Microbes and Infectious Diseases 2023; 4(3): 871-883

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Klebsiella pneumoniae isolated from an Egyptian pediatric hospital: Prevalence, antibiotic resistance, biofilm formation, and genotyping

Nancy M. Attia *[1] , Ebtisam F. El-Ghazzawi [1], Fayek S. Elkhwsky [2], Dalia Metwally [1], Asmaa A. Ramadan [3, 4]

1- Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt.
2- Department of Biomedical Informatics and Medical Statistics, Medical Research Institute, Alexandria University, Alexandria, Egypt.
3- Department of Microbiology and Biotechnology, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT), Alexandria, Egypt.
4- Ministry of Health and Population, Alexandria, Egypt.

ARTICLE INFO

Article history:
Received 8 May 2023
Received in revised form 19 June 2023
Accepted 21 June 2023

Keywords:
K. pneumoniae
Antibiotic resistance
Biofilm formation
Genotyping
ERIC-PCR

ABSTRACT

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is a significant contributor to nosocomial infections in neonates and children. This is due to a variety of virulence mechanisms, such as the ability to form biofilms and antibiotic resistance. Hence, this study aimed to evaluate the prevalence of *K. pneumoniae* infections among children, to determine the association of their antimicrobial resistance patterns, biofilm formation ability, and their molecular genotypes. **Methods:** Forty-six *K. pneumoniae* isolates were collected from a pediatric hospital in Alexandria, Egypt. After being identified by conventional methods, they were tested for their susceptibility to different classes of antibiotics, biofilm formation ability, and were genotyped by enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). **Results:** *Klebsiella pneumoniae* was the most prevalent bacteria isolated from children (36%). Antimicrobial susceptibility testing revealed that the majority of our isolates (47.8%) were extensively drug-resistant (XDR) and 41.3 % were multi-drug resistant (MDR). Among the isolates, 80.4% were biofilm producers. No statistically significant association was noticed between the biofilm-producing ability and the drug-resistance type. Based on ERIC-PCR profile results, isolates were classified into 3 main clusters and 30 different ERIC genotypes. Moreover, no statistically significant association was found between ERIC-PCR clusters and neither the ability to produce biofilm nor the drug-resistance type. **Conclusions:** The results of this study indicate an alarming increase in the antimicrobial resistance patterns among *K. pneumoniae* isolated from neonates and children. ERIC-PCR typing showed high

DOI: 10.21608/MID.2023.209787.1520
* *Corresponding author:* Nancy M. Attia
E-mail address: nancy.attia@alexu.edu.eg

genetic diversity among *K. pneumoniae* isolates, indicating a polyclonal distribution of *K. pneumoniae* isolates within the hospital averments.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a significant contributor in severe morbidity and mortality, especially in immunocompromised patients, children and neonates [1]. They are more vulnerable to infections due to their immature immune systems, as well as the repeated use of antibiotics and invasive devices. As reservoirs for the transmission of infection, the hands of healthcare workers and the gastrointestinal tract of hospitalized infants play an important role and are responsible for multiple hospital outbreaks [2]. In Egypt, *K. pneumoniae* is considered to be the most frequently isolated microorganism from different pediatric infections [3,4].

Infections caused by *K. pneumoniae* that are resistant to various antibiotics have become a serious public health problem in recent decades, making these infections extremely difficult to treat. The continual horizontal transfer of antimicrobial resistance genes through mobile elements are critical factors for *K. pneumoniae* to thrive in the hospital environment [5].

The ability of *K. pneumoniae* to form biofilms on the surfaces of medical devices and diseased tissues is thought to be one of the virulence factors required for the pathogenesis of the bacteria, allowing them to persist for long periods despite the presence of an immune system response as well as antimicrobial therapy. Antibiotic resistance is manifested in biofilm-producing bacteria through a variety of mechanisms, including limited antibiotic penetration into the complex biofilm structure, lower bacterial growth rate within the biofilm, and the exchange of resistance genes. Bacterial biofilms also represent a significant risk of dissemination between patients and throughout the hospital environment [6].

Thereby, understanding the clonal relatedness between *K. pneumoniae* isolates causing infections is crucial to determine the source and routes of infection. It is also essential to confirm or rule-out outbreaks, recognize virulent strains, and evaluate the effectiveness of infection-control measures [7]. It has been reported that molecular typing methods would be helpful. Rapid and discriminative typing methods are currently available for the characterization of isolates or strains [8]. Several methods were described for *K. pneumoniae* typing. These include biotyping, serotyping, ribotyping, pulsed-field gel electrophoresis (PFGE), as well as the polymerase chain reaction (PCR)-based typing methods, such as random amplified polymorphic DNA-PCR (RAPD-PCR) and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). Other methods, such as microarrays and sequencing-based methods, are also available, but are more expensive [9].

ERIC-PCR is a PCR-based fingerprinting method that is widely applied for genetic typing and analyzing the genetic diversity among the members of family *Enterobacteriaceae* such as *K. pneumoniae*. ERIC are 127bp sequences that occur as repetitive palindromic units; their position and number vary in bacteria. Because ERIC patterns and numbers differ among the bacterial isolates, they can be employed as genetic markers to permit comparative analysis in a broad range of bacterial species [10].

