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Molecular Characterization of Carbapenem-resistant *K. pneumoniae* Causing Respiratory Tract Infections, and The Antimicrobial Impact of Two Herbal Essential Oils

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Abstract: *Klebsiella pneumoniae* (*K. pneumonia*) is a key pathogen that can cause severe infections such as pneumonia, catheter-associated pneumoniae, and respiratory failure, especially in immunocompromised individuals. Multidrug-resistant (MDR) *K. pneumoniae* has become more common due to the overuse of antibiotics that led to a major threat to public health. The current study aimed to determine the resistance profile of *K. pneumoniae* causing respiratory tract infections (RTI) and detect the frequency of carbapenem resistance encoding genes, along with the determination of the antibacterial activity of thyme (TEO) and marjoram essential oils (MEO) on the MDR *K. pneumoniae* isolates. The antibiotic susceptibility profiles of the *K. pneumoniae* isolates were determined using the Kirby Bauer disc diffusion agar method. The frequency of carbapenem resistance encoding genes including (*bla$_{KPC}$*, *bla$_{OXA}$*, *bla$_{IMP}$*, *bla$_{NDM}$*, and *bla$_{VIM}$*) was determined using conventional PCR. The antimicrobial activity of TEO and MEO was assessed by determination of their minimum inhibitory concentration (MIC). Phenotypic detection of the resistance profile of 19 *K. pneumoniae* isolates causing RTI revealed that 100% of isolates were MDR. The frequency of carbapenem resistance encoding genes was 100 % for *bla$_{NDM}$*, 94.7 % for *bla$_{OXA}$*, and 31.6 % for *bla$_{IMP}$*, while the frequency of *bla$_{VIM}$*, and *bla$_{KPC}$* was 21% and 5.26%. MEO showed higher antimicrobial activity than TEO compared to meropenem as a standard antibiotic. MIC of MEO was 0.244 µL/mL for 10.5% of MDR *K. pneumoniae* isolates while MIC of TEO was 3.9 µL/mL for 5.3% of isolates.

Keywords: Carbapenem resistance; *Klebsiella pneumoniae*; multidrug resistance; respiratory tract; Thyme essential oil; Marjoram essential oil.

1. INTRODUCTION

K. pneumoniae is an opportunistic pathogen that mostly affects hospitalized and immunocompromised patients causing pneumonia, severe catheter-associated pneumonia, respiratory failure, urinary tract infections, bacteremia, meningitis, endocarditis, and cellulitis [1]. The respiratory tract is the main site of *K. pneumoniae* infection [2]. The human oropharynx is usually colonized by *K. pneumoniae* which can cause bacterial pneumonia, it usually affects the upper lobes of the lungs but can involve the lower lobes as well. Patients usually suffer from cough, fever, pleuritic chest pain, and shortness of breath. *K. pneumoniae* results in significant inflammation and necrosis of the surrounding lung tissue [3]. The emergence of MDR *K. pneumoniae* is a global health concern, especially for developing nations, where resources are scarce [4–6]. The pathogenic nature of *K.*

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pneumoniae is attributed to its capacity to develop resistance to numerous antibiotics through various mechanisms and its possession of numerous virulence factors [7]. These mechanisms include antibiotic target alteration, enzymatic antibiotic inactivation and modification, increased expression of the efflux pump system, and porin mutation [6,7]. The most important resistance mechanism is the enzymatic antibiotic inactivation and modification. β-lactamases are classified into carbapenemases, extended-spectrum β-lactamases (ESBLs), and cephalosporinases (AmpC) [8–11]. Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamases that includes extended-spectrum penicillins (e.g. piperacillin), monobactams (e.g. aztreonam), and one or more of the third- and fourth-generation cephems (e.g. cefotaxime, ceftriaxone, etc.). Clavulanic acid or tazobactam are inhibitor of a variety of β-lactamases [12]. Carbapenems have been suggested as the last-line treatment for life-threatening infections caused by *Enterobacteriaceae*. However, selection pressure on carbapenem resistance and the proliferation of carbapenemases has been exerted by the overuse of carbapenems [13].

