International Journal of Infectious Diseases 120 (2022) 103–112

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

The first nationwide surveillance of carbapenem-resistant *Enterobacterales* in the United Arab Emirates – increased association of *Klebsiella pneumoniae* CC14 clone with Emirati patients☆

Ágnes Sonnevend[1,2], Najiba Abdulrazzaq[3], Akela Ghazawi[1], Jens Thomsen[4], Greeshma Bharathan[1,5], Lilla Makszin[6], Tahir A. Rizvi[1,7], Tibor Pál[1,2,*], for the UAE CRE Study Group[#]

[1] *Department of Medical Microbiology and Immunology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates*
[2] *Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Pécs, Hungary*
[3] *Department of Medicine, Al Kuwait Hospital, Dubai, United Arab Emirates*
[4] *Abu Dhabi Public Health Center, Abu Dhabi, United Arab Emirates*
[5] *Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, United Arab Emirates*
[6] *Institute of Bioanalysis, Faculty of Medicine, and Szentágothai Research Center, University of Pécs, Pécs, Hungary*
[7] *Zayed Center for Health Sciences, United Arab Emirates University, United Arab Emirates*

ARTICLE INFO

Article history:
Received 25 January 2022
Revised 13 April 2022
Accepted 16 April 2022

Keywords:
Carbapenem-resistant *Enterobacterales*
Metallo-beta-lactamases
Klebsiella pneumoniae ST14
Country-wide surveillance
Middle East

ABSTRACT

Objectives: To assess the current prevalence, distribution, and main clonal types of carbapenem-resistant *Enterobacterales* (CRE) in the United Arab Emirates.
Methods: A total of 504 CRE collected over a 9-month period in 15 hospitals were studied. Antibiotic susceptibility and the presence of common carbapenemase, 16S methylase, and mobile colistin resistance genes were assessed. Selected strains forming larger clusters by pulsed field gel electrophoresis were subjected to whole genome sequencing to identify their sequence types and core genome MLST.
Results: Strains expressing OXA and NDM type carbapenemases and 16S methylases were present in all major hospitals. Considerable interhospital differences were noticed, suggesting the role of specific clones. A total of three major *Klebsiella pneumoniae* clones (CC14, ST231, and CC147) were identified, accounting for 48.6% of all CRE. All clones were significantly more resistant than sporadic isolates. CC14 strains exhibited a significant association with Emirati patients.
Conclusions: Nearly half of CRE infections in the country are due to a limited number of clones. The data indicate the possibility of interhospital transmission, combined in some hospitals with inadequate stewardship practices. The study also revealed an association of the largest, most resistant clone (CC14) with Emirati patients. The specific reasons for it should be clarified by further investigations.

☆ Present address: Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, United Arab Emirates.

* Corresponding author at: Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Pécs, Szigeti út 12, Pécs 7624, Hungary.
E-mail addresses: pal.agnes@pte.hu (Á. Sonnevend), najiba.abdulrazzaq@ehs.gov.ae (N. Abdulrazzaq), akelag@uaeu.ac.ae (A. Ghazawi), jthomsen@adphc.gov.ae (J. Thomsen), greeshmabharathan@gmail.com (G. Bharathan), lilla.makszin@aok.pte.hu (L. Makszin), tarizvi@uaeu.ac.ae (T.A. Rizvi), pal.tibor2@pte.hu (T. Pál).

[#] **UAE CRE Study Group members**: Adnan Alatoom (Cleveland Clinic, Abu Dhabi); Karen Lawlor (Cleveland Clinic, Abu Dhabi); Anju Nabi (Rashid Hospital, Dubai); Deeba Jafri (Fujairah Hospital, Fujairah); Fouzia Jabeen (Sheikh Khalifa Hospital, Ajman); Hala Ahmed Fouad Ismail (Al Baraha Hospital, Dubai); Handan Celiloglu (Mediclinic City Hospital, Dubai and Mohammed Bin Rashid University, Dubai); Ibrahim Sayed Mustafa Alhashmi (Qassimi Hospital, Sharjah); Manal Abdelfattah Ahmed Aly (Saqr Hospital, Ras Al-Khaimah); Moeena Zain, (American Hospital, Dubai); Aaron Han (American Hospital, Dubai and Mohammed Bin Rashid University, Dubai); Mubarak Alfaresi (Sheikh Khalifa General Hospital, Umm Al-Quwain); Riyaz Amirali Husain (Dubai Hospital, Dubai); Shaikha Al Kaabi (Tawam Hospital, Al Ain); Somansu Basu (NMC Hospital, Al Ain); Stefan Weber (Sheikh Khalifa Medical Center, Abu Dhabi); Zulfa Omar Al Deesi (Latifa Hospital, Dubai).