The current work aimed to study the prevalence of *K. pneumoniae* among bacterial infections in a governmental pediatric hospital in Alexandria, Egypt and to determine the association of their antimicrobial resistance patterns, biofilm formation ability, and their molecular genotypes.

Materials and methods

Over a period of one-year, bacterial isolates were collected from various types of clinical samples (blood, sputum, urine, endotracheal tube (ETT) aspirates, bronchoalveolar lavage (BAL), and cerebrospinal fluid (CSF)) among pediatric inpatients that were admitted in the paediatric hospital. Out of the total number of bacterial isolates (n=153), forty-six *K. pneumoniae* isolates were included in this study.

Identification of *K. pneumoniae* was done by conventional methods, including morphology, culture characteristics and biochemical tests [11]. The antibiotic susceptibility testing was carried out as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2020 [12]. The Kirby-Bauer disc diffusion method was used to test the susceptibility of *K. pneumoniae* isolates to 19 different antibiotics. As for colistin, broth

microdilution method was performed. Phenotypic detection of biofilm formation ability was determined by using the modified microtiter plate test method as described by **Stepanović et al.** [13].

Bacterial DNA of the included isolates was extracted by using the boiling method [14]. For genotyping, the extracted DNA was amplified by ERIC-PCR method using Veriti Thermal Cycler (Applied Biosystems, USA), using the primers ERIC-1 (5′-ATGTAAGCTCCTGGGGATTCAC-3′), and ERIC-2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) as previously described [15]. The amplification reactions were executed under the following conditions: initial denaturation at 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 2.5 minutes, and a final extension cycle at 72°C for 15 minutes. Amplified samples (10 µl) were analyzed by electrophoresis on a 1.7 % agarose gel, stained with ethidium bromide (0.5 µg/mL), and visualized by using UV transillumination. The ERIC-PCR banding patterns of the isolates were first compared for similarity by visual inspection of the band patterns. Clustering of the isolates was performed using the Unweighed Pair Group Method with Arithmetic mean (UPGMA) and Dice similarity coefficient through TotalLab analysis computer software (Version.1.0.1), https://www.totallab.com.

Results

During the study period, a total of 142 samples were culture positive, out of which, (n=131, 92.3%) revealed a single bacterial infection, while (n=11, 7.7%) were mixed-infections. Regarding the samples that revealed single bacteria, *K. pneumoniae* was the most commonly isolated (n=47, 36%). Other pathogens isolated during this period were *Escherichia coli* (n=36, 27%), *Enterobacter spp*. (n=5, 4%), *Citrobacter spp*. (n=1, 1%), *Pseudomonas spp*. (n=7, 5%), *Acinetobacter spp*. (n=4, 3%), *Staphylococcus aureus* (n=10, 8%), Coagulase-negative *Staphylococci* (n=18, 14%) and *Enterococcus* spp. (n=3, 2%). *K. pneumoniae* contributed to (n= 8, 72.7%) of mixed infections. The overall prevalence of *K. pneumoniae* among the isolated bacteria (single and mixed) was (n=55, 36%)

Among the total isolated *K. pneumoniae*, forty-six isolates were included in the present study. They were isolated from different clinical samples, including blood (n=21, 45.6%), sputum (n=11, 23.9%), urine (n=7, 15.2%), ETT aspirate (n=5, 10.9 %), BAL (n=1, 2.2 %) and CSF (n=1, 2.2 %). The majority of the *K. pneumoniae* isolates were obtained from male patients (n=32, 69.6 %). Regarding its distribution according to the hospital unit, most of the isolates were collected from neonatal intensive care unit (NICU) (n=26, 56.5%), followed by the pediatric intensive care unit (PICU), inpatient unit, and surgical unit: (n=13, 28.3%), (n=5, 10.9%) and (n=2, 4.3%) respectively.

By testing the susceptibility of the *K. pneumoniae* isolates towards different antibiotics, the highest level of resistance was encountered for each of cefadroxil, ceftriaxone, and cefepime (n=41, 89.1%), followed by ceftazidime (n=40, 87%), ampicillin/sulbactam (n=37, 80.4%), amoxicillin/clavulanic acid (n= 36, 78.3%), piperacillin/tazobactam, gentamicin (n=35, 76.1%), cefoxitin (n= 34, 74%), and aztreonam (n=34, 73.9%). Resistance towards the carbapenems; imipenem and meropenem was (n=24, 52.2%) and (n=26, 56.5%) respectively. Meanwhile, the highest level of sensitivity was encountered for colistin (n=33, 71.7%) and tigecycline (n=31, 67.4%) **(Figure 1).** By classifying isolates according to drug-resistance type, (n= 22, 47.8%) of the isolates were extensively drug-resistant (XDR), (n= 19, 41.3%) were multi-drug resistant (MDR), and the remaining (n=5, 10.9%) were sensitive (S). Isolates were defined as MDR when they were resistant to at least one agent in three or more antibiotic groups, and as XDR when they were resistant to at least one agent in all but two or less groups [16].

Regarding the ability and the degree of biofilm formation among the *K. pneumoniae* isolates, it was found that (n=5, 10.9%), (n=17, 36.9%), (n=15, 32.6%), (n=9, 19.6%) were strong, moderate, weak, and non-biofilm producers respectively.

The relation between biofilm-producing ability and the drug-resistance type is shown in **table (1).** No statistically significant association was noticed.