K. pneumoniae carbapenemase (KPC), the best known of class A carbapenemases which spread all around the world and reduce susceptibility to carbapenems and other β-lactams [14]. Clinically, the most relevant carbapenemase is class B of the Ambler group, which includes the most commonly found New Delhi metalobetalactamase MBL (NDM), imipenemase (IMP), and Verona integron-encoded MBL (VIM) [15]. Oxatype β-lactamases, also known as class D carbapenemases, are a group of enzymes that have been found in human strains of *K. pneumoniae* including the OXA-48 [16].

Scientific researches highlight the development of new antibacterial drugs derived from natural sources [17,18]. To combat the problem of rising antibiotic resistance, alternative and complementary therapies that have been demonstrated to produce antibacterial action may be used. Phytochemicals differ from conventional antibiotics in their target sites and mechanisms [19]. The primary mechanism of the antibacterial action of essential oils (EOs) is mostly linked to the increase in cell permeability and hence the rupture of the plasma membrane. Among several essential oils that may be useful as antimicrobial agents, are *Origanum majorana* (MEO) belonging to the family Lamiaceae, and *Thymus vulgaris* (TEO) belonging to the family Labiateae. Many studies reported the antimicrobial activity of MEO [20,21] and its leaves have been traditionally used to alleviate bronchial asthma [22], therefore, it was used in traditional medicine to treat respiratory tract infections [23]. The microbiological investigations of the MEO have shown antibacterial properties against different pathogenic bacteria including *K. pneumonia*. The antibacterial action of MEO includes multiple mechanisms such as increased membrane permeability, leaking of vital cell contents and quorum sensing inhibition [24–26]. The thyme herb and its extracts are used orally to treat bronchitis, laryngitis, and tonsillitis. Thyme is considered to be broncho spasmolytic, an expectorant, and an antimicrobial agent in cold infections [29].

This research aimed to determine the antimicrobial susceptibility testing of *K. pneumoniae* causing RTI and to detect the frequency of carbapenem resistance encoding genes by PCR, in addition to determining the antibacterial action of TEO and MEO on the MDR *K. pneumoniae* isolates.

2. METHODS

2.1. Materials and Methods should be described Study design and identification of bacterial isolates

All experiments were carried out at the research lab of the Microbiology & Immunology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, and the Genomics Lab at Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, from October 2020 to June 2023.

A total number of 19 clinical isolates of *K. pneumoniae* isolated from sputum specimens were obtained from the Microbiology lab at Al-Demerdash Hospital (Ain Shams University), Cairo-Egypt.

K. pneumoniae isolates were cultivated aerobically at 37◦C on nutrient agar and MacConkey agar plates (Oxoid, London, UK) for 24–48 h and were identified biochemically using standard microbiological tests as growth on triple sugar iron agar [30], Simmon's citrate agar [31], indole test [32], urease tests [33], etc according to identification scheme of Patel et al., (2017) [30]. Identification of *K. pneumonia* was confirmed using the Vitek-2 automated system (Biomérieux, Marcy-LÉtoile,

Paris, France) following the manufacturer's instructions. All *K. pneumoniae* isolates were preserved at −20 ◦C in brain–heart infusion broth with 20% glycerol (Oxoid, London, UK) until use.

2.2. Essential oils

TEO (*Thymus vulgaris*, Labiateae) and MEO (*Origanum majorana*, Lamiaceae) were kindly provided in highly pure form (1000 µl/mL) by Dr. M. Eissa (Biotechnology department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt). All the oils were stored in a refrigerator (4°C) in the dark.