https://doi.org/10.1016/j.ijid.2022.04.034

Introduction

Data on carbapenem-resistant *Enterobacterales* (CRE) in countries of the Arabian Peninsula (AP) are limited to small-scale, local studies that investigated the mechanism of carbapenem resistance, but seldom applied molecular typing techniques to reveal the clonality of the strains (Abd El Ghany et al., 2018, Al-Agamy et al., 2018, Al-Baloushi et al., 2018, Alotaibi et al., 2017, Jamal et al., 2016, Memish et al., 2015, Moubareck et al., 2018, Pal et al., 2017, Sonnevend et al., 2015b, Zaman et al., 2018, Zowawi et al., 2014). These studies used varied techniques and studied disproportionate number of isolates from different hospitals collected during different time periods. Recently, a study on strains from 13 hospitals across the Kingdom of Saudi Arabia revealed regional and interhospital differences regarding species and mechanisms of carbapenem resistance, but prevalent clones were not identified (Al-Abdely et al., 2021). Consequently, little is known about the comparative prevalence and clonal distribution of CRE strains in countries and hospitals in the AP. The lack of comparable, typing-based studies limits the understanding of the dynamics of any endemic situation.

In the United Arab Emirates (UAE), data showed that the rate of ertapenem nonsusceptibility in 2019 was 5.5% among clinical isolates of *Klebsiella pneumoniae* and 1.8% among *Escherichia coli* (MOHP, 2021). Limited scale studies revealed that although the most prevalent carbapenemases were members of the OXA-48-like group, metallo-beta-lactamase (MBL) producers represented a considerable proportion (>40%), limiting the use of beta-lactam/beta-lactamase inhibitor combinations (Al-Baloushi et al., 2018, Pal et al., 2017, Sonnevend et al., 2020, Sonnevend et al., 2012, Sonnevend et al., 2015b). KPC-producers have been encountered only occasionally (Sonnevend et al., 2015a). Colistin resistance rate as high as over 20% among CRE was recorded and, at least in Dubai, a few highly resistant *K. pneumoniae* clones, particularly ST14, dominated (Moubareck et al., 2018, Sonnevend et al., 2020). Although these data suggest a different epidemiology of CRE than in several other parts of the world (Hansen 2021), systematic surveillance of CRE in the UAE is required for meaningful comparison. The UAE has one of the highest and most diverse expatriate population and the country is a highly frequented commercial and touristic hub (CIA, 2022). Consequently, local prevalence of antibiotic resistant pathogens is likely to affect global epidemiology. In the current study, for the first time, we studied the nationwide epidemiology of CRE, collecting isolates from 15 hospitals of different service levels. The antibiotic resistance, its genetic background, and the molecular types of the isolates were studied, identifying the most common clones present regionally and nationwide. A possible association of the dominant clones with particular patient groups were also analyzed.

Materials and Methods

Strain collection

Between July 1, 2018 and March 31, 2019, the laboratories of 15 hospitals in the UAE (Supplementary Table 1) were requested to submit all *Enterobacterales* strains exhibiting nonsusceptibility to any carbapenems isolated from human clinical and screening samples. Data obtained were age, sex, nationality (ie, Emirati or non-Emirati), days of hospitalization before sample collection, and specimen types (blood, respiratory specimen, wound and tissue samples, urine, and screening samples). Patients' identities were masked and known only to submitting hospitals. Repeat isolates were marked and the only first ones expressing any distinct carbapenem resistance mechanisms were included in the study. For analysis, the country was divided into 3 major regions of strain collection, which included Abu Dhabi Emirate (ABD), Dubai Emirate (DUB) and Sharjah and the Northern Emirates, which included Ajman, Umm Al-Quwain, Ras Al-Khaimah, and Fujairah (SNE). Hospitals contributing ≥10 isolates were considered individually; others were combined in each region as "smaller hospitals".

