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RESEARCH ARTICLE

Biofilm Formation and its Correlation with Antimicrobial Resistance in *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) is an opportunistic pathogen capable of causing a wide range of diseases in humans and animals. The increase, emergence, and spread of antimicrobial resistance among *K. pneumoniae* are the most important health problems worldwide. The production of biofilms by bacterial pathogens exacerbates the complexity of bacterial resistance and prolongs the treatment time. This study analyzed the possible relationship between antimicrobial resistance and biofilm formation in *K. penumoniae* isolated from different sources. Eleven *K. pneumoniae* isolates were recovered from 100 samples comprising 6 (12%) from chicken respiratory organs, 3 (12%) from meat products and 2 (8%) from milk products. All *K. pneumonia* isolates were resistant to ampicillin and amoxacillin-clavulinic acid (100%) followed by cefepime (72.72%), tetracycline, trimethoprime and trimethoprime/sulphamethaxole (54.54% each), while they were sensitive to imipenem (82 %) followed by aztreonam (55%) then amikacin and azithromycin (45% each). It is noteworthy that 10 (90.90%) *K. pneumonia* isolates were multidrug resistant (MDR) and their multiple antibiotic resistance (MAR) indices were far greater than 0.2 (0.846- 9.307). Of note, 81.81% of *K. pneumonia* isolates could produce biofilms, those categorized as strong (33.33%), moderate (22.22%) or weak (44.44%) biofilm producers, whereas 18.18% of the isolates were non-biofilm producers. Interestingly, resistance pattern of *K. pneumoniae* recovered from chicken source was higher than those from milk and meat products. Moreover, there is a non-significant ($P > 0.05$) positive correlation (r= 0.38) between the antimicrobial resistance and biofilm formation in *K. pneumoniae* isolates recovered from animal sources. In conclusion, our results emphasized that biofilm formation may be an important factor that influences the antimicrobial resistance in *K. pneumoniae*, and strict measures of antimicrobial usage should be done in both animal husbandry and humans globally.

Keywords: *Klebsiella pneumonia*; Biofilm; Multidrug resistance; MAR index

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) has become a significant healthcare-associated pathogen. It causes upper respiratory tract infection and `pneumonia in animals housed under stress factors and unhygienic conditions [1]. In dairy animals, it is a common environmental cause of clinical and subclinical mastitis [2].

K. pneumoniae infections are mainly a concern among neonates, old and immunocompromised individuals. This microorganism is also responsible for a major number of community acquired infections such as pneumonia and sepsis [3, 4]. Several virulence factors including fimbriae, antiphagocytic capsule (CPS), lipopolysaccharides (LPS), siderophores, and membrane transporters could help *K. pneumoniae* to survive and escape the innate immune mechanism during infection [5]. *K. pneumoniae* ability to form biofilms can protect the pathogen from the host immune responses and from antibiotics, enhancing its persistence on epithelial tissues and medical device surfaces [6-8]. Interestingly, *cps* gene cluster (capsule),

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mrk genes (type 3 fimbriae) and *wbbM* and *wzm* (belonging to the six-gene wb cluster encoding enzymes for the biosynthesis of O-antigen, which constitutes LPS) contribute to biofilm formation in *K. pneumoniae* [9-11]. In addition, *luxS* (type2 quorum-sensing regulatory system) and *pgaABCD* operon (responsible for synthesis and translocation of poly-β-1,6-N-acetyl-D-glucosamine (PGA) adhesin) affect biofilm development by promoting cell–cell communication process as well as abiotic surface binding and intercellular adhesion, respectively [12,13]. Several studies stated that multidrug resistant (MDR) pathogens isolated from hosts with persistent infections are often biofilm producers [14].Also, it was noted that intrinsic resistance to antimicrobial agents dramatically increases when *K. pneumoniae* isolates grow as biofilms [8]. Despite the reported information available on the correlation between biofilm formation and multidrug resistant *K. pneumoniae* in humans, there have been limited data on this issue from a veterinary overview. Herein, the study was conducted to investigate the prevalence of MDR Klebsiella isolates in respiratory infections in chickens as well as milk and meat products. Further, to evaluate the possible correlations between the antibiotic resistance of Klebsiella isolates and their ability to form biofilms.