2.3. Antimicrobial susceptibility testing using Kirby–Bauer disc diffusion method

Following the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2021), the antimicrobial susceptibility of all *K. pneumoniae* isolates was determined using the Kirby-Bauer disc diffusion method on Muller-Hinton agar plates (Oxoid, London, UK) [34]. All antibiotic discs were supplied by Oxoid, London, UK and included amoxicillin/clavulanic acid (20/10µg), ceftriaxone (30µg), ceftazidime (30µg), cefuroxime (30µg), cefoxitin (30µg), imipenem(10µg), Amikacin (30µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), ciprofloxacin(5µg), gentamicin (10µg), chloramphenicol (30µg) and colistin (10µg). *K pneumoniae* ATCC700603 was used as a positive control. The results of the disc diffusion assay were interpreted following CLSI 2021 guidelines [34].

MDR K. pneumoniae isolates were identified as isolates that were resistant to ≥3 antimicrobial classes [35].

2.4. DNA extraction and primer design

Total genomic DNA was extracted using G-spinTM Total DNA Extraction Kit (Intron Biotechnology, Seonganam, South Korea) according to the manufacturer's instructions. The extracted DNA was kept at −20 °C till the following stage. Primers for carbapenem resistance encoding genes (*bla$_{KPC}$, bla$_{OXA}$, bla$_{IMP}$, bla$_{NDM}$, and bla$_{VIM}$*) were designed according to the conserved region of the *Klebsiella pneumoniae* subsp. pneumoniae HS11286. The primer specificity was checked using Primer-BLAST tools in NCBI https://www.ncbi.nlm.nih.gov/tools/primer-blast/. All designed primers are listed in (Table 1).

2.1. Molecular detection of resistance genes by the polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) reaction mixture was performed in 25 µL, including 10 µL of PCR master mix (Willofort, Nutingamshire, England) 1 µL of each forward and reverse specific primer (25 nanomoles), 5 µL (250 ng) of DNA template and 8 µL of RNAse free distilled water. The timetable and thermal schedule for each gene are presented in (Table 2). The amplified products were run on 1% agarose gel (Genetix Biotech, New Delhi, India) using ethidium bromide stain (Bioshop, Ontario, Canada) and showed under UV light. For sizing the PCR products, a 100-1000 bp standard DNA ladder (Bengaluru, Karnataka, India) was used.

Table 1. Sequences of PCR oligonucleotide primers

Gene	Primer sequence 5' - 3'	Amplicon size (bp)
Carbapenem resistance encoding genes		
bla$_{KPC}$	F: CGT CTA GTT CTG CTG TCT TG	798
	R: CTT GTC ATC CTT GTT AGG CG	
bla$_{OXA-48}$	F: GCG TGG TTA AGG ATG AAC AC	438
	R: CAT CAA GTT CAA CCC AAC CG	
bla$_{IMP}$	F: CTA CCG CAG CAG AGT CTT TGC	589
	R: ACA ACC AGT TTT GCC TTA CC	
bla$_{NDM}$	F: GCA GCT TGT CGG CCA TGC GGG C	782
	R: GGT CGC GAA GCT GAG CAC CGC AT	
bla$_{VIM}$	F: AAA GTT ATG CCG CAC TCA CC	865
	R: TGC AAC TTC ATG TTA TGC CG	

Table 2. PCR test conditions for the amplification of tested genes.

Stage / Gene	Primary denaturation	Denaturation	Annealing	Extension	Terminal extension
bla$_{KPC}$	95°C- 5 min	94°C- 30 sec	51°C-30 sec	72°C- 30 sec	72°C- 7 min
		30 cycles			
bla$_{OXA}$	95°C- 5 min	94°C- 30 sec	55°C-30 sec	72°C- 1 min	72°C- 7 min
		30 cycles			
bla$_{IMP}$	95°C- 5 min	95°C- 30 sec	60°C- 30 sec	72°C- 30 sec	72°C- 7 min
		35 cycles			
bla$_{NDM}$	95°C- 5 min	95°C- 30 sec	55°C-30 sec	72°C- 50 sec	72°C- 5 min
		30 cycles			
bla$_{VIM}$	95°C- 2 min	95°C- 15 sec	52°C-20 sec	72°C- 15 sec	72°C-2 min
		30 cycles			