Antibiotic susceptibility testing

The panel of antibiotics, methods of quantitative susceptibility testing and the interpretation criteria applied were described earlier (Sonnevend et al., 2020). Strains exhibiting susceptibility to a single drug were considered extremely drug resistant (XDR), and those resistant to all as pan resistance (PDR). Because aztreonam/avibactam is still investigational and fosfomycin is not available for parenteral application in the UAE, susceptibility to these 2 antibiotics was not considered when setting XDR and PDR categories. A resistance index (R index) was calculated as the number of drugs of the 17 tested a strain was nonsusceptible to.

Assessing the genetic background of antibiotic resistance

The phenotypic assay for carbapenemase activity, the PCR methods detecting the 5 common carbapenemase (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$) and 16S ribosomal methylase genes and the case of strains exhibiting colistin MIC >1 mg/L of the *mcr 1–5* genes were described earlier (Sonnevend et al., 2020). In colistin-resistant strains that were subjected to whole genome sequencing (WGS), the presence of *mcr 6–10* was established using ResFinder 4.0 (Zankari et al. 2012). In the same group of strains, the integrity of chromosomal genes *mgrB, phoP, phoQ, pmrA*, and *pmrB* were compared with the respective genes of *K. pneumoniae* MGH78578 (GenBank Acc. No. CP000647) (Olaitan et al., 2014).

Molecular typing

Pulsed field gel electrophoresis (PFGE) patterns of *K. pneumoniae* and *E. coli* strains (Gautom, 1997) were analyzed by the GelCompar II software (Applied Maths, Belgium). Clusters exhibiting ≥80% similarity were further divided into subclusters with ≥90% similarity, and multiple members of those with different carbapenem resistance mechanisms were subjected to WGS. Genomic DNA extracted using the Wizard® Genomic DNA Purification Kit (PROMEGA, USA) was sequenced, applying the 150 bp paired-end approach on Illumina HiSeq platform (Novogene Company Limited, Hong Kong). Contigs assembled using CLC Genomic Workbench v20.0 (QIAGEN Aarhus, Denmark) were uploaded to PathogenWatch (https://pathogen.watch/) to confirm species identification and for sequence type (ST) determination according to the Pasteur *K. pneumoniae* (Diancourt et al., 2005) and the Warwick *E. coli* MLST (Wirth et al., 2006) schemes. WGS reads were uploaded to the European Nucleotide Archives under the study number PRJEB50277. STs and clonal complexes (CC), including single-locus variants (SLV) with >10 members were considered major clones, whereas other strains were considered sporadic. Strains within the same PFGE subclusters of major clones (ie, exhibiting ≥90% similarity) were considered as part of the respective clones. The STs of 40 tentatively assigned, randomly selected isolates were confirmed by traditional MLST typing (Diancourt et al., 2005). For creating minimum spanning trees on the basis of the core genome MLST (cgMLST), the SeqSphere+ software (Ridom© GmbH, Münster, Germany) was used. cgMLST clusters were considered "major" if containing ≥4 members. Capsular loci, siderophore, and hypermucoidity-determining genes of *K. pneumoniae* strains were deduced from the WGS data using Kleborate on the PathogenWatch site.

Statistical analysis

This was performed using the SPSS Statistics Version 28.0 (IBM, 2021). Differences in categorical variables were evaluated by the Pearson chi-square test. Continuous variables were compared by independent samples *t* test or, in case of non-normal distribution, by the Mann-Whitney *U* test. The impact of nationality, age, sex, days of hospitalization, and types of samples on the isolation of particular species or STs were analyzed by univariate and by the multivariate logistic regression models.