The relation between antibiotic resistance profiles in biofilm producer and non-producer isolates is shown in **table (2).**

The genotyping profiles of the 46 *K. pneumoniae* isolates according to ERIC-PCR fingerprinting are presented in **figure (2).** Banding patterns showed 1 to 6 major bands per isolate, with varying molecular sizes ranging from 90 bp to 950 bp. The position of the amplified PCR products

differed, indicating a genetic variation between isolates.

The genetic relatedness among all ERIC-PCR patterns of *K. pneumoniae* based on the collected data is represented in the dendrogram **(Figure 2).** Based on 70% similarity, the isolates were divided into three main clusters: A (11 isolates), B (17 isolates), and C (18 isolates). Each cluster was then further sub-divided into different ERIC genotypes at the individual isolate level based on 100% similarity between the isolates. Isolates showing differences in one or more bands were classified into different ERIC genotypes. In this manner, 30 ERIC genotypes (E1-E30) were hence obtained from 3 ERIC clusters among the 46 *K. pneumoniae* isolates.

The result of the ERIC-PCR cluster classification and ERIC-PCR genotypes in concordance with the isolates, specimen types, hospital wards, drug-resistance types, and the degree of biofilm formation is summarized in **table (3).**

After studying the distribution of the three ERIC-PCR clusters among biofilm-producers and non-producers, it was found that all isolates among cluster A were biofilm producers. No statistically significant association was detected **(Table 4).**

Table 1. Relation between biofilm-producing ability and drug-resistance type (n=46).

	Non-biofilm producer		Biofilm producer		χ^2	^{MC}p
	No.	%	No.	%		
Drug-resistance type						
MDR	2	4.3	17	37.0	1.904	0.375
XDR	6	13.0	16	34.8		
S	1	2.2	4	8.7		

χ^2: Chi-square test.
MC: Monte Carlo.
p: p-value for association between different categories.

Table 2. Percentage resistance against different antimicrobial agents among biofilm-producers and non-producers of *K. pneumoniae* clinical isolates.

	% Resistance				χ^2	^{FE}p
	Biofilm Non-producers (n=9)		Biofilm producers (n=37)			
	No.	%	No.	%		
Amoxicillin/Clavulanic acid	8	88.9	28	75.7	0.743	0.659
Ampicillin/Sulbactam	7	77.8	30	81.1	0.050	1.000
Piperacillin/Tazobactam	8	88.9	27	73.0	1.008	0.421
Cefadroxil	8	88.9	33	89.2	0.001	1.000
Cefoxitin	8	88.9	26	70.3	1.301	0.409
Ceftazidime	8	88.9	32	86.5	0.037	1.000
Ceftriaxone	8	88.9	33	89.2	0.001	1.000
Cefepime	8	88.9	33	89.2	0.001	1.000
Amikacin	3	33.3	17	45.9	0.469	0.711
Gentamicin	7	77.8	28	75.7	0.018	1.000
Ciprofloxacin	7	77.8	22	59.5	1.043	0.450
Levofloxacin	7	77.8	18	48.6	2.476	0.151
Ofloxacin	7	77.8	18	48.6	2.476	0.151
Aztreonam	7	77.8	27	73.0	0.087	1.000
Trimethoprim/Sulphamethoxazole	2	22.2	18	48.6	2.057	0.262
Doxycycline	6	66.7	17	45.9	1.243	0.459
Tigecycline	0	0.0	1	2.7	0.249	1.000
Imipenem	7	77.8	17	45.9	2.940	0.139
Meropenem	7	77.8	19	51.4	2.057	0.262
Colistin	5	55.6	8	21.6	4.112	0.092

χ^2: Chi-square test
FE: Fisher Exact
p: p-value for association between different categories

Table 3. Results of ERIC-PCR genotyping of the studied clinical isolates (n=46).

Cluster	ERIC-Genotype	Isolate No.	Specimen	Ward	Drug-resistance type	Biofilm formation degree
A	E1	41	Blood	NICU	MDR	Moderate
		42	Urine	PICU	MDR	Weak
		44	Blood	Inpatient	XDR	Moderate
		46	Urine	NICU	MDR	Weak
	E2	43	ETT	NICU	MDR	Weak
	E3	40	Blood	NICU	XDR	Strong
	E4	39	Blood	NICU	XDR	Weak
	E5	37	Urine	PICU	XDR	Strong
	E6	29	Blood	NICU	MDR	Strong
	E7	9	Blood	NICU	S	Moderate
		23	Blood	NICU	MDR	Moderate
B	E8	45	BAL	PICU	MDR	Strong
	E9	32	Blood	PICU	S	Moderate
	E10	38	ETT	NICU	XDR	Moderate
	E11	33	Sputum	PICU	MDR	Weak
		34	Blood	NICU	MDR	–
		36	Sputum	NICU	MDR	Moderate
	E12	19	Sputum	PICU	XDR	Moderate
		22	Sputum	PICU	XDR	Moderate
		35	Blood	NICU	S	Moderate
	E13	13	ETT	NICU	XDR	Moderate
		14	Blood	PICU	XDR	–
		16	Urine	NICU	XDR	–
		17	Sputum	PICU	XDR	–
	E14	20	Blood	NICU	S	–
	E15	12	Sputum	Inpatient unit	MDR	Weak
	E16	11	Blood	NICU	XDR	Weak
	E17	10	Blood	NICU	XDR	–
C	E18	30	Blood	Inpatient unit	XDR	Moderate
		31	Sputum	NICU	XDR	Moderate
	E19	24	Sputum	PICU	MDR	Strong
	E20	27	Sputum	PICU	XDR	Weak
	E21	18	Sputum	Inpatient unit	MDR	Weak
	E22	2	Urine	Inpatient unit	XDR	Weak
		3	ETT	NICU	XDR	Weak
	E23	4	Blood	NICU	XDR	Weak
		28	CSF	NICU	XDR	–
	E24	8	ETT	NICU	MDR	Weak
	E25	7	Blood	NICU	MDR	Weak
	E26	21	Blood	NICU	XDR	–
	E27	15	Sputum	PICU	XDR	Weak
	E28	5	Urine	Surgical unit	S	Moderate
		26	Blood	NICU	MDR	Moderate
	E29	6	Urine	Surgical unit	MDR	–
		25	Blood	PICU	MDR	Moderate
	E30	1	Blood	NICU	MDR	Moderate