Table 3. Results of The Kirby–Bauer disc diffusion sensitivity test for *K. pneumonia* clinical isolates

Antibiotics	**Sensitive No. (%)**	**Intermediate No. (%)**	**Resistant No. (%)**
Amoxicillin-clavulanate	0 (0%)	0 (0%)	19 (100%)
Ceftriaxone	0 (0%)	0 (0%)	19 (100%)
Cefoxitin	0 (0%)	1 (5.3%)	18 (94.7%)
Cefuroxime	0 (0%)	0 (0%)	19 (100%)
Ceftazidime	0 (0%)	1 (5.3%)	18 (94.7%)
Imipenem	4 (21.1%)	1 (5.3%)	14 (73.6%)
Meropenem	2 (10.5%)	0 (0%)	17 (89.5%)
Piperacillin-tazobactam	1 (5.26%)	1 (5.26%)	17 (89.5%)
Gentamicin	4 (21.1%)	0 (0%)	15 (78.9%)
Amikacin	3 (15.8%)	3 (15.8%)	13 (68.4%)
Ciprofloxacin	2 (10.5%)	1 (5.3%)	16 (84.2%)
Colistin	6 (31.6%)	0 (%)	13 (68.4%)
Chloramphenicol	7 (36.8%)	1 (5.3%)	11 (57.9%)

Table 4. Molecular detection of antimicrobial resistance encoding genes among *K. pneumoniae* isolates

Carbapenem resistance genes	**Positive isolates No. (%)**
bla$_{NDM}$	19 (100%)
bla$_{OXA}$	18 (94.7%)
bla$_{IMP}$	6 (31.6%)
bla$_{VIM}$	4 (21%)
bla$_{KPC}$	1(5.26%)

3. RESULTS

3.1. Antimicrobial susceptibility of *K. pneumoniae* using Kirby–Bauer disc diffusion method

The results of the Kirby–Bauer disc diffusion method revealed that 19 (100%) of isolates were resistant to amoxicillin/clavulanic acid, cefuroxime, and ceftriaxone, 18 (94.7%) were resistant to ceftazidime and cefoxitin, 17 (89.5%) were resistant to meropenem and piperacillin-tazobactam while, 13 (68.4%) were resistant to both colistin and amikacin, and 11 (57.9%) showed resistance to chloramphenicol (Table 3). A percentage of 100% of *K. pneumoniae* isolates were MDR being resistant to at least one antimicrobial agent in three or more antimicrobial classes.

3.2. Molecular detection of carbapenem resistance encoding genes

PCR assays revealed that 19 (100%) of MDR *K. pneumoniae* isolates harbored bla_{NDM} gene and 18 (94.7%) harbored $bla_{\text{OXA 48}}$ gene, while 6 (31.6%) harbored bla_{IMP} gene. Four isolates (21%) harbored bla_{VIM} gene and 1(5.26%) isolate harbored bla_{KPC} gene as illustrated in (Table 4) and Figures 1, 2, 3, 4, and 5.

3.3. The antimicrobial effect of MEO and TEO on MDR *K. pneumonia*

The MIC results of TEO and MEO showed that both EOs exhibited different levels of antibacterial activities against the *K. pneumoniae* isolates, the results are shown in Table 5 and indicated that MEO has higher antimicrobial activity than TEO, The MIC of both EOs were compared to breakpoints of MEM as a standard antibiotic. MIC of MEO was 0.244 µL/mL for 10.5% of MDR *K. pneumoniae* isolates while MIC of TEO was 3.9 µL/mL for 5.3% of isolates.

4. DISCUSSION

K. pneumoniae pathogen has been classified as a threat to public health by the World Health Organization [37] and by the Centers for Disease Control and Prevention [38]. It became un-curable despite last-resort antibiotics [39]. It is often linked to healthcare-associated infections that are associated with higher death rates and longer hospital stays [40].