Materials and methods

Samples

This study was conducted during the period from November 2018 to April 2019. A total of 100 samples including lung and trachea specimens (n=25 each) from broiler chickens showing respiratory manifestations, milk products [n=25; Rumi cheese (n=10), and cooked, feta and mozerella cheese (n= 5 each)] and meat products [n=25; minced meat (n=10), lunchon (n=10) and sausage (n=5)] were collected from various outlets, Zagazig city, Sharkia Governorate, Egypt. All samples were collected under aseptic conditions and transferred to the laboratory without delay for further bacteriological investigations.

Isolation and identification of Klebsiella species

For isolation of *Klebsiella* species, the samples were inoculated onto HiCrome Klebsiella selective agar media (Himedia, India) followed by incubation at 37°C for 24h. Suspected purple colonies were transferred onto eosin methylene blue (EMB; Oxoid, Uk) and MacConkey`s agar (Oxoid, UK) then incubated at 37°C for 24h for further confirmation. The presumptive isolates were confirmed as Klebsiella based on biochemical tests including IMViC (indole, methyl red, Voges-Proskauer and citrate), lysine decarboxylase as well as their characteristic reactions on triple sugar iron (TSI; Oxoid, UK) agar media [15].

PCR confirmation of Klebsiella isolates

Genomic DNA was extracted from presumptive isolates by the QIAamp DNA Mini kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. Oligonucleotide primers for Klebsiella *gyrA* gene; 5'-CGCGTACTATAC GCCATGAACGTA-3' and 5'-ACCGTTGATCACTTCGGTCAGG-3' [16] and *K. pneumonia* 16S-23S ITS*;* 5'-ATTTGAAGAGGTTGCAAACGAT3' and 5'TTCACTCTGAAGTTTTCTTGTGTTC-3' [17] were used. PCR amplifications were performed with a PTC-100 TM programmable thermal cycler (MJ Research Inc., Waltham, USA) in a total reaction volume of 50 µL consisting of 25µL of Dream Taq TM Green Master Mix (2X) (Fermentas, USA), 1µL of each primer (20 pmole) (Sigma-Aldrich, USA), 5µL template DNA and the volume was completed to 50 µL by nuclease-free water. The amplification conditions for Klebsiella *gyrA* gene were performed as the following: 94°C for 30 s, 55°C for 40 s and 72 °C for 45 s. *K. pneumonia 16S-23S ITS* gene amplification conditions were 94°C for 30 s, 55°C for 30 s and 72 °C for 30 s. An aliquot of each amplicon (8 µL) was loaded on 1.5% agarose gel (Sigma-Aldrich, USA) containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, USA). A100 bp DNA ladder (Fermentas, USA) was used as a molecular weight marker. The amplified DNAs were electrophoresed at 100 V for 60 min on a mini horizontal electrophoresis unit (Bio-Rad, USA). The gel was then visualized and photographed under an UV transilluminator (Spectroline, Westbury, USA). The positive control (*K. pneumoniae* reference strain was kindly obtained from Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza, Egypt) and the negative control (PCR reaction mixture without DNA) were included in each run.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of all *K. pneumoniae* isolates against a panel of 13 widely used antimicrobial agents (Oxoid, Hampshire, England, UK) was conducted according to the standardized disk diffusion method **[**18].The following antimicrobials were tested: ampicillin (10 µg), amoxicillin/ clavulinic acid (20/10 µg), amikacin (30 µg), imipenem (10 µg), azithromycin (15 µg), aztreonam (30 µg), cefepime (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30µg), trimethoprim (5 µg) and trimethoprime/sulphamethoxazole (1.25/23.75 µg). The inhibition zones` diameters were interpreted following the criteria published by Clinical and Laboratory Standards Institute guidelines **[**19]. The isolates showing resistance to at least 3 different antimicrobial classes were categorized as MDR **[**20]. The multiple antibiotic resistance (MAR) index for each isolate was calculated as following: Number of antimicrobials to which the isolate showing resistance / Number of antimicrobials to which the isolate had been tested; while the MAR index for each antimicrobial = Total number of resistance detected / (total number of antimicrobials tested × Total number of isolates) [21].

