Journal of Global Antimicrobial Resistance 17 (2019) 227–232

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

The emergence of colistin-resistant *Klebsiella pneumoniae* strains from swine in Malaysia

Golnaz Mobasseri[a], Cindy Shuan Ju Teh[b], Peck Toung Ooi[c], Kwai Lin Thong[a,*]

[a] Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
[b] Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[c] Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia

ARTICLE INFO

Article history:
Received 30 September 2018
Received in revised form 20 December 2018
Accepted 21 December 2018
Available online 3 January 2019

Keywords:
Colistin
Genetic diversity
Klebsiella pneumoniae
Multidrug resistance
Swine

ABSTRACT

Objective: Colistin is the last line of therapy for infections caused by multidrug-resistant Gram-negative bacteria. The objective of this study was to determine the phenotypic and genotypic characteristics of colistin-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) isolated from swine samples in Malaysia.
Methods: A total of 46 swine *K. pneumoniae* strains isolated from 2013–2015 in Malaysia were analysed for the production of extended-spectrum β-lactamases and carbapenemase. The resistance traits and genetic diversity of these strains were characterised by polymerase chain reaction, conjugation, plasmid analysis, and pulsed-field gel electrophoresis.
Results: Nineteen of 46 strains were multidrug resistant while 13 were resistant to colistin. The majority of colistin-resistant strains harboured bla_{TEM} gene (92.3%), followed by bla_{SHV} (69.23%), bla_{CTXM-1} (38.46%), and bla_{MCR-1} (23.08%). All three colistin-resistant strains had transferable plasmids and the colistin resistance gene bla_{MCR-1}. Genotyping by pulsed-field gel electrophoresis showed high genetic diversity among the *K. pneumoniae* and that the colistin-resistant *K. pneumoniae* strains were heterogenous.
Conclusion: It is believed that this is the first report of colistin-resistant *K. pneumoniae* among swine strains associated with *mcr-1* plasmid in Malaysia. Due to the emergence of β-lactam, carbapenem and colistin resistance, the use of colistin in animal husbandry and agriculture should be avoided to prevent treatment failure.

1. Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative bacterium that belongs to the Enterobacteriaceae family. It is a common pathogen that is responsible for hospital-acquired and community-acquired infections such as bacteraemia, pneumonia, soft tissue infection, and urinary tract infections [1,2].

In 1983, the first extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* were reported in Germany. Since then, the numbers of ESBL-producing *K. pneumoniae* have increased and rapidly spread across the world causing a serious problem in antibiotic management. Furthermore, ESBL-producing *K. pneumoniae* are also resistant to several groups of antibiotics, often resulting in treatment failure [2].

Carbapenems are used as last-resort antibiotics for severe infections caused by ESBL-producing bacteria; cases of carbapenem resistance have been reported since 1996. Carbapenem resistance has become a critical concern in the healthcare sector due to increased mortality and limited therapeutic options. Under this situation, colistin and tigecycline are the 'last-line therapy' for these resistant organisms [2–4]. However, the excessive use of colistin has resulted in increased colistin resistance in bacterial pathogens reported worldwide [2]. The main cause of resistance to polymyxins is lipopolysaccharide (LPS) modification and is associated with two-component systems: *phoPQ, pmrAB* and the regulator *mgrB* [5].

The plasmid-mediated colistin resistance due to *mcr-1* resistance gene was first reported by Liu et al. from food, animals and patients in China [6]. This was followed by reports of animals and humans from other countries such as Denmark, Thailand, Laos, Algeria, Portugal, United Kingdom, France, and Germany [7–11].

Infections caused by colistin-resistant pathogens are a serious issue because there are no available suitable antimicrobial options,

* Corresponding author at: Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur, 50603, Malaysia.
E-mail addresses: thongkl@um.edu.my, q5thong@yahoo.com (K.L. Thong).

https://doi.org/10.1016/j.jgar.2018.12.015
2213-7165/

and they are associated with high mortality rates among infected humans and animals [12]. Food-producing animals play an important role in the transfer of antibacterial resistance among animals and humans, and several studies have focused on this possible transmission with regard to public health [6–8,11].

Therefore, the objective of this study was to determine the genotypic and phenotypic characteristics of colistin-resistant *K. pneumoniae* strains isolated from swine farms in Malaysia from 2013–2015. It also analysed the prevalence and transferability of colistin-associated gene and antimicrobial resistance among *K. pneumoniae* strains.

2. Materials and methods

2.1. Bacterial isolation and identification

Forty-six *K. pneumoniae* strains were isolated from 41 pigs in seven different farms located in Penang (Farms A–E) and Selangor (Farms F–G), Malaysia between 2013–2015 (Fig. 1). The strains were isolated using a basic microbiological culture method and identified as *K. pneumoniae* by the Vitek-MS system and polymerase chain reaction (PCR) targeting *mdh* gene (http://bigsdb.web.pasteur.fr/klebsiella/primers_used.html). The PCR products were verified via DNA sequencing. The 46 confirmed strains were isolated from swine rectum (n = 20), swine faeces (n = 6), swine nasal cavities (n = 8), and swine oral cavities (n = 12). About 26% of the strains were isolated from Farm F, followed by eight strains each from Farms B and C, five strains each from Farms D and G, and four strains each from Farms A and E.