The phenotypic Kirby–Bauer disc diffusion method has been the gold standard for testing antibiotic susceptibility [41]. Kirby–Bauer disc diffusion results revealed that 100 % and 89.5% of *K. pneumoniae* isolates were resistant to amoxicillin-clavulanate, and piperacillin-tazobactam, respectively. Our resistance results were greater than other previous studies. The resistance rate to amoxicillin-clavulanate in two studies in Ethiopia was 85.4%, and 74.1% [42,43], while the resistance to piperacillin-tazobactam in a study conducted in Bangladesh was 51.2% [44]. Additionally, our study showed high antimicrobial resistance to 2nd and 3rd generation cephalosporins; cefoxitin (94.7%), cefuroxime (100%), ceftriaxone (100%), and ceftazidime (94.7%).

Compared to our results, other studies conducted in Ethiopia and Korea also found significant rates of resistance where the Ethiopian study showed that the resistance rate was 86.4% to cefotaxime and 85.4% to both ceftazidime and cefepime [45]. According to a Korean study, the resistance rates to ceftazidime, cefepime, and ceftriaxone were 97.6%, 94.1%, and 88.2%, respectively [43]. Nevertheless, Badamchi et al.(2018) from Bangladesh stated that the resistance rate was 60.2% to ceftriaxone, 32.6% to cefotaxime, and 54.8% to cefepime[46]. The extensive use of broad-spectrum β-lactams may cause the development of high resistance to these antimicrobials that exert a selective pressure. Third-generation cephalosporins are now mostly ineffective against Gram-negative bacteria, including *K. pneumoniae* because of the development of ESBL-producing pathogens. [45]

Our study revealed significant carbapenem resistance, 89.5% of our isolates were resistant to meropenem, and 73.6% were resistant to imipenem. This resistance rate was comparable to an earlier study in Egypt that reported a resistance rate of 68.5% to imipenem and 71.2% to meropenem [47]. Another study in Egypt showed that 31.3% of isolates were resistant to imipenem and 30% were resistant to meropenem, which is a lower rate than our study [48]. In the current study, *K. pneumoniae isolates* showed moderate incidences of resistance to amikacin and chloramphenicol (68.4% and 57.9%, respectively), which is consistent with the findings of Abdelhamid et al., (2020) from Egypt who reported that the frequency of resistance to amikacin, and chloramphenicol was 54% and 40% [49].

The low resistance to amikacin may be due to its limited usage as empirical therapy and the lack of considerable cross-resistance with β-lactam antibiotics [50]

On the other hand, our isolates had an elevated rate of resistance to gentamycin(78.9%) which was comparable to a study in Canada which reported that 66.3 % of their *K. pneumoniae* isolates were resistant to gentamycin [51]; however, Abdelhamid et al., (2020) in Egypt reported a lower percentage (28%) of resistance to gentamycin [49]. The frequency of resistance to aminoglycosides could vary in different

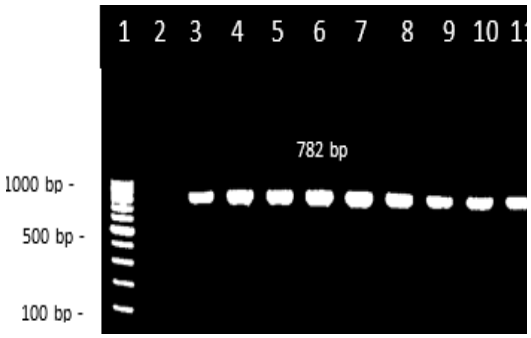

Figure 1. Gel picture of amplified products of *bla*$_{NDM}$ at 782 bps.
Lane 1: Marker, Lane 2: Negative control, Lane 3-11: Positive amplification of the gene.