Detection of biofilm formation by K. pneumoniae

Qualitative Congo red agar method

K. pneumoniae isolates were incubated for 24 - 48 h at 37°C in brain heart infusion agar (BHI; Oxoid, UK) supplemented with 5% (w/v) sucrose and 0.08% (w/v) Congo red dye (Oxoid, UK).The isolates showing red colonies with a dry crystalline consistency were considered exopolysaccharides producers, while white/pink colonies reflected weak exopolysaccharides production [22].

Quantitative microtitre plate method

An overnight culture at 37 °C in trypticase soy broth (TSB; Oxoid, UK) was prepared for each *K. pneumoniae* isolate. Subsequently, 2 µL of cell suspension was inoculated in sterile 96 well-flat bottom polystyrene microtitre plates contained 198 µL of TSB. Negative control wells that contained 200 µL of un-inoculated TSB were included in each test. Incubation was done at 37 °C for 24 h. The wells were gently washed 3 times with 200 µL phosphate-buffered saline (PBS). The wells were dried in an inverted position. The biofilm mass was stained with 50 µL of 0.1% crystal violet (Oxoid, UK). The wells were gently washed with 200 µL of distilled water 3 times and dried in an inverted position. Finally, the wells were dissolved in 200 µL of 5% isopropanol acid to solubilize the stain. Biofilm mass optical density (OD) measurement was done by using a microplate reader (Stat Fax 2100, USA) at 570 nm**.** The OD cut-off (ODc) was defined as three standard deviations above the mean OD of the negative control. All the isolates were classified on the basis of the adherence capabilities into the following categories: non-biofilm producers (OD ≤ ODc), weak biofilm producers (ODc < OD ≤ 2xODc), moderate biofilm producers (2ODc < OD ≤ 4xODc), and strong biofilm producers (4xODc < OD) [23].

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp, 2013, Armonk, USA) was used for statistical analysis of data. Fisher's exact two-tailed test was used to study the antimicrobial resistance of *K. pneumoniae* isolates from different sources. Spearman's correlation coefficient test was performed to measure the strength of a monotonic relationship between paired data i.e. antimicrobial resistance and biofilm formation. The *P* values of < 0.05 were considered statistically significant.

Results

Prevalence of Klebsiella species in different sources

In the present study, 11 Klebsiella isolates were recovered from 100 analyzed samples (11%), comprising 6 (12%) from chicken respiratory organs, 3 (12%) from meat products and 2 (8%) from milk products. Klebsiella isolates were identified by their morphological and biochemical characteristics. They were characterized by purple-magenta mucoid colonies on HiCrome Klebsiella selective agar base, lactose fermenting mucoid colonies on MacConkey's agar, and large mucoid, pink-to-purple colonies on EMB agar medium. The biochemical characters could identify *Klebsiella* species simply. They were positive for Voges-Proskauer, citrate utilization, lysine decarboxylase and urease tests. However, they produce negative reactions with indole and methyl red tests. On the TSI agar media,

Klebsiella isolates produced acid slant / acid butt with gas production and no H_2S production. The genus and species identification of the isolates were further confirmed by the PCR-based detection of Klebsiella *gyrA* gene (441 bp) and the species specific *K. pneumoniae* 16S-23S ITS (130 bp) gene (Figure1 A, B).