2.2. Antimicrobial susceptibility testing

The susceptibilities of *K. pneumoniae* strains to 16 antimicrobial agents — including amikacin (30 μg), amoxicillin-clavulanate (20/10 μg), nalidixic acid (30 μg), aztreonam (30 μg), ceftazidime (30 μg), cefoperazone (30 μg), cefixime (5 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), colistin (10 μg), gentamycin (10 μg), ampicillin (10 μg), tazobactam (10 μg), imipenem (10 μg), meropenem (10 μg), and tetracycline (30 μg) (Oxoid) — were determined by using standard disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [13].

Extended-spectrum β-lactamase production was confirmed using the disk diffusion method as described in the CLSI guidelines [13]. Briefly, antibiotic disks containing cefotaxime (30 μg), ceftazidime (30 μg), cefotaxime-clavulanate (30/10 μg) and ceftazidime-clavulanate (30/10 μg) were placed 20 mm apart from the centre of the Mueller-Hinton Agar plate that was inoculated with a cell suspension of *K. pneumoniae* at 0.5 McFarland standard. An increase of 5 mm in the diameter of the inhibition zone of cefotaxime or ceftazidime, in combination with clavulanate when compared with the diameter of cefotaxime or ceftazidime in the absence of clavulanate, was considered as a positive sign for positive ESBL production. *Escherichia coli* (*E. coli*) strain ATCC 25922 and *K. pneumoniae* strain ATCC 700603 were used as quality controls.

