mcr-3 in *Escherichia coli*. MBio 8:e00543–17.
13. Carattoli, A. 2001. Importance of integrons in the diffusion of resistance. Vet. Res. 32:243–259.
14. Borowiak, M., J. Fischer, J.A. Hammerl, R.S. Hendriksen, I. Szabo, and B. Malorny. 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. enterica serovar Paratyphi B. J. Antimicrob. Chemother. 72:3317–3324.
15. Partridge, S.R., V. Di Pilato, Y. Doi, M. Feldgarden, D.H. Haft, W. Klimke, S. Kumar-Singh, J.-H. Liu, S. Malhotra-Kumar, and A. Prasad. 2018. Proposal for assignment of allele numbers for mobile colistin resistance (mcr) genes. J. Antimicrob. Chemother. 73:2625–2630.
16. Wang, X., Y. Wang, Y. Zhou, J. Li, W. Yin, S. Wang, S. Zhang, J. Shen, Z. Shen, and Y. Wang. 2018. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing *Klebsiella pneumoniae*. Emerg. Microbes Infect. 7:122.
17. Litrup, E., K. Kiil, A.M. Hammerum, L. Roer, E.M. Nielsen, and M. Torpdahl. 2017. Plasmid-borne colistin resistance gene mcr-3 in Salmonella isolates from human infections, Denmark, 2009–2017. Euro. Surveill. 22: pii: 30587.
18. Peleg, A.Y., H. Seifert, and D.L. Paterson. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21:538–582.
19. Kareem, S.M., I.M. Al-Kadmy, M.H. Al-Kaabi, S.N. Aziz, and M. Ahmad. 2017. *Acinetobacter baumannii* virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB). Microb. Pathog. 110:568–572.
20. Cai, Y., D. Chai, R. Wang, B. Liang, and N. Bai. 2012. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J. Antimicrob. Chemother. 67:1607–1615.
21. AL-Kadmy, I.M., A.N.M. Ali, I.M.A. Salman, and S.S. Khazaal. 2018. Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment. New Microbes New Infect. 21:51–57.
22. Henry, R., N. Vithanage, P. Harrison, T. Seemann, S. Coutts, J.H. Moffatt, R.L. Nation, J. Li, M. Harper, and B. Adler. 2012. Colistin-resistant, lipopolysaccharide-deficient *Acinetobacter baumannii* responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids, and

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poly-β-1, 6-N-acetylglucosamine. Antimicrob. Agents Chemother. 56:59–69.