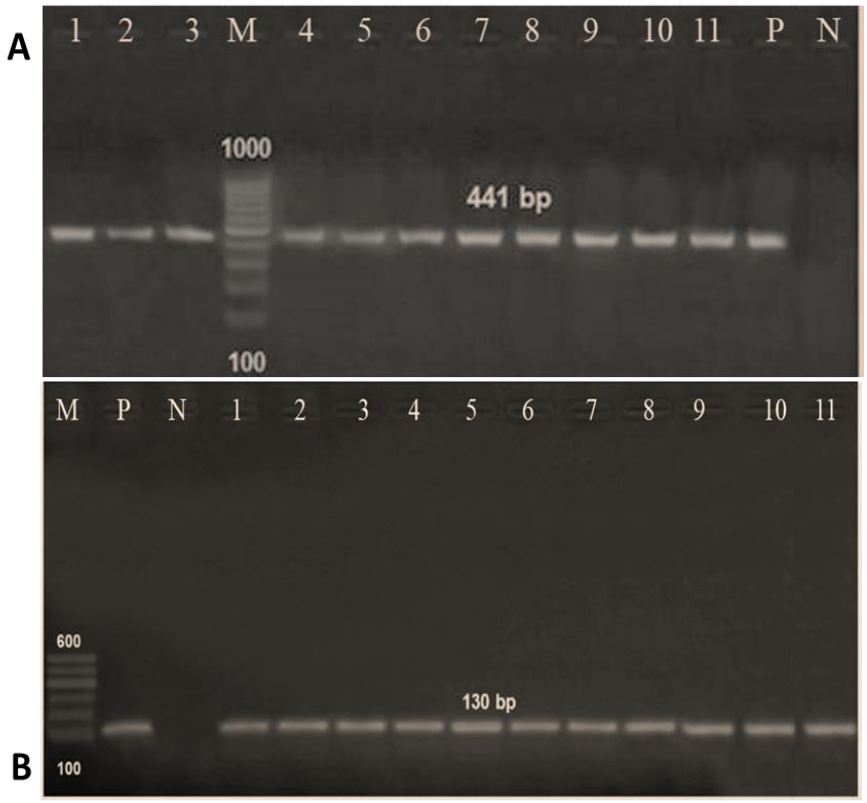

Figure 1 (A, B): PCR amplification of genus specific *Klebsiella* species *gyrA* (A) and species-specific *K. pneumoniae16S-23S ITS* genes (B). Lane M: 100 bp molecular weight marker, Lanes 1-11: positive isolates. Lane P: positive control, Lane N: negative control.

Antimicrobial susceptibility results

The *in vitro* antibiogram of *K. pneumoniae* against 13 antimicrobial agents is depicted in Table 1. All *K. pneumonia* isolates were resistant to ampicillin and amoxacillin-clavulinic acid (100% each) followed by cefepime (72.72%), tetracycline, trimethoprime and trimethoprime/ sulphamethaxole (54.54% each). On the other hand, the tested isolates were sensitive to imipenem (82%) followed by aztreonam (55%) then amikacin and azithromycin (45% each). Statistical analysis revealed non-significant differences in the levels of resistance of *K. pneumoniae* isolated from different sources to the most tested antimicrobials ($P > 0.05$) except for trimethoprime/sulphamethaxole that showed significant variation ($P < 0.05$).

MAR index

The MAR indices of *K. pneumoniae* isolates recovered from chicken, milk and meat products are given in Table1.Analysis of the results showed that *K. pneumoniae* isolates were resistant to at least 2 to 9 of 13 antimicrobials. MAR indices for tested antimicrobials ranged from 0.006 to 0.076. Majority of the isolates (90.90%) were MDR generating MAR indices far greater than 0.2 (0.846- 9.307).

The resistance pattern in K. pneumoniae isolates from different sources

The antimicrobial resistance patterns of *K. pneumoniae* isolates are illustrated in Table 2. Analysis of the results revealed that 4 (36.36%) *K. pneumonia*e isolates from chicken origin were resistant to 8-9 antimicrobial agents. However, 5 (45.45%) *K. pneumonia* recovered from meat and milk products showed resistance to ≤ 6 antimicrobials tested. Thus, the resistance pattern of *K. pneumoniae* of chicken origin was higher than those isolated from milk and meat products.