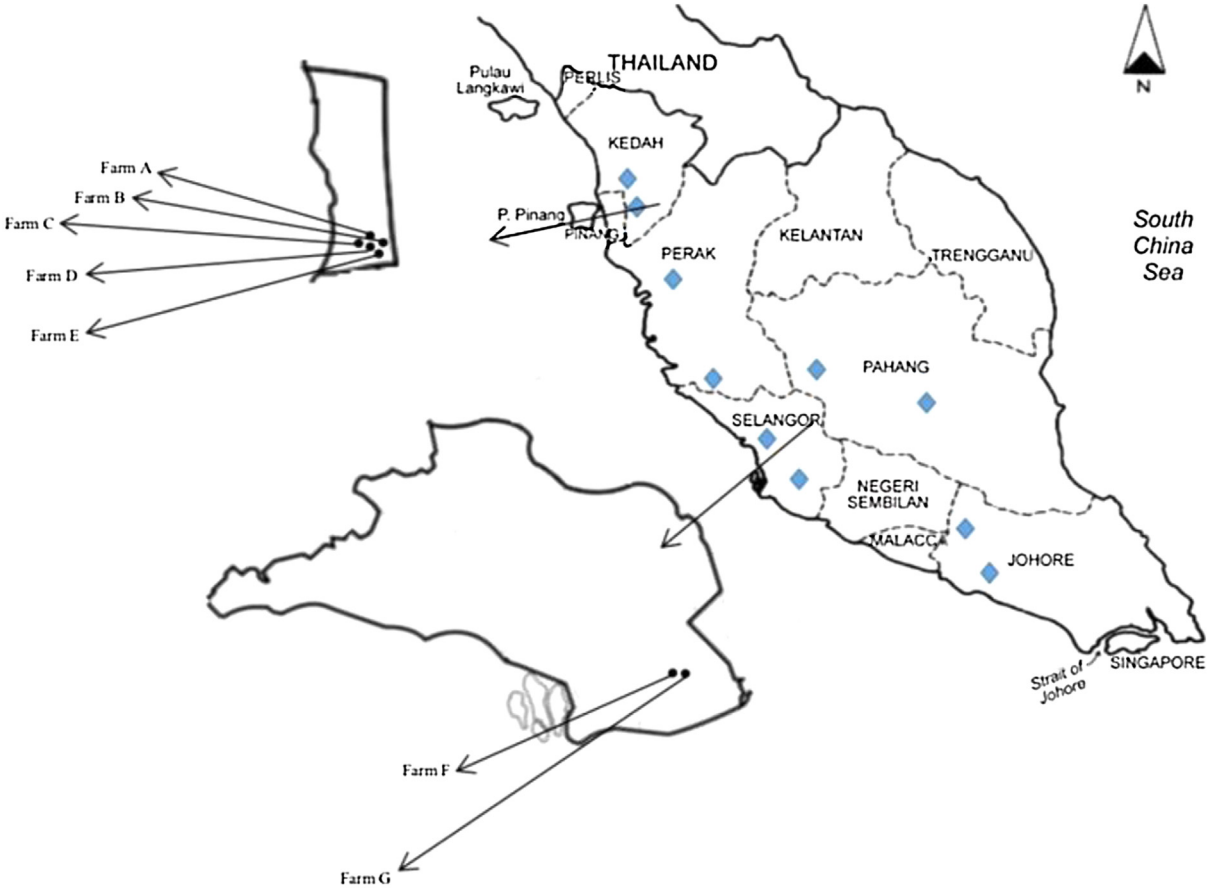

Fig. 1. The map of Peninsular Malaysia showing the locations of the seven swine farms.
Number of strains in each farm: Farm A (swine oral cavities, n = 3; swine rectum, n = 1); Farm B (swine rectum, n = 2; swine faeces, n = 6); Farm C (swine rectum, n = 8); Farm D (swine rectum, n = 5); farm E (swine rectum, n = 4); Farm F (swine oral cavities, n = 5; swine nasal cavities, n = 7); and Farm G (swine oral cavities, n = 4; swine nasal cavities, n = 1).

Modified Hodge Test was carried out to detect carbapenemase production in *K. pneumoniae* strains according to the CLSI guidelines [14]. The carbapenem-susceptible strain (*E. coli* ATCC 25922) was streaked as a lawn on MHA and dried for 3–5 min. The meropenem disk (10 μg) was then placed in the centre of the plate, and strains were streaked from the edge of the disk to the edge of the plate in a straight line. After 16–24 h of incubation, a clover leaf-type indentation at the intersection of the strains and *E. coli* ATCC 25922 was considered as a positive carbapenem resistance. *K. pneumoniae* strains ATCC BBA1705 and ATCC BBA1706 were used as positive and negative quality controls, respectively.

The minimum inhibitory concentration (MICs) for meropenem, imipenem, ceftazidime and cefotaxime (bioMerieux) was determined by Etest according to the CLSI guidelines, and broth microdilution method was performed for colistin. *E. coli* ATCC 25922 was used as the quality control strain [13].

2.3. Detection of extended-spectrum β-lactamase, carbapenem and colistin resistance genes

Polymerase chain reaction detection of β-lactamase genes (bla_{SHV}, bla_{TEM}, bla_{OXA-1}, bla_{CTXM-1}, bla_{CTXM-2}, bla_{CTXM-9}) and bla_{OXA-9}), carbapenem genes (bla_{VIM}, bla_{KPC}, bla_{IMP}, bla_{NDM} and bla_{OXA-48}) and colistin resistance genes (bla_{MCR-1}, bla_{MCR-2}, bla_{MCR-3}, bla_{MCR-4} and bla_{MCR-5}) was performed as previously described [6,15–17]. The PCR products were sequenced to confirm their identity, and the strains with the confirmed amplicon products were used as positive controls for subsequent PCR analysis.

2.4. Conjugation assay and plasmid analysis

For estimation of plasmid sizes, plasmid DNA extracted from *K. pneumoniae* strains, by using the alkaline lysis method [18], were separated on a 0.8% agarose gel for 4.5 h at 100 V. A 1 kb DNA ladder and lambda DNA/*Hind*III (Promega, Madison, USA) were used as DNA markers. Detection of selected β-lactamase genes (bla_{SHV}, bla_{TEM}, bla_{OXA-1} and bla_{CTXM-1}) and colistin resistance gene (bla_{MCR-1}, bla_{MCR-2}, bla_{MCR-3}, bla_{MCR-4} and bla_{MCR-5}) were performed using extracted plasmid DNA via PCR [6,15–17]. The PCR products were sequenced to confirm their identity.

Transfer of colistin resistance trait by conjugation was performed in Luria-Bertani broth using *E. coli* DH5α, which is resistant to nalidixic acid, as the recipient. Transconjugants were selected on Luria-Bertani agar supplemented with a combination of colistin (1 mg/L) and nalidixic acid (100 mg/mL) (Sigma Aldrich, St. Louis, USA) as previously described by Anjum et al. [19]. Presence of plasmids in the transconjugants was confirmed by agarose gel electrophoresis.

2.5. Genotyping by pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was carried out according to a previously published study, with minor modification [20]. In brief, an equal volume of standardised cell suspensions (OD_{610} = 0.6) was mixed with 1% Seakem Gold agarose and allowed to solidify. The cell lysis buffer (50 mM Tris, 50 mM EDTA (pH = 8), 1% Sacrosine, 1 mg/mL proteinase K (Promega, Madison, WI USA) was used to lyse the cell-plug at 54 °C for 4 h. The lysed plug was washed twice with sterile deionised water and six times with TE buffer at room temperature. Then, a slice of the DNA plug was digested with 10 U of *Xba*I (Promega, Madison, WI USA) at 37 °C for at least 4 h. The restricted DNAs were separated by using a CHEF-DR III with pulse times of 2.25–54.2 s at 200 V for 24 h, and gels were viewed under ultraviolet light after staining with Gel-Red. Analysis of the PFGE banding patterns was based on the unweighted pair group method with arithmetic mean at 1.5 position tolerance using the BioNumerics 6.0 software (Applied Maths, Ghent, Belgium).