23. Moffatt, J.H., M. Harper, P. Harrison, J.D. Hale, E. Vinogradov, T. Seemann, R. Henry, B. Crane, F.S. Michael, and A.D. Cox. 2010. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob. Agents Chemother. 54: 4971–4977.
24. Matuschek, E., J. Åhman, C. Webster, and G. Kahlmeter. 2018. Antimicrobial susceptibility testing of colistin–evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter spp.* Clin. Microbiol. Infect. 24:865–870.
25. Adams, M.D., G.C. Nickel, S. Bajaksouzian, H. Lavender, A.R. Murthy, M.R. Jacobs, and R.A. Bonomo. 2009. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. Antimicrob. Agents Chemother. 53:3628–3634.
26. Yin, W.-F., K. Purmal, S. Chin, X.-Y. Chan, C.-L. Koh, C.-K. Sam, and K.-G. Chan. 2012. N-acyl homoserine lactone production by *Klebsiella pneumoniae* isolated from human tongue surface. Sensors 12:3472–3483.
27. Hasen Esaa, R., E. N N Naji, H. Sami. 2016. Comparison of three diagnostic methods for *Acinetobacter baumannii* Isolated from Baghdad Hospitals. Adv. Environ. Biol. 10: 87–93.
28. Balaji, V., S. Jeremiah, and P. Baliga. 2011. Polymyxins: antimicrobial susceptibility concerns and therapeutic options. Indian J. Med. Microbiol. 29:230.
29. Farhan, S.M., R.A. Ibrahim, H.F. Hetta, K.M. Mahran, and R.M. Abdelbaky. 2018. Prevalence of OXA-23 in multidrug resistance *Acinetobacter baumannii* Isolated From Different Infections At Minia University Hospital. N. Egypt. J. Microbiol. 50:34–43.
30. El-Mokhtar, M.A., and H.F. Hetta. 2018. Ambulance vehicles as a source of multidrug-resistant infections: a multicenter study in Assiut City, Egypt. Infect. Drug Resist. 11: 587.
31. Ahmed, S., S. Ahmed, W. Mohamed, M. Feky, E. Daef, M. Badary, and H. Hetta. 2011. Nosocomial vancomycin and methicillin resistant staphylococcal infections in intensive care units in Assiut University Hospitals. Egypt. J. Med. Microbiol. 20:127–140.
32. Farhan, S.M., R.A. Ibrahim, K.M. Mahran, H.F. Hetta, and R.M.A. El-Baky. 2019. Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing *Pseudomonas aeruginosa* isolated from hospitals in Minia, Egypt. Infect. Drug Resist. 12:2125.
33. El-Baky, R.M.A., T. Sandle, J. John, G.E.-D.A. Abuo-Rahma, and H.F. Hetta. 2019. A novel mechanism of action of ketoconazole: inhibition of the NorA efflux pump system and biofilm formation in multidrug-resistant *Staphylococcus aureus*. Infect. Drug Resist. 12:1703.
34. Al-Saryi, N., S.A. Ibrahim, I.M. AL-Kadmy, and H.F. Hetta. 2019. Whole genome sequencing of *Streptococcus pneumoniae* serotype 33C causing fatal sepsis in a hospitalized patient with nephrotic syndrome. Gene Rep. 16: 100434.
35. Hinchliffe, P., Q.E. Yang, E. Portal, T. Young, H. Li, C.L. Tooke, M.J. Carvalho, N.G. Paterson, J. Brem, and P.R. Niumsup. 2017. Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1. Sci. Rep. 7:39392.
36. Wang, Y., G.-B. Tian, R. Zhang, Y. Shen, J.M. Tyrrell, X. Huang, H. Zhou, L. Lei, H.-Y. Li, and Y. Doi. 2017. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect. Dis. 17:390–399.
37. Rajaei, B., S.D. Siadat, M.R. Razavi, M.R. Aghasadeghi, N.S. Rad, F. Badmasti, S.K. Jafroodi, T. Rajaei, A. Moshiri, and S. Javadian. 2011. Expanding drug resistance through integron acquisition in Salmonella spp. isolates obtained in Iran. Afr. J. Microbiol. Res. 5:2249–2253.
38. Tamang, M.D., J.Y. Oh, S.Y. Seol, H.Y. Kang, J.C. Lee, Y.C. Lee, D.T. Cho, and J. Kim. 2007. Emergence of multidrug-resistant Salmonella enterica serovar Typhi associated with a class 1 integron carrying the dfrA7 gene cassette in Nepal. Int. J. Antimicrob. Agents 30:330–335.
39. Yang, B., J. Zheng, E.W. Brown, S. Zhao, and J. Meng. 2009. Characterisation of antimicrobial resistance-associated integrons and mismatch repair gene mutations in Salmonella serotypes. Int. J. Antimicrob. Agents 33:120–124.
40. Hall, R.M., and C.M. Collis. 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol. Microbiol. 15:593–600.
41. Hall, R.M. In Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria, Ciba Foundation Symposium 207-Antibiotic Resistance: Origins, Evolution, Selection and Spread: Antibiotic Resistance: Origins, Evolution, Selection and Spread: Ciba Foundation Symposium 207, John Wiley & Sons, Chichester, UK, 2007; pp. 192–205.
42. Mazel, D. 2006. Integrons: agents of bacterial evolution. Nat. Rev. Microbiol. 4:608.
43. Stokes, H.T., and R.M. Hall. 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. 3:1669–1683.
44. Nešvera, J., J. Hochmannová, and M. Pátek. 1998. An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium *Corynebacterium glutamicum*. FEMS Microbiol. Lett. 169:391–395.
45. Nandi, S., J.J. Maurer, C. Hofacre, and A.O. Summers. 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proc. Natl. Acad. Sci. USA. 101:7118–7122.
46. Tauch, A., S. Götker, A. Pühler, J. Kalinowski, and G. Thierbach. 2002. The 27.8-kb R-plasmid pTET3 from Corynebacterium glutamicum encodes the aminoglycoside adenyltransferase gene cassette aadA9 and the regulated tetracycline efflux system Tet 33 flanked by active copies of the widespread insertion sequence IS6100. Plasmid 48: 117–129.
47. Yang, L. 2009. *Pseudomonas aeruginosa* Quorum-Sensing: A Factor in Biofilm Development, and an Antipathogenic Drug Target (Doctoral dissertation). Technical University of Denmark, Kongens Lyngby, Denmark.
48. Kalia, V.C., and H.J. Purohit. 2011. Quenching the quorum sensing system: potential antibacterial drug targets. Critic. Rev. Microbiol. 37:121–140.
49. Bose, S., and A.K. Ghosh. 2016. Understanding of quorum–sensing: a possible solution for drug resistance in bacteria. Int. J. Curr. Microbiol. App. Sci. 5:540–546.
50. LaSarre, B., and M.J. Federle. 2013. Exploiting quorum sensing to confuse bacterial pathogens. Microbiol. Mol. Biol. Rev. 77:73–111.

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51. Labbate, M., S.Y. Queck, K.S. Koh, S.A. Rice, M. Givskov, and S. Kjelleberg. 2004. Quorum sensing-controlled biofilm development in Serratia liquefaciens MG1. J. Bacteriol. 186:692–698.
52. Cady, N.C., K.A. McKean, J. Behnke, R. Kubec, A.P. Mosier, S.H. Kasper, D.S. Burz, and R.A. Musah. 2012. Inhibition of biofilm formation, quorum sensing and infection in *Pseudomonas aeruginosa* by natural products-inspired organosulfur compounds. PLoS One 7:e38492.
53. Jakobsen, T.H., S.K. Bragason, R.K. Phipps, L.D. Christensen, M. van Gennip, M. Alhede, M. Skindersoe, T.O. Larsen, N. Høiby, and T. Bjarnsholt. 2012. Food as a source for quorum sensing inhibitors: iberin from horseradish revealed as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 78: 2410–2421.
54. Brackman, G., P. Cos, L. Maes, H.J. Nelis, and T. Coenye. 2011. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob. Agents Chemother. 55:2655–2661.
55. Koh, K.H., and F.-Y. Tham. 2011. Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. J. Microbiol. Immunol. Infect. 44:144–148.
56. Smith, R.S., and B.H. Iglewski. 2003. *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. J. Clin. Invest. 112:1460.
57. Siehnel, R., B. Traxler, D.D. An, M.R. Parsek, A.L. Schaefer, and P.K. Singh. 2010. A unique regulator controls the activation threshold of quorum-regulated genes in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA. 107: 7916–7921.

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