Table 1: Antimicrobial resistance in *K. pneumoniae* isolated from different sources

Antimicrobial agent	No of resistant *K. pneumoniae*(%)			Total No (%)	MAR index	*P* Value
	Chicken respiratory organs (6)	Milk products (2)	Meat products (3)			
AMP	6 (100.00)	2 (100.00)	3(100.00)	11 (100)	0.076	NE
AMC	6 (100.00)	2 (100.00)	3(100.00)	11 (100)	0.076	NE
AK	1 (16.60)	0 (0.00)	0 (0.00)	1 (9.00)	0.006	0.999
IPM	1 (16.60)	1 (50.00)	0 (0.00)	2 (18.18)	0.013	0.400
AZM	2 (33.30)	0 (0.00)	1 (33.33)	3 (27.27)	0.020	0.999
ATM	1 (16.60)	0 (0.00)	0 (0.00)	1 (9.00)	0.006	0.999
FEB	6 (100.00)	1 (50.00)	1 (33.33)	8 (72.72)	0.055	0.097
NA	3 (50.00)	0 (0.00)	1 (33.33)	4 (36.36)	0.027	0.727
CIP	2 (33.30)	1 (50.00)	0 (0.00)	3 (27.27)	0.020	0.509
C	3 (50.00)	0 (0.00)	0 (0.00)	3 (27.27)	0.020	0.327
TE	4 (66.66)	1 (50.00)	1 (33.33)	6 (54.54)	0.041	0.740
W	5 (83.33)	0 (0.00)	1 (33.33)	6 (54.54)	0.041	0.113
SXT	5 (83.33)	1 (50.00)	0 (0.00)	6 (54.54)	0	0.041

MAR, multiple antibiotic resistance; AMP, ampicillin; AMC, amoxicillin-clavulinic acid; AK, amikacin; IPM, imipenem; AZM, azithromycin; ATM, aztreonam; FEB, cefepime; NA, nalidixic acid**;** CIP, ciprofloxacin; C, chloramphenicol; TE, tetracycline; W, trimethoprim; SXT, trimethoprim/sulphamethaxole; NE, not estimated.
MAR index was calculated for each tested antimicrobial agent.
P-values (Fisher's exact two-tailed test) < 0.05 were considered statistically significant

Table 2: Antimicrobial resistance patterns and biofilm forming ability of *K. pneumoniae* isolated from different sources

Isolate code no	Source	Antimicrobial resistance pattern	Biofilm production		MAR index
			OD	Degree	
1	**Chicken**	AM,AMC,TE,CIP,C,AZM,FEB,SXT, NA	0.558	+	0.692
2	**Chicken**	AM,AMC,TE,C,FEB,SXT	1.48	+++	0.461
3	**Chicken**	AM,AMC,TE,IPM, FEB,SXT,ATM,AK	0.43	++	0.538
4	**Chicken**	AM,AMC,TE,FEB	1.091	+++	`0.307
5	**Chicken**	AM,AMC,TE,C,AZM,FEB,SXT,ATM	1.616	+++	0.615
6	**Milk product**	AM,AMC,TE,C,AZM,FEB	0.27	+	0.461
7	**Chicken**	AM,AMC,CIP,TE,C,FEB,SXT,NA	0.367	++	0.615
8	**Milk product**	AM,AMC	0.261	-	0.153
9	**Meat product**	AM,AMC,AZM	0. 29	+	0.230
10	**Meatproduct**	AM,AMC,TE,C,AZM,SXT	0.324	+	0.461
11	**Meatproduct**	AM,AMC,TE,AZM,FEB	0.227	-	0.384

MAR, multiple antibiotic resistance; AMP, ampicillin; AMC, amoxicillin-clavulinic acid; AK, amikacin; IPM, imipenem; AZM, azithromycin; ATM, aztreonam; FEB, cefepime; NA, nalidixic acid; CIP, ciprofloxacin; C, chloramphenicol; TE, tetracycline;W, trimethoprim; SXT, trimethoprim/sulphamethaxole; +, weak biofilm; ++, moderate biofilm; +++, strong biofilm.