3. Results

3.1. Bacterial isolation and identification

A total of 46 *K. pneumoniae* strains were previously isolated by the basic microbiological culture method and identified by using the Vitek-MS system. All the strains were confirmed as *K. pneumoniae* based on the presence of the 477 bp DNA band of the *mdh* gene by using PCR, and sequence analyses of the amplicons confirmed them as *K. pneumoniae* (Gen bank accession no; NC_016845).

3.2. Antibiograms

The antimicrobial resistance of the 46 *K. pneumoniae* strains to 16 different antibiotics are as follows: tetracycline (91.3%), ampicillin (91.3%), cefoperazone (15.2%), cefotaxime (41.3%), ceftazidime (41.3%), aztreonam (37.0%), gentamycin (37.0%), cefixime (37.0%), amoxicillin-clavulanate (34.5%), imipenem (32.6%), amikacin (30.4%), colistin (28.3%), tazobactam (28.3%), ciprofloxacin (19.6%), nalidixic acid (15.2%), and meropenem (8.69%). Nineteen (41.3%) strains were multidrug resistant (MDR), as they showed resistance to more than three classes of antibiotics. According to the disk diffusion test and Modified Hodge Test, all these MDR strains were detected as ESBL producers but none was a carbapenemase producer. Among these 19 MDR, 13 (28.3%) were resistant to colistin and β-lactam antibiotics. Eleven out of 13 colistin-resistant strains (23.9%) showed resistance to at least one carbapenem antibiotic. The MIC for colistin, meropenem, imipenem, ceftazidime, and cefotaxime for the 13 colistin-resistant strains are summarised in Table 1. The MIC results of 13 colistin-resistant strains showed that all were resistant to cefotaxime and ceftazidime (MIC range, >256 μg/mL to >32 μg/mL), imipenem and meropenem (MIC range, <0.06 μg/mL to >16 μg/mL) and colistin (MIC range, >32 μg/mL to >8 μg/mL).

3.3. Detection of resistance genes

The bla_{TEM} gene was present in 92.3% of 13 colistin-resistant strains (12 of 13), followed by bla_{SHV} (9 of 13) and bla_{CTXM-1} (5 of 13). Three colistin-resistant strains harboured bla_{MCR-1} (23.1%). Two strains (KP2013Z28 and KP2015Z09) harboured bla_{TEM}, bla_{SHV}

Table 1
The minimal inhibitory concentrations (MIC) of 13 colistin-resistant strains.

Strains	Minimal inhibitory concentration (MIC) (μg/mL)[a]				
	CTX (30 μg)	CAZ (30 μg)	MEM (10 μg)	IPM (10 μg)	CT (10 μg)
KP2013Z27	64/R	256/R	1.5/S	0.06/S	8/R
KP2013Z28	256/R	128/R	0.5/S	0.47/S	16/R
KP2015Z01	32/R	32/R	4/R	0.32/S	16/R
KP2015Z02	128/R	32/R	2/I	0.12/S	8/R
KP2015Z04	32/R	128/R	8/R	2/I	16/R
KP2015Z05	64/R	32/R	4/R	0.32/S	32/R
KP2015Z07	256/R	64/R	2/I	0.32/S	16/R
KP2015Z09	32/R	128/R	16/R	0.47/S	32/R
KP2015Z10	64/R	128/R	6/R	4/R	8/R
KP2015Z11	64/R	64/R	8/R	0.16/S	16/R
KP2015Z14	128/R	32/R	16/R	0.23/S	8/R
KP2015Z15	32/R	64/R	4/I	0.06/S	8/R
KP2015Z17	32/R	128/R	6/R	0.16/S	16/R

CTX: cefotaxime (S ≤ 1; I = 2; R ≥ 4); CAZ: ceftazidime (S ≤ 4; I = 8; R ≥ 16); MEM: meropenem (S ≤ 1; I = 2; R ≥ 4); IMP: imipenem (S ≤ 1; I = 2; R ≥ 4); CT: colistin ((S ≤ 2; R ≥ 4) [13].

[a] Minimal inhibitory concentration interpretive criteria (μg/mL).

and bla_{MCR-1}, while one strain (KP2015Z02) harboured bla_{MCR-1} and bla_{SHV}. No carbapenem-resistant gene was found among these strains and no PCR amplicon was observed for primers targeting bla_{CTXM-2}, bla_{CTXM-9}, bla_{OXA-1}, bla_{OXA-9}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{MCR-2}, bla_{MCR-3}, bla_{MCR-4}, and bla_{MCR-5} genes. Sequence analyses of the PCR amplicons of bla_{TEM}, bla_{SHV}, bla_{CTXM-1}, and bla_{MCR-1} indicated 97-99% identity to their respective sequences in the NCBI database (Gen bank accession no; AAP43782, CAI30650, X92506 and NG_051170).