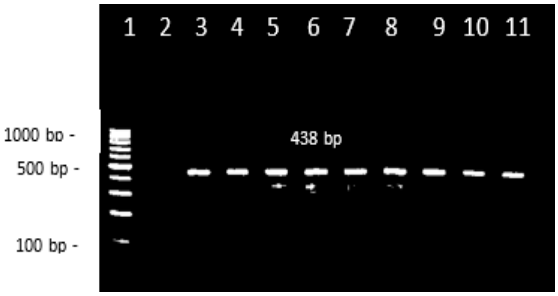

Figure 2. Gel picture of amplified products of *bla*$_{OXA}$ at 438 bps
Lane 1: Marker, Lane 2: Negative control, Lane 3-11: Positive amplification of the gene.

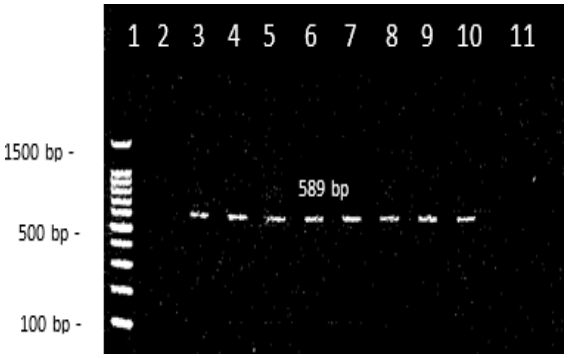

Figure 3. Gel picture of amplified products of *bla*$_{IMP}$ at 589 bps.
Lane 1: Marker, Lane 2: Negative control, Lane 3-10: Positive amplification of the gene.

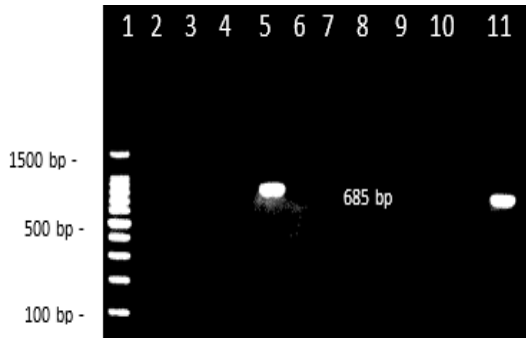

Figure 4. Gel picture of amplified products of *bla*$_{VIM}$ at 685 bps.
Lane 1: Marker, Lane 2: Negative control, Lane 5,11: Positive amplification of the gene.

Figure 5. Gel picture of amplified products of *bla*$_{KPC}$ at 798 bps.
Lane 1: Marker, Lane 2: Negative control, Lane **11:** Positive amplification of the gene.

Table 5. MIC of TEO and MEO against *k. pneumoniae* isolates.

MIC range	MEO No.(%)	TEO No.(%)
≥ 500	0 (0%)	0 (0%)
250	0 (0%)	4 (21.05%)
125	0 (0%)	5 (26.3%)
62.5	0 (0%)	5 (26.3%)
31.25	1 (5.3%)	3 (15.8%)
15.6	2 (10.5%)	0 (0%)
7.8	4 (21.05%)	1 (5.3%)
3.9	3 (15.8%)	1 (5.3%)
1.95	1 (5.3%)	0 (0%)
0.975	4 (21.05%)	0 (0%)
0.48	2 (10.5%)	0 (0%)
< 0.244	2 (10.5%)	0 (0%)
% of resistance compared to MEM (Sensitive ≤ 1µg/mL, Intermediate =2, Resistant ≥ 4µg/mL)	57.9 %	100%

Values are expressed as µL/mL

countries and even hospitals. The interplay of aminoglycoside resistance genes and the expression of aminoglycoside-modifying enzymes contributes to the observed variations in gentamicin resistance among *K. pneumoniae* isolates [52].

Although colistin is now used as a last-line treatment for infections of the most virulent multidrug-resistant Gram-negative bacteria [53], the current investigation has shown a concerning state of *K. pneumoniae* resistance to colistin which was 68.4%. Reports from the Sentry Antimicrobial Surveillance Program conducted in the Philippines stated that colistin resistance existed in 50% of their *K. pneumoniae* isolates [54].