Table 4. Distribution of *K. pneumoniae* ERIC-PCR clusters according to the biofilm-producing ability.

Cluster \ Biofilm-producing ability	N	Negative		Positive		χ^2	^{FE}p
		No.	%	No.	%		
A	**11**	0	0.0	11	100.0	3.517	0.089
B	**17**	6	35.3	11	64.7	4.239	0.058
C	**18**	3	16.7	15	83.3	0.158	1.000
$\chi^2(^{MC}p)$		5.114(0.062)					

χ^2: Chi-square test
MC: Monte Carlo
^{FE}p Fisher-exact *P* value

Figure 1: Antimicrobial susceptibility testing of the *K. pneumoniae* isolates (n=46); **(SDD:** Susceptible dose-dependent).

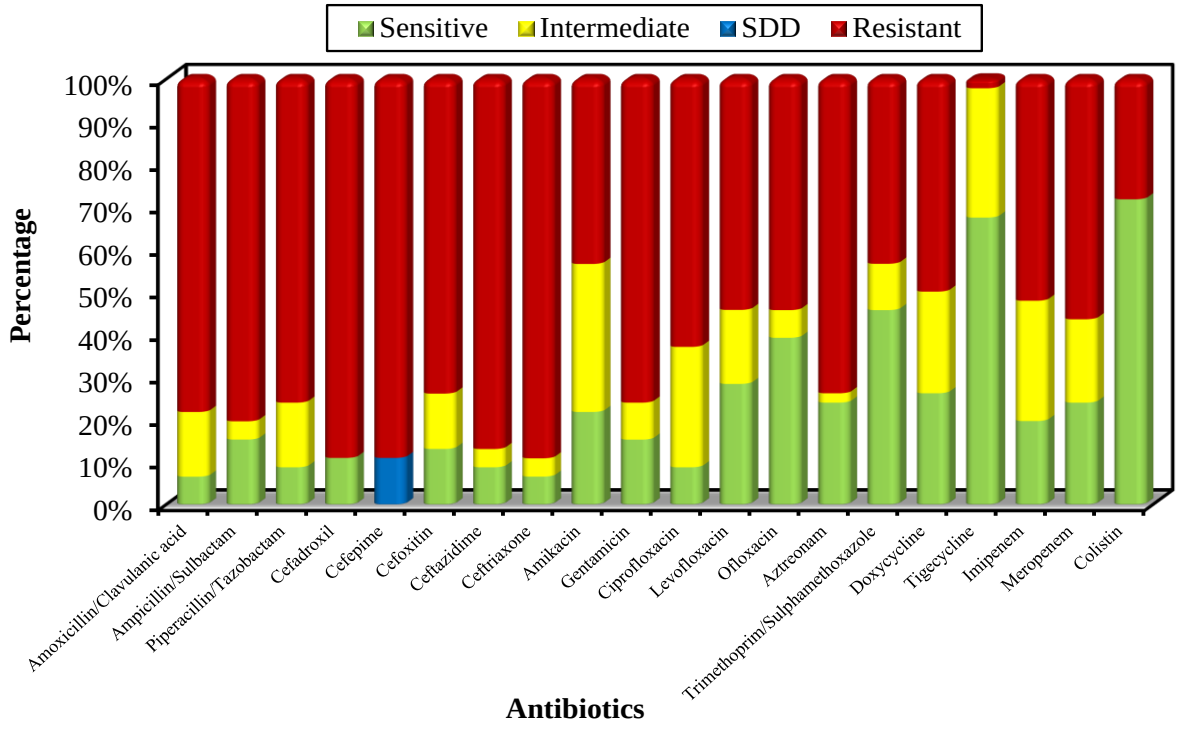
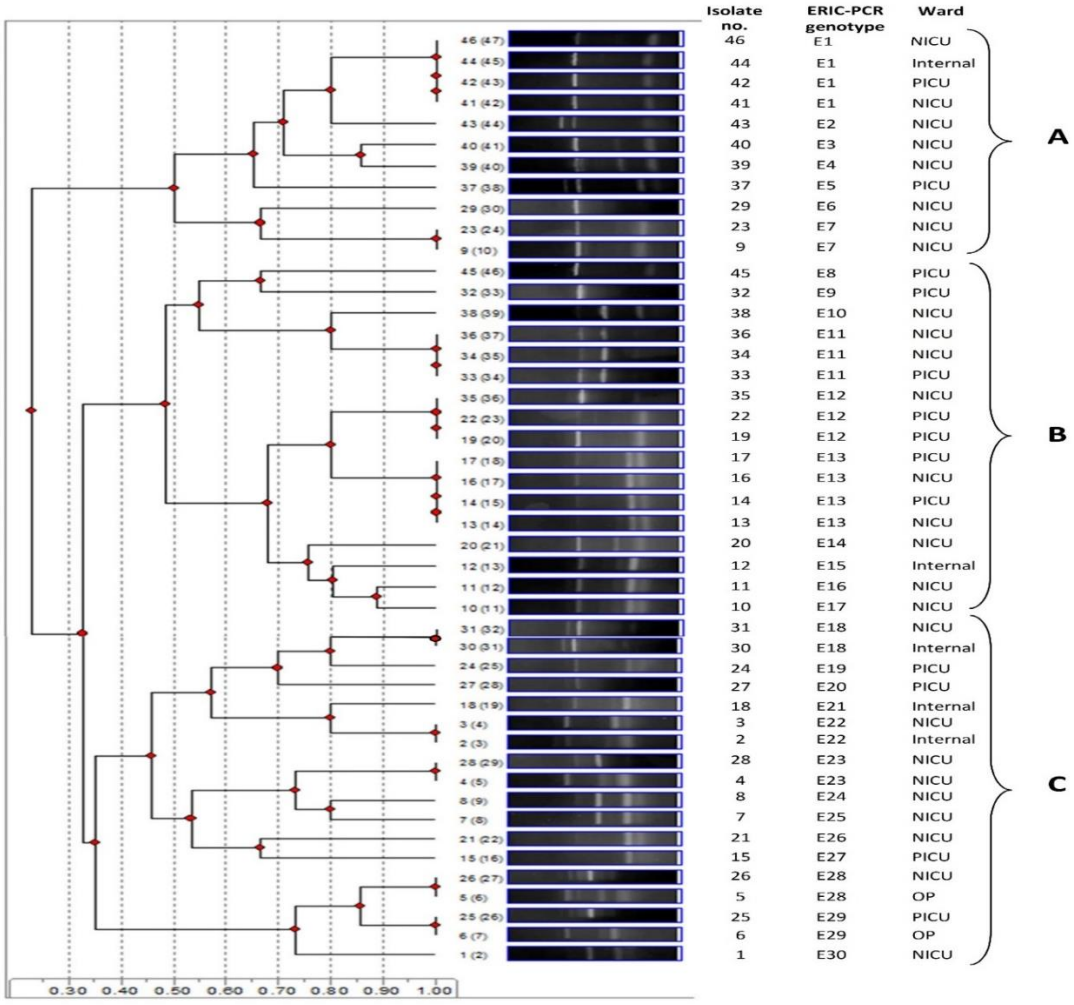