MAR index was calculated for each *K. pneumoniae* isolate.

The biofilm formation ability of K. pneumoniae isolates

K. pneumoniae isolates (n=11) were cultured onto Congo red agar for testing their ability to produce biofilms. Biofilm producer isolates (9/11; 81.81%) could convert the red color of the media into black due to consumption of sucrose. Thereafter, biofilm production by11 *K. pneumoniae* isolates was evaluated using the crystal violet staining method and the results showed a range of absorbance values from 0.227 to 1.616. Nine (81.81%) analyzed isolates were biofilm producers, among them 3 (33.33%), 2 (22.22%) and 4 (44.44%) were strong, moderate and weak biofilm producers, respectively, whereas two (18.18%) isolates were non-biofilm producers (Table 2).

The correlation between biofilm formation and antibiotic resistance in K. pneumoniae isolates

With the exception of a *K. pneumonia* isolate (code No. 1) that showed resistance to 9 antimicrobial agents and weak biofilm producing ability, our results revealed a non-significant ($P > 0.05$) positive correlation (r = 0.38) between the antimicrobial resistance and biofilm formation (Table 3). As presented in Table 2, it was noted that *K. pneumonia* isolate code No. 5 exhibited resistance to 8 antimicrobials with strong biofilm forming ability. Another isolate (code No. 7) that showed resistance to 9 antimicrobial agents was moderate biofilm producer. Moreover, the MDR *K. pneumonia* code No. 4 (resistant to 4 antimicrobials) was categorized as a strong biofilm producer. Also, a *K. pneumonia* isolate (code No. 8) has no biofilm forming ability and the lowest rate of antimicrobial resistance. It was noted in Table 2 that all *K. pneumoniae* isolates producing strong and moderate biofilms showed resistance to ampicillin, amoxicillin-clavulinic acid, tetracycline and cefepime.

Table 3: Spearman's correlation coefficient results showing the correlation between antimicrobial resistance and biofilm formation

Variables	Antibiotic resistance	Biofilm formation
Antimicrobial resistance	**1**	0.376
Biofilm formation	0.376	**1**

Discussion

Klebsiella pneumoniae is a significant opportunistic pathogen causing both human and animal infections. This pathogen became uncontrollable all over the world due to the emergence of MDR isolates [24]. A lot of studies have isolated MDR *K. pneumonia* from a variety of animals as well as humans [25]. However, the correlation between biofilm formation and antibiotic resistance in *K. pneumoniae* is not fully understood. Herein, *K. pneumonia* was recovered from 11 out of 100 samples with an overall prevalence of 11%. It was detected with percentages of 12% in each of chicken respiratory organs and meat products and 8% in milk products. These results are higher than Hayati *et al*. [26] (9.2 %) in Indonesia, and Hossain *et al.* [27] (6%) and Khalda *et al.* [28] (8.69%) in Egypt. On the contrary, our results are lower than Younis and coauthors [29] who reported that *Klebsiella* species were recovered from 33 out of 90 diseased chickens with an isolation rate of 36.67%. In meat products, Messaoudi *et al*. [30] found that the prevalence of *Klebsiella* species in marketed meat samples was 33.33%, out of them 10.52% were identified as *K. pneumoniae*. In contrary to our results, Gaffer and coworkers [31] could isolate extended-spectrum beta-lactamase (ESBL) producing *K. pneumoniae* from dairy samples with a percentage of 13.5%. The variable results of Klebsiella prevalence could be attributed to differences of hygiene and sanitary measures in the examined areas.