Results

The collection

Altogether, 752 strains were received. After excluding non-*Enterobacterales,* non-CRE, and repeat isolates, the study included 504 strains, of which 75.2% were *K. pneumoniae*, 17.9% *E. coli*, and 6.9% represented other species (Supplementary Table 2). The characteristics and contribution of hospitals are shown in Supplementary Table 1, and sample types and basic demographic data of patients are provided in Supplementary Table 2. According to the figures reported to the National Antimicrobial Resistance Surveillance Program of UAE, the strains investigated represented 45.9% of all nonrepeat CRE isolates encountered in the participating hospitals during the study period (Jens Thomsen – unpublished).

A total of 45% of the samples from patients with known nationality (N = 500) were obtained from Emirati patients (Supplementary Table 3). Emiratis were significantly older, and the ratio of male patients were significantly lower among them. No specific sample type was significantly more common in either of the nationality groups. The average time of hospitalization before isolating the strains was 48.5 ± 95.6 days, with no significant difference between the Emirati and non-Emirati patients. Nevertheless, significantly more samples were taken after >30 days of hospitalization from Emirati patients (Supplementary Table 3).

Antibiotic resistance

The nonsusceptibility rate, MIC_{50} and MIC_{90} values of the collection, as well as those of the 2 major species and the rest of the strains are presented in Table 1. Overall, 35 (6.9%) of the isolates were XDR and 17 (3.4%) were PDR. The resistance rate of the collection to ceftazidime-avibactam was 44.6%; this value reached 74.3% among the 35 XDR strains (data not shown). On the other hand, when aztreonam was combined with this inhibitor, 6.2% of all isolates and only 5.7% of the XDR strains were nonsusceptible (data not shown). Altogether, 119 (23.6%) strains were resistant to colistin and the rate was even higher (28.8%) among the 379 *K. pneumoniae* isolates.

Antibiotic resistance genes

The distribution of different carbapenemase genes is presented in Table 2. Interestingly, although in *K. pneumoniae* $bla_{\text{OXA-48-like}}$ was the most common carbapenemase gene detected, in *E. coli* and in other species, bla_{NDM} was dominating. bla_{KPC} was encountered in 7 *K. pneumoniae*, forming 4 distinct PFGE patterns (Supplementary Figure 1). The rate of multiple carbapenemase producers, mostly $bla_{\text{OXA-48-like}}$ and bla_{NDM} coproducers, was particularly notable in *K. pneumoniae*. Overall, 223 of the 504 isolates produced an MBL enzyme (44.2%). A total of 69 strains (13.7%) in the collection did not carry the carbapenemase genes targeted, and none of them exhibited any carbapenemase activity. At least one 16S modifying enzyme gene was detected in 271 of the 504 isolates (53.8%), with *armA* being the most common (Table 2). Mobile

Table 1
Antibiotic resistance and related characteristics of the strains

Characteristics	The collection (*N=504*)			*Klebsiella pneumoniae (N=379)*			*Escherichia coli (N=90)*			Other species (*N=35*)		
	NS* (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	NS* (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	NS* (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	NS* (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Ceftazidime	93.8	>128	>128	95.5	>128	>128	95.6	>128	>128	68.6	>128	>128
Ceftazidime/Avibactam	44.6	≤0.25	>128	50.7	2	>128	52.2	128	>128	51.4	16	>128
Cefotaxime	97.8	>128	>128	98.2	>128	>128	98.9	>128	>128	91.4	>128	>128
Ertapenem	100.0	256	32	100.0	64	256	100.0	16	128	100.0	16	64
Imipenem	79.2	8	64	82.6	4	64	62.2	2	8	85.7	8	64
Meropenem	80.0	128	128	84.7	16	128	62.2	2	32	74.3	8	32
Aztreonam	91.5	>128	>128	94.5	>128	>128	75.6	>128	>128	62.9	>128	>128
Aztreonam/Avibactam	6.2	0.5	2	0.8	0.5	1	30.0	2	16	2.9	<0.25	2
Ciprofloxacin	93.8	>64	>64	95.5	>64	>64	94.4	>64	>256	74.3	4	64
Gentamicin	68.8	256	>256	76.8	>256	>256	45.6	2	>256	42.9	4	>256
Amikacin	56.7	>256	>256	69.7	>256	>256	16.7	4	>256	20.0	8	>256
Trimethoprim-Sulfamethoxazole	80.4	>256	>256	83.6	>256	>256	82.2	256	>256	40.0	1	>256
Tetracycline	53.0	16	>256	47.2	8	>256	81.1	256	256	42.9	8	256
Chloramphenicol	78.8	32	>256	83.9	64	>256	53.3	16	256	88.6	16	256
Colistin	23.6	≤0.5	32	28.8	<0.5	32	3.3	<0.5	<0.5	20.0	<0.5	>256
Tigecycline	41.5	1	4	50.9	2	4	8.9	0.25	0.5	22.9	1	4
Fosfomycin	21.6	16	128	26.1	16	128	5.6	0.5	8	14.3	16	64
Other resistance-related characteristics												
MER MIC >8 mg/L (%)	49.2			56.5			23.3			34.3		
XDR (%)	6.9			8.7			1.1			2.9		
PDR (%)	3.4			4.5			0.0			0.0		
R index (X ± SD)	11.105 ± 2.62			11.59 ± 2.46			9.83 ± 2.24			9.08 ± 3.20		