Significantly, Aris et al., (2020) reported that colistin-resistant *K. pneumoniae* were extremely common and dispersed throughout the Middle East, particularly in Turkey and Iran, where a prevalence of up to 75.6% was recorded[55]. Colistin resistance is considered a serious worldwide antimicrobial problem. Many studies conducted in Egypt reported increased frequencies of colistin resistance over the years; Zafer et al.,(2019) indicated that the percentage of colistin-resistant *K. pneumoniae* isolates was just 4.9%[56]. Also, Emara et al., (2019) reported that 7.5% of their *K. pneumonia* isolates were colistin resistant [57], while Rabie & Abdallah, (2020) showed that the percent of colistin resistance among their *K. pneumoniae* isolates was 17.2% [58]. This could be because colistin therapy which was discontinued many years ago is now used as a last-resort antibiotic for carbapenem-resistant *K. pneumoniae* (CRKP) infections.

In the current investigation, all *K. pneumoniae* isolates were MDR. Our results were in accordance with Shivannavar, (2014) and Abdelhamid et al., (2020) who found that 90.2% and 94% of their *K. pneumoniae* isolates were MDR [49,59]. Additionally, Kot et al., (2023) reported that the MDR isolates were highly isolated from respiratory tract infections (93.7%) [60]. Furthermore, Erami et al., (2015) recorded that 46.6% of the isolates of *K. pneumoniae* were MDR [61]. The high MDR percentage in our study could be a result of misuse and abuse of antibiotics in Egypt.

Molecular identification techniques offer several advantages over traditional methods, such as being more accurate, sensitive, and rapid [62,63]. The high prevalence of antibiotic resistance for many classes is associated with the simultaneous existence of most resistance-encoding genes [64]. Various carbapenem resistance encoding genes were investigated in the present study. Our study reported high frequencies of the bla_{NDM} and bla_{OXA-48} (100%, and 94.7%, respectively) among *K. pneumoniae* isolates. These high frequencies were significantly greater than those of several studies, including one from India that found that the frequencies of bla_{OXA-48} and bla_{NDM} were 80.4%, and 31.4% [65].

In contrast, the frequencies of bla_{IMP}, and bla_{VIM} in our study were 31.6%, and 21%, respectively. The prevalence of bla_{IMP} and bla_{VIM} was greater than those reported by several studies; Mohamed et al., (2018) from Egypt showed that the prevalence of bla_{VIM} and bla_{IMP} were 11.1% and 0%, respectively [66]. Another study by Urmi et al., (2020) in Bangladesh showed that the prevalence of bla_{VIM} and bla_{IMP} were 19% and 10.3% [67]. Leila et al., (2018) from Iran showed that the prevalence rates of bla_{VIM} and bla_{IMP} were 2.18% and 0.5%[68]. On the other hand, the prevalence of the bla_{KPC} in the current study was 5.26% which was less than that reported by Onyedibe et al., (2015) in Nigeria(16.7%) [69] and Urmi et al, (2020) in Bangladesh (15.5%) [67]. While Leila et al., (2018) showed the absence of bla_{KPC} 0%, among their isolates [68]. This variation in resistance rates might be due to vast differences in geographical areas, sample sizes, different strains, and different types of antibiotics used in the treatment of infections [70,71].

Globally, the rise of MDR *K. pneumoniae* harboring several antimicrobial resistance genes represents a significant threat. The meta-analysis by Heidary et al., (2018) revealed a comparatively high frequency of drug-resistant *K. pneumoniae* isolates [72]. Geographical distance, hospital prescription patterns for antibiotics, and patient hygiene standards could all be contributing factors to this variance. Antibiotic exposure is the main factor contributing to antimicrobial resistance [73]. Numerous causes, including the use of antibiotics in healthcare facilities, communities, farm animals, agriculture, and the environment, all contribute to the rise of antibiotic resistance. Antibiotics are overused because they may be purchased easily over the counter without a prescription. Prolonged and heavy antibiotic use in the healthcare context is most likely the primary cause of the widespread nosocomial infections that are resistant to antibiotics and difficult to treat [74].