K. pneumonia isolates showed absolute resistance to ampicillin and amoxacillin-clavulinic acid followed by cefepime (72.72%), while they were sensitive to imipenem (82%) followed by aztreonam (55%), amikacin and azithromycin (45% each). Similar findings have been reported in a

recent study [32] in which most of *K. pneumoniae* isolates were resistant to ampicillin and cefazolin, while amikacin, piperacillin-tazobactam, and meropenem had the most favorable profile. Similarly, Masood *et al.* [33] reported that *K. pneumonia* isolates were 100% resistant to ampicillin and 100% sensitive to amikacin. Excess antibiotic exposure is the most important factor of antimicrobial resistance. The increase in antibiotic resistance could be attributed to the overuse of antibiotics in the hospitals, community, animal production and agriculture, as well as the facility in purchasing antibiotics freely without prescription. In the health service setting, intensive and prolonged use of antibiotics are very likely the main underlying factor in the widespread transmission of difficult-to-cure antibiotic-resistant nosocomial infections [34].The MDR pattern may be attributed to the unregulated use of antibiotics in veterinary and human medicine in Egypt or due to the horizontal or vertical transfer of plasmid encoding antimicrobial resistance genes among different bacterial pathogens or from animals to humans as was reported elsewhere [35].The overall proportion of MDR *K. pneumoniae* isolates in this study was 90.9%. Similarly, Manjula *et al.* [36] and Nirwati *et al.* [32] reported MDR *K. pneumoniae* isolates with high percentages (90.2 and 54.49%, respectively). One of the mechanisms of resistance used by bacteria is the biofilm formation [37]. Herein, 81.81% of the isolates could produce biofilms, those were categorized as strong (33.33%), moderate (22.22%) or weak (44.44%) biofilm producers, whereas 18.18% of the isolates were non-adherent. In the light of the published data, Nirwati *et al.* [32] found that 85.63% of *K. pneumoniae* isolates were biofilm producers. A similar study reported by Hassan *et al.* [38] stated that 64.7% of the isolates were identified as strong biofilm producers.

Many antibiotics are often excessively and unreasonably used in animal clinics for the treatment of multiple infections [39], which increases the selective pressure for antibiotic and multidrug resistance. In modern livestock production systems, antimicrobials are heavily used for treating diseases and promoting animal growth, which has resulted in an environment conducive to the amplification of antibiotic resistance. In this study, we found that *K. pneumoniae* isolated from chickens showed higher rates of resistance than those isolated from milk and meat products. In a recent study in China, Moran [40], the extensive use and abuse of antimicrobials are common in large-scale pig and chicken farms, but antibiotics are less commonly used in cows and sheep farms, which might explain the lower prevalence of antibiotic resistance among the *K. pneumoniae* isolated from cows and sheep compared to that found among those isolated from pigs and chickens. Some expensive and newly synthesized antibiotics are rarely used in animal agriculture and thus, bacteria exhibit less drug resistance against these antibiotics than against traditional antibiotics. For example, gatifloxacin, imipenem and meropenem are rarely used for the treatment of animal infections.

Cepas *et al.* [41] looked for possible relation of antimicrobial resistance and the ability to form biofilms between the collected samples of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. There was no statistically significant relationship between general MDR and biofilm formation in the three Gram-negative species because the MDR isolates did not show any greater disposition to become a strong biofilm producer compared to non-MDR isolates. However, they reported some correlations between biofilm formation and resistance to specific antibiotics. Resistance to gentamicin and ceftazidime were correlated with biofilm formation in *E. coli*, as well as ciprofloxacin in *P. aeruginosa* and piperacillin, tazobactam and colistin in *K. pneumoniae*. This pattern of resistance should raise grave concerns because colistin is now considered to be the last line treatment choice for *K. pneumoniae* [42].