Figure 2. Dendrogram showing the cluster analysis by ERIC-PCR fingerprinting of the 46 *K. pneumoniae* isolates included in this study. The dendrogram shows 30 different patterns and 3 main clusters. Bands were analyzed by applying the Dice similarity coefficient, and the matrix was clustered by the UPGMA method. Isolate number, its corresponding ERIC genotype, and the hospital ward from where it was obtained are also shown. Numbers in brackets refer to lane numbers.



Discussion

K. pneumoniae is one of the most common causes of nosocomial infections in hospitals. It accounts for 3% to 8% of all healthcare associated bacterial infections [17] and is one of the eight most prevalent causes of nosocomial bacterial infections in developed countries[18].

Several studies from Egypt reported a high prevalence of *K. pneumoniae* (73%, 51.35%, 36%, and 33.3%) [19-22]. **Nirwati et al.** found that *K. pneumoniae* was present in 17.36% of all clinical bacterial isolates [23]. On the other hand, **Halim et al.** reported that Coagulase-negative *Staphylococci* (CONS) was the most commonly isolated organism (28.7%), followed by *Klebsiella* (19.7%) [24].

Each hospital setting can have its own unique species. This requires extreme caution when extrapolating data to other hospital settings and intensive care unit (ICUs). The specific pattern of the causative organisms varies between countries and within ICUs depending on the patient's disease, the infection site, hospital antimicrobial protocols, infection control measures, local ecology, and resistance manner [25].

Similar to our study, a high level of resistance to cephalosporins was reported [20, 26, 27]. Regarding resistance to carbapenems, our results are in concordance with **Ghaith et al.** [28]. However, a surprising frequency of carbapenem-resistant *K. pneumoniae* (CRKP) was reported by **Hassuna et al.** (2020) at NICU in Minia, Egypt, with a rate higher than 95% [29]. Resistance to the aminoglycosides was also high in the current study

similar to other studies [26, 29]. Our results also showed moderate resistance to quinolones, whilst the highest sensitivity was to colistin and tigecycline; a similar pattern was also reported by **Tian et al.** [27]. Despite the fact that some antimicrobials (eg: levofloxacin, colistin, tigecycline) are not recommended in the treatment of children and neonates, they were incorporated in this study. Some guidelines state the empiric use of such antimicrobials for the treatment of severe conditions caused by MDR bacteria based on culture results [30, 31].