3.4. Plasmid analysis and transfer of resistance determinants

Plasmid analysis showed that all 13 colistin-resistant *K. pneumoniae* strains harboured plasmids, with sizes ranging 2500–20,000 bp. The PCR detection of selected β-lactamase and colistin genes (bla_{SHV}, bla_{TEM}, bla_{OXA-1}, bla_{CTXM-1}, and bla_{MCR-1}) using extracted plasmid DNA as templates, showed that seven strains harboured bla_{TEM} in plasmids, four strains each harboured bla_{SHV} and bla_{CTXM-1}, and three strains had bla_{MCR-1}. Plasmid analysis of three colistin-resistant strains that harboured bla_{MCR-1} indicated the presence of bla_{TEM}, bla_{SHV} and bla_{MCR-1} on the plasmids of KP2013Z28 and KP2015Z09, while KP2015Z02 carried only bla_{MCR-1} on the plasmid. DNA sequence analyses of these PCR amplicons confirmed the identity of these genes.

Conjugation was carried out for the three colistin-resistant and nalidixic acid-sensitive *K. pneumoniae* strains that harboured plasmids and colistin resistance gene bla_{MCR-1} by using nalidixic acid-resistant *E. coli* DH5α as the recipient. All these three strains had colistin-resistant transconjugants. Plasmids analysis of the transconjugants confirmed that plasmids with sizes of 2500, 3000, 6000, 8000 and 20,000 bp were transferred from the donors to the recipient (Fig. 2).

3.5. Genetic diversity of colistin-resistant K. pneumoniae strains by pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) subtyped the *Xba*I-digested genomic DNA of 46 swine *K. pneumoniae* strains into 38 distinct pulsed field profiles (pulsotypes). The genetic similarity of the strains ranged 51.9–100% and the discriminatory power of PFGE was 0.98 (Simpson's Index of Diversity). The 13 colistin-resistant *K. pneumoniae* strains were subtyped into 13 distinct pulsotypes, indicating that these strains were very heterogenous (Index of similarity: 54–91%) (Fig. 3).

The PFGE dendrogram of 13 colistin-resistant *K. pneumoniae* showed three clusters and four unique pulsotypes at 70% similarity cut-off (Fig. 3). Cluster A contained three strains, KP2015Z05 and KP2015Z04, which were isolated in 2015 from Farm F, with 90.9% similarity to each other, and they showed 71.3% similarity to KP2013Z27 from Farm D, which was isolated in 2013. Cluster B contained three strains isolated in 2015 from Farm G and Farm F with a similarity of 73.2%. Cluster C also grouped three strains, from Farm G and Farm F, which were collected in 2015 with 78.1% similarity.

The 13 colistin-resistant *K. pneumoniae* strains showed high genetic diversity and were heterogeneous. There was no association among these strains from different sources, antibiograms and resistance genes.

4. Discussion

Generally, colistin resistance in *K. pneumoniae* is mainly related to modification of the lipopolysaccharide, which depends on: (i) chromosomally-mediated genes associated with two-component systems, *pho*PQ, *pmr*AB, and with the regulator *mgr*B; and (ii) plasmid-mediated *mcr-1* [5].

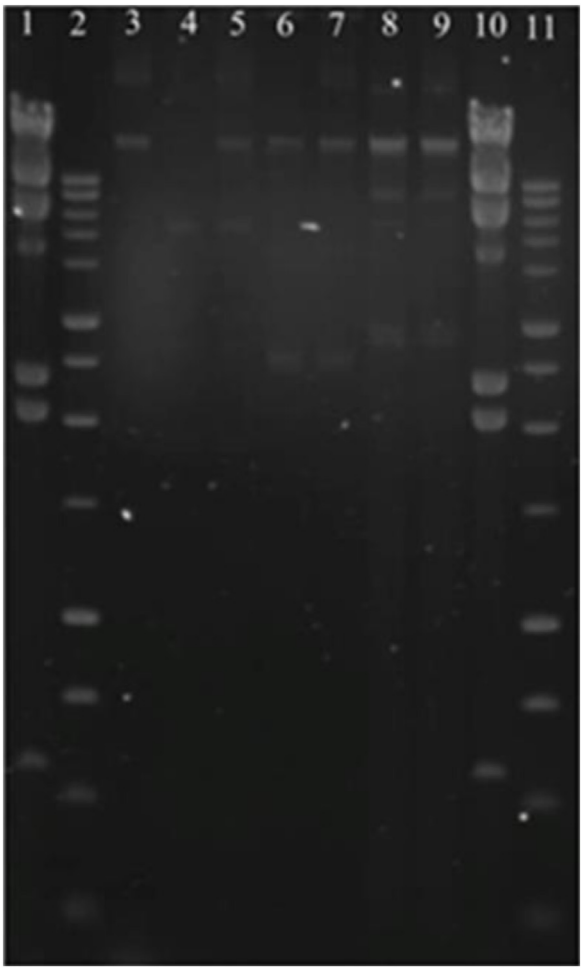

Fig. 2. Plasmid profiles of the donor *Klebsiella pneumoniae* strains. Lanes 4, 6, 8: KP2015Z02, KP201509, KP2013Z28 as donors, lane 3: *E. coli* DH5α as recipient, lanes 5, 7, 9: TKP201502, TKP2015Z09, TKP2013Z28 as transconjugants obtained. Lanes 1, 10: lambda DNA/ *Hind*III and lanes 2,11: 1 kb DNA ladder.