NS – Nonsusceptible

Table 2
Frequency of resistance genes

Genes	Frequency of genes present (%) in The collection *(N=504)*	*Klebsiella pneumoniae (N=379)*	*Escherichia coli (N=90)*	Other species *(N=35)*
$bla_{OXA\text{-}48\text{-}like}$ [S]	40.9	45.4	33.3	11.4
bla_{NDM} [S]	31.5	27.2	47.8	37.1
$bla_{OXA\text{-}48\text{-}like}$ + bla_{NDM}	12.1	14.8	4.4	2.9
bla_{KPC} [S/C]	1.4	1.8	0.0	0.0
bla_{VIM} [S/C]	0.4	0.0	0.0	5.7
No carbapenemase	13.7	10.8	14.4	42.9
Multiple carbapenemases	12.5	15.3	4.4	2.9
Any metallo-beta-lactamases	44.2	42.2	52.2	45.7
arm A [S/C]	33.7	43.3	3.3	8.6
rmtB [S/C]	3.2	2.4	7.8	0.0
rmtC [S/C]	0.6	0.5	0.0	2.9
rmtF	18.1	22.7	3.3	5.7
Any 16S methylase	53.8	66.5	14.4	17.1
Multiple 16S methylase	1.6	1.8	0.0	0.0

[S] Present as a single gene of similar functions (i.e. carbapenemase or 16S methylase)
[S/C] Present as a single gene or in combination with genes of similar functions (i.e. other carbapenemase or 16S methylase)

Table 3
Major clones of *Klebsiella pneumoniae* identified

Clones (N)*	MLST determined by WGS (N)	Strains with ≥90% PFGE similarity (N)	SLVs within cluster (N)	% among *K. pneumoniae**	% in the collection*
CC14 (154)	36	118	ST78 (3) ST2096 (1)	40.6	30.6
ST231 (48)	13	35	0	12.7	9.5
CC147 (43)	18	25	ST392 (2)	11.3	8.5
3 clones (245)	67	178	NA	64.6	48.6

* Including strains tentatively considered to belong to the same ST/CC clone based on ≥90% PFGE similarity

colistin-resistant genes were detected by PCR targeting *mcr 1–5* in the 3 colistin-resistant *E. coli* isolates only, all representing *mcr-1.1*. Two of them exhibited limited similarity by PFGE, whereas the third represented a different pattern (Supplementary Figure 2).

Molecular typing

The 379 *K. pneumoniae* isolates exhibited 58 distinct PFGE patterns, with 1 isolate being nontypable. The largest cluster contained 154 *K. pneumoniae* strains (40.6% of the species), followed by 1 with 48, and 1 with 43 members. Of the 90 *E. coli* isolates, 81 were typable, exhibiting 32 distinct patterns (data not shown).