Hassuna et al. reported that among isolated *K. pneumoniae* 83.3% were XDR, while the rest were MDR isolates [29]. **Mrowiec et al.** reported that all examined strains exhibited MDR phenotype [26], while another study from Indonesia, reported a lower proportion of MDR among *K. pneumoniae* isolates 54.49% [23]. On the contrary, we found that 47.8% of our isolates were XDR, 41.3% were MDR, and only 10.9% were of the S type.

Long-term and excessive usage of various antibiotics frequently results in the development of XDR strains in hospital wards. Furthermore, the longer turnaround time of laboratory diagnosis necessitates the use of empirical antibiotic medication on a regular basis. The utilization of ampicillins and cephalosporins as the first and second lines of empirical treatment in cases of sepsis, meningitis, and pneumonia in neonates and children in Egypt has resulted in substantial resistance levels to these antibiotics [32].

Unfortunately, the alarming level of drug-resistant *K. pneumoniae* isolates has dramatically reduced the treatment options for healthcare associated infections. However, these findings may raise an alert for launching surveillance programs to record the bacterial trends and antibiotic resistance patterns in Egypt [32].

Biofilm production in *K. pneumoniae* has been proposed as a key stage in the pathogenesis of these bacteria [33]. Similarly, another study reported that 85.6% of their isolates were able to produce biofilm [23]. **Seifi et al.** found that an enormous proportion of their *K. pneumoniae* isolates produced biofilms [34]. **Yang et al.** reported that 62.5% of their *K. pneumoniae* isolates had the ability to generate biofilms [35]. This variation could be attributed to changes in geographical location and/or sample size and types.

When comparing the percentages of MDR and XDR properties among biofilm producers and non-biofilm producers, we found a higher percentage than expected among the non-biofilm producers. This is surprising because the resistance profiles are usually enhanced with biofilm production. Consequently, we did not find a significant relationship between biofilm production and antimicrobial resistance.

In agreement with our results, some studies found no relationship between antibiotic resistance and biofilm development in clinical isolates of *K. pneumoniae* [36, 37]. Yet, several studies reported a direct relationship between them [38, 39].

By investigating the discrepancies in the relation between antimicrobial resistance and biofilm formation ability among studies, we could not conclude a discrete reason. However, we suggest that the differences could be attributable to (i) modifications in the methodologies used to test biofilm (the initial bacterial count, media used, glucose supplementation, incubation conditions, fixation and/or elution solvents), (ii) the sample sizes included, (iii) variations in geographical locations of *K. pneumoniae* isolates, and (iv) different antibiotic protocols applied in healthcare facilities.

In the current study, we report a high genetic diversity between the isolates. However, some isolates were related, but when taking a close look at the data concerning them, it was found that most genotypically related isolates were collected from different patients at different time-frames, and from different wards, which most probably negates any cross-contamination/outbreak within the hospital.

It should notably be taken into consideration that the hospital policy, from which we have collected the samples, allows the shifting of patients from one unit to another during the hospital stay. In addition, the isolation of the same *K. pneumoniae* genotype from different patients over a long period of time could indicate the circulation of these strains in the unit they were obtained from. However, the high clonal diversity we have observed among the isolates suggests that some strains could not persist and spread among young patients in the hospital environment. This high diversity observed among *K. pneumoniae* leads to great challenges in the management and/or

treatment of the resultant infections in hospital settings.

Khalil et al. reported that the majority of their isolates were unrelated to each other [40]. **Wasfi et al.** stated that the 28 isolates included in their study were divided into 21 distinct ERIC-genotypes and were highly heterogeneous [41]. Likewise, studies from Malaysia and Turkey reported that their clinical isolates were genetically diverse [42, 43].

In the present study, no association was found between the ERIC-cluster distribution and type of specimen or the place/unit from which the isolate was obtained. This is in agreement with a study from Indonesia [44].

We also did not notice any association between ERIC-cluster distribution and biofilm formation. **Seifi et al.** who also did not observe this relation [34], also support the current study's results. However, these results are in contrast with Diago-**Navarro's et al.** study, in which they observed that strong biofilm-forming *K. pneumoniae* were clustered into a specific type [45].

A significant association between the ERIC-cluster distribution and antibiotic resistance type was not found in this study. This was in agreement with a Malaysian study [43]. In contrast to these findings, an Egyptian study reported significant correlations between antibiotic resistance patterns and the genetic profiles of *K. pneumoniae* isolates [41].

The discrepancies in the observations against typing methods are due to the possibility of genetic changes, that could only be revealed by DNA sequencing or other specific analyses. Thus, the evidence for genetic relatedness and clonality is best-considered relative rather than absolute. It should be emphasized that within the limited number of pediatric studies performed worldwide, our study provides important insights into the epidemiology, resistance, and biofilm-producing ability among *K. pneumoniae* bacteria isolated from neonates and children in Egypt.

In conclusion, *K. pneumoniae* is a highly prevalent pathogen affecting children and neonatal wards in Egypt. A high level of MDR and XDR resistance was detected among the isolates. Although resistance towards colistin and tigecycline is increasing, they are still promising for treating MDR and XDR *K. pneumoniae* isolates, however, extreme care should be taken into consideration when dealing with children/neonatal patients, due to the grave drug toxicities they may cause. *K. pneumoniae* has a profound ability to form biofilms, which enhances its virulence. ERIC-PCR is a rapid, affordable, accessible, and interpretable method that can be easily used for the genetic characterization of bacterial isolates, with a good discriminatory power. *Klebsiella pneumoniae* isolates included in our study were extremely diverse according to cluster analysis, which negates any cross-contamination or outbreak.