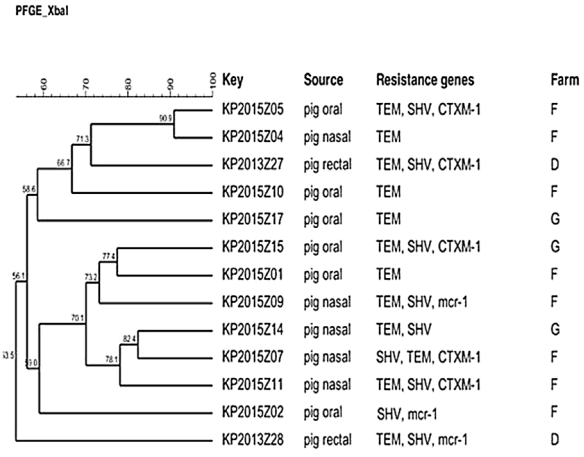

Fig. 3. Dendrogram for the pulsed-field gel electrophoresis of 13 colistin-resistant *Klebsiella pneumoniae* strains.

Since 2016, *mcr-1* has been reported in many parts of the world [2,11]. Following the first report of *mcr-1* in *K. pneumoniae* and *E. coli* from China [6], *mcr-1.2*, a variant of *mcr-1*, was reported in multidrug-resistant *K. pneumoniae* from Italy [21]. In addition, *mcr-2* (76% identity with *mcr-1*) was also reported in Belgium [22]. In

the current study, 19 out of 46 *K. pneumoniae* strains (41.3%) were multidrug-resistant and ESBLs producers, while 13 of these 19 (28.3%) were also resistant to colistin and three strains harboured *mcr-1*.

Since the 1960s, colistin has been used in livestock for prophylactic and therapeutic use for treating infections caused by Enterobacteriaceae. The colistin resistance *mcr-1* gene has been globally identified from a wide range of sources, including foods, humans, animals, and the environment. However, high prevalence of colistin resistance *mcr-1* gene in animals compared with other sources has been identified as the main source for spreading colistin resistance [23].

K. pneumoniae is an opportunistic pathogen in humans and animals that causes several infections, such as pneumonia, urinary tract infections, septicaemia, and wound infections. *K. pneumoniae* causes mastitis, lung infection and septicaemia in pigs and can be fatal to piglets. *K. pneumoniae* is also highly resistant to multiple antibiotics and harbour many resistance determinants such as β-lactamases and carbapenemase [1,24]. Colistin-resistant *K. pneumoniae* strains have been reported in several regions, such as North and South-America, Asia, Europe, and South Africa [2]. High prevalence of colistin resistance rates was reported in clinical *K. pneumoniae* strains in Greece at 10.5–20%, followed by Singapore (6.3%), South Korea (6.8%), and Canada (2.9%) [2].

This is first report of colistin-resistant *K. pneumoniae* strains harbouring *mcr-1* in Malaysian pigs. About 28% of the *K. pneumoniae* strains were resistant to colistin, with MICs ranging from 8–32 μg/mL, while 41.3% were categorised as multidrug resistant and ESBL-producing based on the MICs of CAZ and CTX, which ranged from 32–256 μg/mL. Among the 46 strains, 23.9% were resistant to carbapenems. MICs for MEM and IMP ranged from 0.5–16 and 0.06–4 μg/mL, respectively. On the contrary, *K. pneumoniae* strains isolated from animals and humans had shown a high prevalence of ESBLs and carbapenemase production, as reported in another study [25].

In the current study, bla_{TEM} was the most common resistance gene, as it was detected in 92% of the colistin-resistant *K. pneumoniae* strains. However, one strain (KP2015Z02) did not harbour bla_{TEM} but had bla_{MCR-1} and bla_{SHV}; bla_{SHV} was detected in 69% of the strains, which was previously reported as a more common resistance gene among Malaysian *K. pneumoniae* strains [20]. The most prevalent ESBL gene worldwide is $bla_{CTX-M-1}$ [26] and was found in 38% of *K. pneumoniae* strains in the current study. bla_{MCR-1} was detected in three colistin-resistant *K. pneumoniae* strains, which was previously reported among *K. pneumoniae* strains around the world such as China [6], Italy [21] and Belgium [22]. Recently, a study in Portugal showed a very high rate of prevalence of bla_{MCR-1} among *K. pneumoniae* strains from swine farms [27]. bla_{CTXM-2}, bla_{CTXM-9}, bla_{OXA-1}, bla_{OXA-9}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{MCR-2}, bla_{MCR-3}, bla_{MCR-4} and bla_{MCR-5} genes were not detected in the current strains.