A total of 97 (25.6%) of the *K. pneumoniae* and 27 (30.0%) of the *E. coli* isolates were subjected to WGS analysis. *E. coli* represented a variety of STs (ST38, ST131, ST167, ST205, ST359, ST405, ST410, ST448, ST617, ST648, ST940, ST1158, ST1193, ST1284, ST1303, ST1431, ST1702, ST2659, ST3541, ST8346) without forming any major clusters (Supplementary Table 4). On the other hand, 3 major *K. pneumoniae* clones, corresponding to the 3 large PFGE clusters, were identified, which are CC14 (including SLVs ST78 and ST2096), ST231, and CC147 (including SLV ST392). Together with isolates exhibiting ≥90% PFGE pattern similarities to them, these clones represented 64.6% of the species and 48.6% of the entire collection (Table 3). Strains not submitted to WGS due to forming small PFGE clusters only, singleton STs, and those forming smaller groups (ST11, ST13, ST15, ST48, ST108, ST336, ST353, ST383, ST395, ST441, and ST6085) were considered sporadic (Table 3 and Supplementary Table 5).

Members of the 3 large *K. pneumoniae* clones were resistant to significantly more antibiotics, had a higher meropenem MIC, and significantly more often carried a 16S methylase gene compared with sporadic isolates. Furthermore, CC14 strains were also significantly more resistant to colistin and tigecycline, more often XDR or PDR, and more frequently coproduced NDM and OXA-48-like carbapenemases than their sporadic counterparts (Supplementary Table 6.). All 16 *K. pneumoniae* ST147 and 29 (90.6%) of the 32 *K. pneumoniae* ST14 isolates subjected to WGS carried the genes of the capsular locus KL64, whereas 3 *K. pneumoniae* ST14 harbored those of KL2. All 13 *K. pneumoniae* ST231 strains with the WGS determined carried the genes of KL51. In none of the 36 colistin-resistant *K. pneumoniae* strains subjected to WGS were any *mcr* genes detected, whereas in 30 of them (83.3%), various mutations in the *mgrB, phoP, phoQ, pmrA,* and *pmrB* were identified. The *rpmA1* gene together with the *iucA* gene were present in 4 *K. pneumoniae* ST383 isolates; thus, these isolates could be considered hypervirulent *K. pneumoniae* by genotype. A total of 18 *K. pneumoniae* isolates carried multiple siderophore genes (Supplementary Table 5).

Regional variations of CRE in the UAE

Strains from the SNE region exhibited significantly higher R index values, were more likely XDR or PDR, and exhibited higher rate of tigecycline resistance than those of the other 2 regions. The only parameter in which another region (ABD) exceeded significantly those of the other 2 collection areas was the rate of ceftazidime-avibactam resistance (Figure 1). Within each region, specific hospitals could be identified (ABD-C, DUB-D, SNE-B) exhibiting the highest regional values for most resistance-related parameters (Figure 1). In hospital SNE-B, all figures but the rate of resistance to ceftazidime-avibactam were the highest of all major hospitals nationwide (Figure 1).

Isolates carrying $bla_{\text{OXA-48-like}}$, bla_{NDM}, multiple carbapenemases, MBL, *armA*, and *rmtF genes* were present in all major hospitals, and their distribution between regions was relatively uniform (Figure 2). Within the regions, however, considerable interhospital variability existed. This was noteworthy in the case of NDM- and MBL-producing strains in the DUB and SNE and OXA-48-like expressing isolates in the SNE regions (Figure 2). The nationwide distribution of MBL-producing strains matched that of strains exhibiting ceftazidime-avibactam resistance (Figure 1, 2). The rate of strains carrying *armA* in the SNE region significantly exceeded those in the other 2 collection areas. In hospitals of the DUB and SNE but not in the ABD region, rates of the 2 common 16S methy-