Antibiotic usage must be monitored closely to reduce antibiotic misuse, as antibiotic resistance is spreading quickly [75,76]. It is imperative to continue studying the genes that lead to the rise of antibiotic resistance in bacteria [76,77], to develop new antibiotics and

strategies to combat antibiotic resistance and preserve the effectiveness of antibiotics [78].
Using complementary and alternative therapies that have been demonstrated to produce antimicrobial activity could be one approach to combat the problem of increasing antibiotic resistance [79]. The World Health Organization (WHO) supports, encourages, and facilitates the effective use of herbal medicine for health programs in developing countries [80]. Essential oils were proven to be one of the antibiotic alternatives that have significant antibacterial action against different microorganisms [81]. Our results revealed that the MIC of MEO was 0.244 µL/mL for 10.5% of isolates while the MIC of TEO was 3.9 µL/mL for 5.3% of isolates and the susceptibility rate of MDR *K. pneumoniae* to MEO was 42.1% compared to MEM.

In the current study, MEO has a higher antimicrobial effect than TEO. These results agreed with that reported by El-Tablawy & Araby, (2017), who found that MEO was the most effective EO among all the tested EOs [82]. In the same line Nnenna& Esther et al., (2022) reported that the least antibacterial activity was shown for TEO [83]. Also, Boskovic M et al., (2015) reported that MEO exhibited a greater antimicrobial effect compared to TEO [84]. On the other hand, the antibacterial activity of TEO and MEO was investigated by Gurgulova et al., (2006), and reported high antibacterial activity for both TEO and MEO (MIC=0.012-0.025%) [85].

Furthermore, Diniz et al., (2023) reported that TEO showed antibacterial potential, with a strong inhibitory activity against *K. pneumoniae* with MIC values ranging from 64 to 512 µg/mL [86]. Since the antibacterial activity of essential oils can act on one or more targets, a series of events within the bacterial cell is thought to be the mechanism of action rather than a single site of action. Several theories for the antibacterial properties of essential oils have been suggested, including the disruption of a variety of enzyme systems responsible for the manufacture of structural components and energy [87].

Finally, with the growing prevalence of drug- and multidrug-resistant human infections, it is essential to discover new sources of antibiotics. The current study revealed that MEO could be used as a good alternative therapy since its antimicrobial effect was prominent compared to MEM as a standard antibiotic. Further studies on the antibacterial mechanisms and activity of MEO are required to clarify its mode of action and prove its effectiveness.

5. CONCLUSIONS

In this study, all *K. pneumoniae* isolates were MDR and harbored various carbapenem resistance encoding genes which are alarming signs. A proper surveillance system should be implemented to prevent an impending healthcare disaster. Researchers should find alternative therapies for treating drug-resistant bacterial infections. MEOs might present an opportunity as an alternative or combination therapy with antibiotics for the management of MDR *K. pneumoniae* infections.

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List of abbreviations:

AmpC: Cephalosporinases, **EOs**: Essential oils, **ESBLs**: Extended-spectrum β-lactamases, **HAIs**: Healthcare-associated infections, **IMP**: Imipenemase, ***K. pneumonia:*** *Klebsiella pneumoniae,* **KPC**: *Klebsiella pneumoniae* carbapenemase, **MBLs:** Metalobetalactamase, **MDR**: Multi-drug resistant, **MEM**: Meropenem, **MEO**: Marjoram essential oil, **MIC**: Minimum

inhibitory concentration, **NDM:** New Delhi Metalobetalactamase, **OXA**: Oxatype β-lactamases, **PCR:** Polymerase chain reaction, **RTI**: Respiratory tract infections, **TEO**: Thyme essential oil, **VIM:** Verona integron-encoded MBL.

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