Our results provide additional evidence supporting this hypothesis, there is non-significant positive correlation between biofilm formation and antibiotic resistance, which contradicts a recently published study of Cepas *et al.* [37]. Additionally, Domenico *et*

al. [43] detected a comparable level of biofilm production in both multidrug- and non - multidrug-resistant *K. pneumoniae* isolates with non-significant differences between the two groups. However, our results are consistent with a recently published study [32] in which antibiotic resistance was greater among biofilm producer *K. pneumoniae* than non-biofilm producers. Moreover, Saha *et al.*[44] demonstrated that all the biofilm-producing isolates presented more resistance patterns in comparison to non- biofilm producers.

In conclusion, the overuse of antibiotics in humans, veterinary medicine, and agricultural practice during the last few decades resulted in the emergence of MDR *K. pneumoniae*. The acquisition of antimicrobial resistance may enhance the biofilm formation in *K. pneumonia* isolate. However, MDR isolates do not present a trend of being greater biofilm producers than non-multi resistant ones.

Conflict of interest

None of the authors have any conflict of interest to declare.

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الملـخـص العربى

تكوين البيوفيلم وارتباطه بالمقاومة للمضادات الحيوية فى الكليبسيلاالرئوية

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تعتبر الكليبسيلا الرئوية ميكروب انتهازي مسئول عن العديد من الامراض في الإنسان والحيوان.كما يعد إنتشار عترات مقاومة' المضادات الحيوي لميكروب الكليبسيلا الرئوية من أهم المشاكل علي مستوي العالم. تساعد قدرة الميكروب على تكوين البيوفيلم في مقاومته للمضاد الحيوي مما يطيل من فتره العلاج. فى هذه الدراسة نحن نسلط الضوء على العلاقة المحتملة بين مقاومة الكليبسيلا الرئوية المعزولة من مصادر مختلفة للمضادات الحيوية وتكوين البيوفيلم. تم عزل إحدي عشر عزلة كليبسيلا رئوية من إجمالي 100 عينه شاملة 6(12%) تم عزلها من الأعضاء التنفسية للدجاج,3(12%) من منتجات اللحوم و2(8%) من منتجات الألبان.قد وجد أن كل عزلات الكليبسيلا الرئوية مقاوم' للأمبيسيلين والأموكساسبلين كلافيولنات (100%,لكل منهم) يليها السيفيبيم (72.72%) ثم التيتراسيكلين والتريميثوبريم (54.54%, لكل منهما), بينما يعتبر الميكروب حساس للأميبينيم (82%) يليه الازترونام (55%) ثم الاميكاسين والازيثرومايسين (45%, لكل منهما). من الملحوظ ان 10 (90.90%) عزلات من الكليبسيلا الرئوية تعتبر متعددة المقاومة للمضادات الحيوية كما أعطى مؤشرMAR الخاص بهم قيم أعلى من 0.2 (0.846-9.307).ووجد ايضا ان 81.81% من عزلات الكليبسيلا الرئوية منتجة للبيوفيلمم صنفين كالاتى :منتج بيوفيلم قوى (33.33%), منتج بيوفيلم متوسط (22.22%) ومنتج بيوفيلم ضعيف (44.44%) ,بينما وجد أن 18.18% من العزلات غير منتج للبيوفيلم. ومن المثير للإهتمام أن نمط المقاومة في الكليبسيلا الرئوية المعزولة من الدجاج تعتبر أعلي من نمط المقاومة في الكليبسيلا المعزولة من منتجات الألبان واللحوم .وقد لوحظ أن هناك علاقة غير معنوية ($P\text{-}value > 0.05$) ايجابيه (r= 0.38) بين تكوين البيوفيلم والمقاومة للمضادات الحيوية في الكليبسيلا الرئوية من مصادر حيوانية. نستخلص من هذه الدراسة ان تكوين البيوفيلم يعتبر عامل اساسي في الثأثير علي اليه المقاومه للمضاد الحيوية في الكليبسيلا الرئويه.ويجب اتخاذ تدابير صارمة في استخدام المضادات الحيوية في كل من الحيوانات والانسان على مستوى العالم .