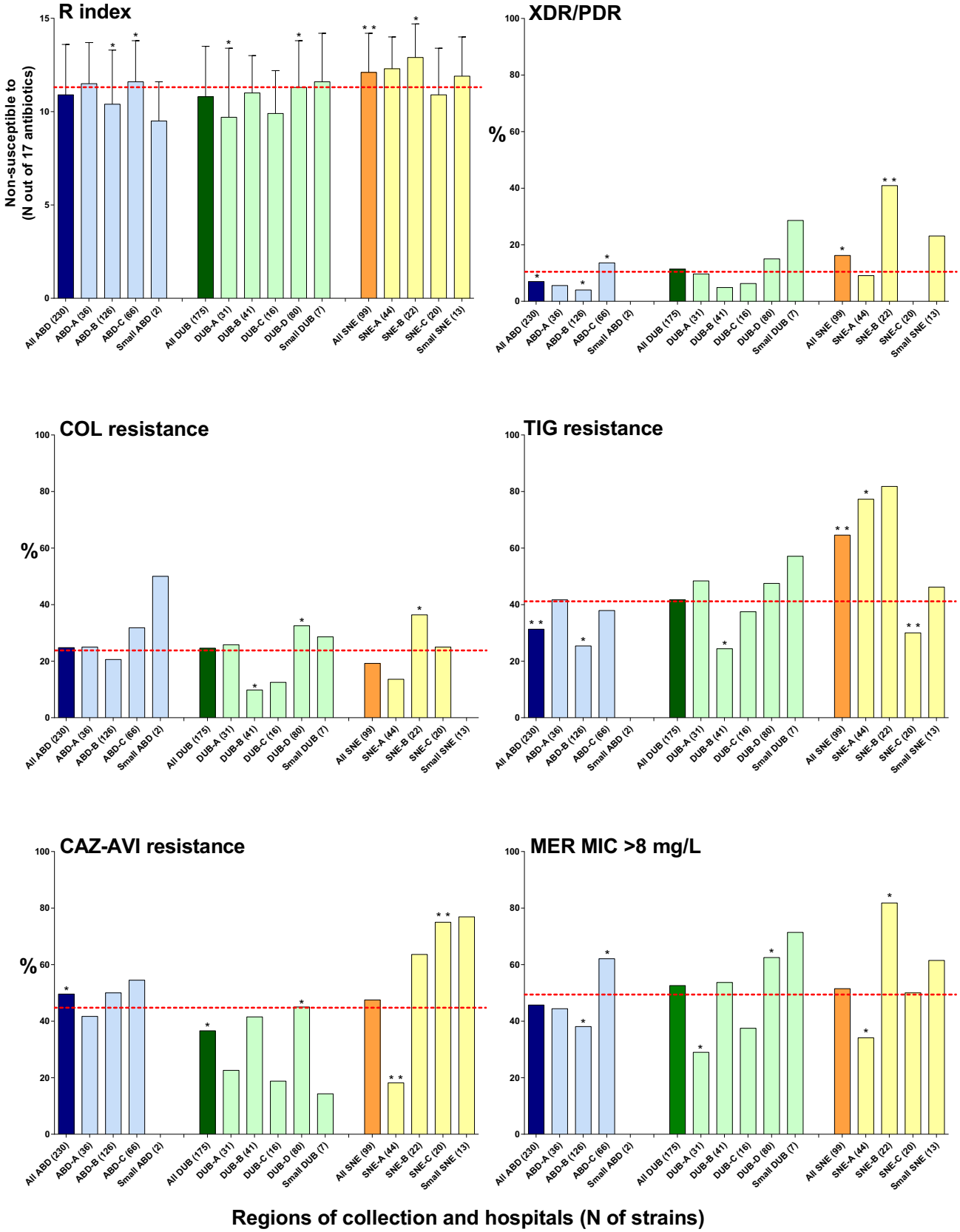

Figure 1. Antibiotic resistance among strains collected in different regions and hospitals.
Legend* indicates significance level *p=0.05-0.001*, ** indicates significance level *p<0.001*. If above columns representing catchment areas (darker colors) they indicate differences from the other 2 regions combined. If above a column of any particular hospital (lighter colors), they indicate that the value is significantly different from those of the other hospitals combined from the same region. Dotted line indicates the average of all hospitals in the UAE.
COL – colistin, TIG – tigecycline, CAZ-AVI – ceftazidime-avibactam, MER - meropenem.