Plasmid analysis showed that all strains harboured plasmids, and seven strains carried bla_{TEM} in their plasmids, followed by four strains with bla_{SHV}, four strains with bla_{CTXM-1}, and three strains with bla_{MCR-1}. From the conjugation experiments, the three colistin-resistant strains were conjugative, which harboured plasmids and bla_{MCR-1} as colistin resistance gene. Other studies also reported the transmission of *mcr* gene between human and animal sources [28,29]. The presence of ESBL genes and the *mcr-1* gene on a unique plasmid has been reported in many strains of animal origin [30].

Subtyping of colistin-resistant *K. pneumoniae* strains by PFGE showed that these strains were highly diverse and heterogeneous. This was in concordance with previous studies, which showed high genetic diversity among *K. pneumoniae* strains from animals and humans [20,31–32]. This finding was not surprising, as the strains were from different pigs, farms and years.

5. Conclusions

In general, the use of polymyxin antibiotics in animal husbandry and agricultural practices should be closely regulated by authorities. The plasmid-mediated *mcr-1*, which can spread among patients in hospitals, is the main concern among people in communities, and between animals in farms and environment [33]. This study is the first report of colistin-resistant *K. pneumoniae* among swine farms in Malaysia and showed a high prevalence of MDR and production of ESBLs. The spread of these resistant organisms might be a threat to public health. Therefore, the authorities need to closely monitor the situation and regulate the usage of colistin to prevent an antimicrobial resistance issue [27].

Acknowledgments

We thank University of Malaya for financial support and facilities.

Funding

This work was supported by University of Malaya Postgraduate Research Grant — (PG072-2014B) and High Impact Research (HIR) Grant (UM.C/625/1/HIR/MOHE/CHAN/11/02).

Competing interests

None.

Ethical approval

The swine sample collection was approved by the Animal Care and Use Committee (ACUC), UPM (UPM/IACUC/FYP- AUP-T006/2013).

References

[1] Vading M, Nauclér P, Kalin M, Giske CG. Invasive infection caused by *Klebsiella pneumoniae* is a disease affecting patients with high comorbidity and associated with high long-term mortality. PLoS One 2018;13(4)e0195258, doi:http://dx.doi.org/10.1371/journal.pone.0195258.

[2] Ah YM, Kim AJ, Lee JY. Colistin resistance in *Klebsiella pneumoniae*. Int J Antimicrob Agents 2014;44(1):8–15, doi:http://dx.doi.org/10.1016/j.ijantimicag.2014.02.016.

[3] Van Duin D, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant *Enterobacteriaceae* a review of treatment and outcomes. Diagn Microbial Infect Dis 2013;75(2):115–20, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2012.11.009.

[4] Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant *Enterobacteriaceae* epidemiology and prevention. Clin Infect Dis 2011;53(1):60–7, doi:http://dx.doi.org/10.1093/cid/cir202.

[5] Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 2014;26(5):643, doi:http://dx.doi.org/10.3389/fmicb.2014.00643.

[6] Liu Y-Y, Wang Y, Walsh TR, Yi L-X, 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. Lancet Infect Dis 2016;16(2):161–8, doi:http://dx.doi.org/10.1016/S1473-3099(15)00424-7.

[7] Olaitan AO, Chabou S, Okdah L, Morand S, Rolain JM. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis 2015;16(2):147–9, doi:http://dx.doi.org/10.1016/S1473-3099(15)00540-X.

[8] Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro Surveill 2015;20(49):261–2, doi:http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085.

[9] Hu Y, Liu F, Lin IY, Gao GF, Zhu B. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis 2016;16(2):146–7, doi:http://dx.doi.org/10.1016/S1473-3099(15)00533-2.