Limitations of the study

Azithromycin was intended to be included among the antimicrobial susceptibility test panel as an important antibiotic in treatment of some clinical conditions among children and neonates. However, the discs were not available in the market during the study period.

Ethics approval

The study was approved by the Ethics Committee of the Medical Research Institute, Alexandria University (IORG#: IORG0008812), The ethical approval number issued by the ethics committee is T36/2018.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1- **Branger C, Lesimple A, Bruneau B, Berry P, Lambert-Zechovsky N.** Long-term investigation of the clonal dissemination of Klebsiella pneumoniae isolates producing extended-spectrum β-lactamases in a university hospital. Journal of medical microbiology 1998;47(3):201-9.

2- **Gupta A**. editor Hospital-acquired infections in the neonatal intensive care unit-Klebsiella pneumoniae. Seminars in perinatology 2002; Elsevier.

3- **Azab SF, Sherbiny HS, Saleh SH, Elsaeed WF, Elshafiey MM, Siam AG, et al.** Reducing ventilator-associated pneumonia in

neonatal intensive care unit using "VAP prevention Bundle": a cohort study. BMC infectious diseases 2015;15(1):1-7.

4- **Zakariya B, Vishnu Bhat B, Harish B, Arun Babu T, Joseph N.** Risk factors and outcome of Klebsiella pneumoniae sepsis among newborns. Curr Pediatr Res 2012;16(2):115-8.

5- **Navon-Venezia S, Kondratyeva K, Carattoli A.** Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS microbiology reviews 2017;41(3):252-75.

6- **Percival SL, Suleman L, Vuotto C, Donelli G.** Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of medical microbiology 2015;64(4):323-34.

7- **MacCannell D.** Bacterial strain typing. Clinics in laboratory medicine 2013;33(3):629-50.

8- **Ramadan AA.** Bacterial typing methods from past to present: A comprehensive overview. Gene Reports 2022;101675.

9- **Hansen D, Skov R, Benedi J, Sperling V, Kolmos H.** Klebsiella typing: pulsed-field gel electrophoresis (PFGE) in comparison with O: K-serotyping. Clinical microbiology and infection 2002;8(7):397-404.

10- **Bakhshi B, Afshari N, Fallah F.** Enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis as a reliable evidence for suspected Shigella spp. outbreaks. brazilian journal of microbiology 2018;49:529-33.

11- **Tille P. Bailey & Scott's.** diagnostic microbiology-E-Book: Elsevier Health Sciences; 2015.

12- **Clinical and Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100 Wayne PCaLSI. 2020.

13- **Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M.** A modified microtiter-plate test for quantification of staphylococcal biofilm formation. Journal of microbiological methods 2000;40(2):175-9.

14- **Mullis KB.** The unusual origin of the polymerase chain reaction. Scientific American 1990;262(4):56-61, 4-5.

15- **Bishi D, Verghese S, Verma R.** Molecular typing of colonizing Streptococcus agalactiae strains by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) in a Chennai based hospital. Indian journal of microbiology 2008;48(2):291-6.

16- **Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas M, Giske C, et al.** Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012;18(3):268-81.

17- **Ashurst JV, Dawson A.** Klebsiella Pneumonia. [Updated 2023]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Available at: https://www.ncbi.nlm.nih.gov/books/NBK519004/ 2018.

18- **Khan HA, Ahmad A, Mehboob R.** Nosocomial infections and their control strategies. Asian pacific journal of tropical biomedicine 2015;5(7):509-14.

19- **Gaballah AH, Shawky S, Amer AN.** Microbiological profiles of neonatal sepsis in northern Egypt. Microbes and Infectious Diseases 2022;3(3):645-56.

20- **Elwakil BH, Ali SM, Hafez SF, Bekhit AA, El-Naggar MY, Olama ZA.** Resistance prevalence profile of Klebsiella pneumoniae in the Intensive Care Units of Al-Shatby pediatric hospital, Alexandria, Egypt. Novel Research in Microbiology Journal 2019;3(6):535-45.

21- **Labib JR, Ibrahim SK, Salem MR, Youssef MRL, Meligy B.** Infection with gram-negative bacteria among children in a tertiary pediatric hospital in Egypt. American journal of infection control 2018;46(7):798-801.

22- **Abdel-Wahab F, Ghoneim M, Khashaba M, El-Gilany AH, Abdel-Hady D.** Nosocomial infection surveillance in an Egyptian neonatal intensive care unit. Journal of Hospital Infection 2013;83(3):196-9.

23- **Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, et al.** Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC proceedings 2019; BioMed Central.

24- **Halim MM, Eyada IK, Tongun RM.** Prevalence of multidrug drug resistant organisms and hand hygiene compliance in surgical NICU in Cairo University Specialized Pediatric Hospital. Egyptian Pediatric Association Gazette 2018;66(4):103-11.

25- **Ylipalosaari P, Ala-Kokko T, Laurila J, Ohtonen P, Syrjälä H.** Epidemiology of intensive care unit (ICU)-acquired infections in a 14-month prospective cohort study in a single mixed Scandinavian university hospital ICU. Acta anaesthesiologica scandinavica 2006;50(10):1192-7.

26- **Mrowiec P, Klesiewicz K, Małek M, Skiba-Kurek I, Sowa-Sierant I, Skałkowska M, et al.** Antimicrobial susceptibility and prevalence of extended-spectrum beta-lactamases in clinical strains of Klebsiella pneumoniae isolated from pediatric and adult patients of two Polish hospitals. The new microbiologica 2019;42(4):197-204.

27- **Tian D, Pan F, Wang C, Sun Y, Zhang H.** Resistance phenotype and clinical molecular epidemiology of carbapenem-resistant Klebsiella pneumoniae among pediatric patients in Shanghai. Infection and drug resistance 2018;11:1935.

28- **Ghaith DM, Zafer MM, Said HM, Elanwary S, Elsaban S, Al-Agamy MH, et al.** Genetic diversity of carbapenem-resistant Klebsiella Pneumoniae causing neonatal sepsis in intensive care unit, Cairo, Egypt. European Journal of Clinical Microbiology & Infectious Diseases 2020;39(3):583-91.

29- **Hassuna NA, AbdelAziz RA, Zakaria A, Abdelhakeem M**. Extensively-drug resistant Klebsiella pneumoniae recovered from neonatal sepsis cases from a major NICU in Egypt. Frontiers in microbiology 2020;11:1375.

30- **Barson WJ, Edwards M, Mallory G, Torkya M.** Pneumonia in children: Inpatient treatment. UpToDate Waltham, MA: UpToDate Inc [Internet]. 2017.

31- **Taketomo C, Hodding J, Kraus D.** Pediatric and neonatal dosage handbook: a comprehensive resource for all clinicians treating pediatric and neonatal patients. Lexi-Comp. Inc, Hudson, OH. 2013.

32- **Shehab El-Din EMR, El-Sokkary MMA, Bassiouny MR, Hassan R.** Epidemiology of neonatal sepsis and implicated pathogens: a

study from Egypt. BioMed research international 2015;2015.

33- **Kania RE, Lamers GE, van de Laar N, Dijkhuizen M, Lagendijk E, Tran Ba Huy P, et al.** Biofilms on tracheoesophageal voice prostheses: a confocal laser scanning microscopy demonstration of mixed bacterial and yeast biofilms. Biofouling 2010;26(5):519-26.

34- **Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saee Y, Shirvani F, et al.** Evaluation of biofilm formation among Klebsiella pneumoniae isolates and molecular characterization by ERIC-PCR. Jundishapur journal of microbiology 2016;9(1): e30682.

35- **Yang D, Zhang Z.** Biofilm-forming Klebsiella pneumoniae strains have greater likelihood of producing extended-spectrum β-lactamases. Journal of Hospital Infection 2008;68(4):369-71.

36- **Alcántar-Curiel MD, Ledezma-Escalante CA, Jarillo-Quijada MD, Gayosso-Vázquez C, Morfín-Otero R, Rodríguez-Noriega E, et al.** Association of antibiotic resistance, cell adherence, and biofilm production with the endemicity of nosocomial Klebsiella pneumoniae. BioMed research international 2018;2018.

37- **Cepas V, López Y, Muñoz E, Rolo D, Ardanuy C, Martí S, et al.** Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. Microbial Drug Resistance 2019;25(1):72-9.

38- **Sanchez CJ, Mende K, Beckius ML, Akers KS, Romano DR, Wenke JC, et al.** Biofilm formation by clinical isolates and the implications in chronic infections. BMC infectious diseases. 2013;13(1):1-12.

39- **Vuotto C, Longo F, Balice MP, Donelli G, Varaldo PE.** Antibiotic resistance related to biofilm formation in Klebsiella pneumoniae. Pathogens 2014;3(3):743-58.

40- **Khalil MA, Elgaml A, El-Mowafy M.** Emergence of multidrug-resistant New Delhi metallo-β-lactamase-1-producing Klebsiella pneumoniae in Egypt. Microbial Drug Resistance 2017;23(4):480-7.

41- **Wasfi R, Elkhatib WF, Ashour HM.** Molecular typing and virulence analysis of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from Egyptian hospitals. Scientific reports 2016;6(1):1-11.

42- **Avcioglu NH, Bilkay IS.** Antibiotic resistance, multidrug resistance and enterobacterial repetitive intergenic consensus polymerase chain reaction profiles of clinically important Klebsiella species. Asian Biomedicine.2016;10(1):41-7.

43- **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. Journal of medical microbiology 2009;58(11):1463-9.

44- **Barus T, Hanjaya I, Sadeli J, LAY BW, Suwanto A, Yulandi A.** Genetic diversity of Klebsiella spp. isolated from tempe based on enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR). HAYATI Journal of Biosciences 2013;20(4):171-6.

45- **Diago-Navarro E, Chen L, Passet V, Burack S, Ulacia-Hernando A, Kodiyanplakkal RP, et al.** Carbapenem-resistant Klebsiella pneumoniae exhibit variability in capsular polysaccharide and capsule associated virulence traits. The

Journal of infectious diseases 2014;210(5):803-13.

Attia NM, El-Ghazzawi EF, Elkhwsky FS, Metwally D, Ramadan AA. Klebsiella *pneumoniae* isolated from an Egyptian pediatric hospital: Prevalence, antibiotic resistance, biofilm formation, and genotyping. Microbes Infect Dis 2023; 4(3): 871-883.