[10] Falgenhauer L, Waezsada S-E, Yao Y, Imirzalioglu C, Käsbohrer A, Roesler U, et al. Colistin resistance gene *mcr-1* in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis 2016;16(3):282–3, doi:http://dx.doi.org/10.1016/S1473-3099(16)00009-8.
[11] Ye H, Li Y, Li Z, Gao R, Zhang H, Wen R, et al. Diversified *mcr-1*-harbouring plasmid reservoirs confer resistance to colistin in human gut microbiota. MBio 2016;7(2)e00177-00116, doi:http://dx.doi.org/10.1128/mBio.00177-16.
[12] Cassir N, Rolain J-M, Brouqui P. A new strategy to fight antimicrobial resistance: the revival of old antibiotics. Front Microbiol 2014;5:551, doi:http://dx.doi.org/10.3389/fmicb.2014.00551.
[13] CLSI. Performance standards for antimicrobial susceptibility testing; 28th informational supplement, document M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
[14] CLSI. Performance standards for antimicrobial susceptibility testing; 26th informational supplement, document M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
[15] Gheorghe I, Czobor I, Chifiriuc MC, Borcan E, Ghiță C, Banu O, et al. Molecular screening of carbapenemase-producing Gram-negative strains in Romanian intensive care units during a one-year survey. J Med Microbiol 2014;63(10):1303–10, doi:http://dx.doi.org/10.1099/jmm.0.074039-0.
[16] Barguigua A, El Otmani F, Talmi M, Bourjilat F, Haouzane F, Zerouali K, et al. Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 2011;60(9):1344–52, doi:http://dx.doi.org/10.1099/jmm.0.032482-0.
[17] Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* for surveillance purposes. Euro Surveill 2018;23(6)17-00672. https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672.
[18] Feliciello I, Chinali G. A modified alkaline lysis method for the preparation of highly purified plasmid DNA from *Escherichia coli*. Analyt Biochemist 1993;212:394–401, doi:http://dx.doi.org/10.1006/abio.1993.1346.
[19] Anjum MF, Duggett NA, AbuOun M, Randall L, Nunez-Garcia J, Ellis RJ, et al. Colistin resistance in *Salmonella* and *Escherichia coli* isolates from a pig farm in Great Britain. J Antimicrob Chemother 2016;71(8):2306–13, doi:http://dx.doi.org/10.1093/jac/dkw149.
[20] Lim KT, Yeo CC, Yasin RM, Balan G, Thong KL. Characterization of multidrug-resistant and extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strains from Malaysian hospitals. J Med Microbiol 2009;58(11):1463–9, doi:http://dx.doi.org/10.1099/jmm.0.011114-0.
[21] Di Pilato V, Arena F, Tascini C, Cannatelli A, De Angelis LH, Fortunato S, et al. *mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512. Antimicrob Agents Chemother 2016;60(9):5612–5, doi:http://dx.doi.org/10.1128/AAC.01075-16.
[22] 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. Euro Surveill 2016;21(27):30280, doi:http://dx.doi.org/10.2807/1560-7917.ES.2016.21.27.30280.
[23] Rhouma M, Beaudry F, Thériault W, Letellier A. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front Microbiol 2016;7:1789, doi:http://dx.doi.org/10.3389/fmicb.2016.01789.
[24] Brisse S, van Duijkeren E. Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. Vet Microbiol 2005;105(3):307–12, doi:http://dx.doi.org/10.1016/j.vetmic.2004.11.010.
[25] Hiroi M, Yamazaki F, Harada T, Takahashi N, Iida N, Noda Y, et al. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. J Vet Med Sci 2012;74(2):189–95, doi:http://dx.doi.org/10.1292/jvms.11-0372.
[26] Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59(2):165–74, doi:http://dx.doi.org/10.1093/jac/dkl483.
[27] Kieffer N, Aires-de-Sousa M, Nordmann P, Poirel L. High rate of MCR-1–producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal. Emerg Infect Dis 2017;23(12):2023–9. https://dx.doi.org/10.3201/eid2312.170883.
[28] Carnevali C, Morganti M, Scaltriti E, Bolzoni L, Pongolini S, Casadei G. Occurrence of *mcr-1* in colistin-resistant *Salmonella enterica* isolates recovered from humans and animals in Italy, 2012 to 2015. Antimicrob Agents Chemother 2016;60(12):7532–4, doi:http://dx.doi.org/10.1128/AAC.01803-16.
[29] Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2017;2:16260, doi:http://dx.doi.org/10.1038/nmicrobiol.2016.260.
[30] Caspar Y, Maillet M, Pavese P, Francony G, Brion J-P, Mallaret M-R, et al. *mcr-1* colistin resistance in ESBL-producing *Klebsiella pneumoniae*, France. Emerg Infect Dis 2017;23(5):874–6, doi:http://dx.doi.org/10.3201/eid2305.161942.
[31] Ben-Hamouda T, Foulon T, Ben-Cheikh-Masmoudi A, Fendri C, Belhadj O, Ben-Mahrez K. Molecular epidemiology of an outbreak of multiresistant *Klebsiella pneumoniae* in a Tunisian neonatal ward. J Med Microbiol 2003;52(5):427–33, doi:http://dx.doi.org/10.1099/jmm.0.04981-0.
[32] Pai H, Kang C-I, Byeon J-H, Lee K-D, Park WB, Kim H-B, et al. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2004;48(10):3720–8, doi:http://dx.doi.org/10.1128/AAC.48.10.3720-3728.2004.
[33] Pragasam AK, Shankar C, Veeraraghavan B, Biswas I, Nabarro LE, Inbanathan FY, et al. Molecular mechanisms of colistin resistance in *Klebsiella pneumoniae* Causing Bacteremia from India—a first report. Front Microbiol 2017;7:2135, doi:http://dx.doi.org/10.3389/fmicb.2016.02135.