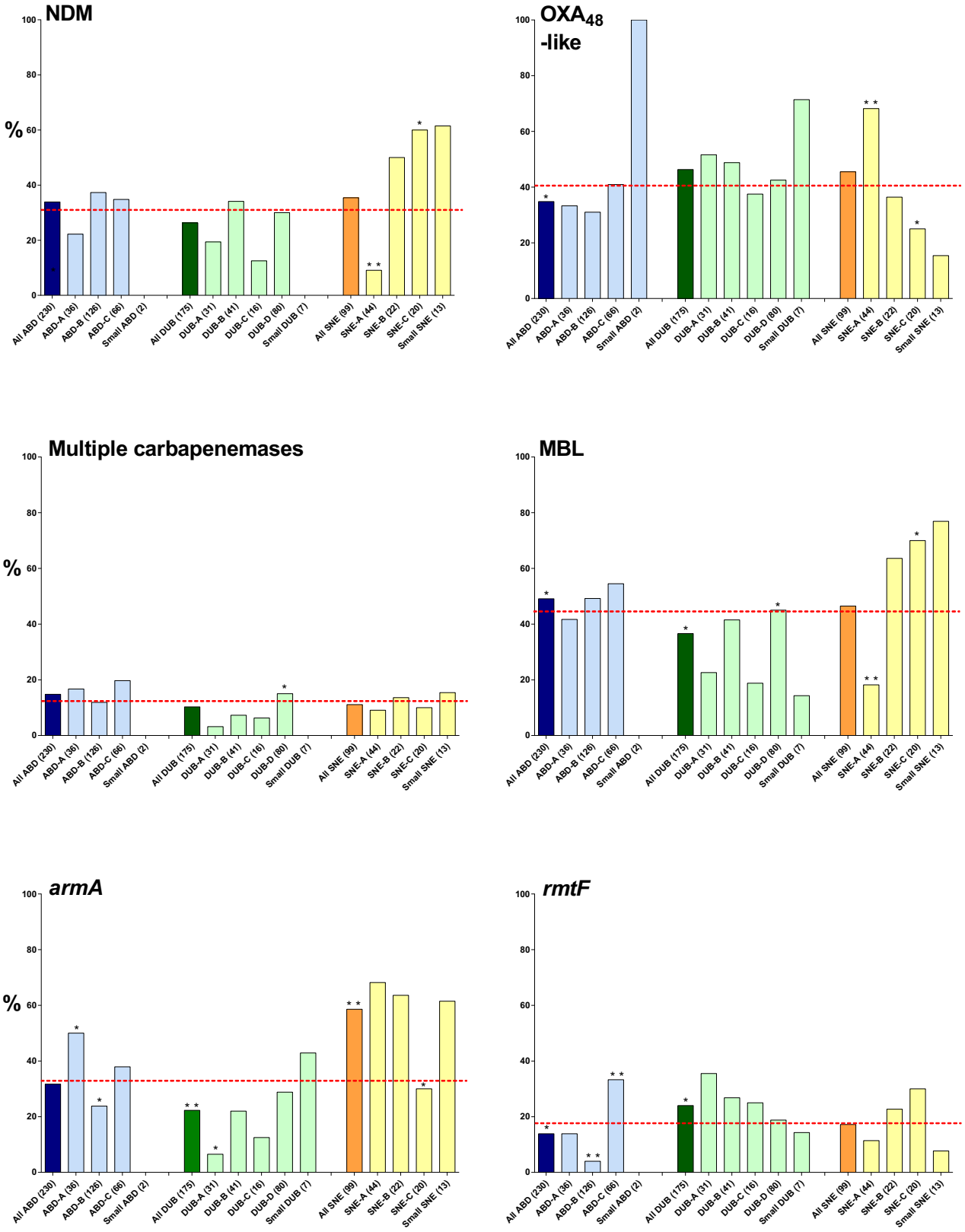

Figure 2. Antibiotic resistance genes strains collected in different regions and hospitals.
Legend: * indicates significance level *p=0.05-0.001*, ** indicates significance level *p<0.001*. If above columns representing catchment areas (darker colors) they indicate differences from the other 2 regions combined. If above a column of any particular hospital (lighter colors), they indicate that the value is significantly different from those of the other hospitals combined from the same region. Dotted line indicates the average of all hospitals in the UAE.
MBL – metallo-beta-lactamase.

lase genes (*armA* and *rmtF*) exhibited opposite trends; a higher figure of 1 was usually associated with a lower rate of the other (Figure 2).

Representatives of CC14 were encountered in all, CC147 strains were isolated in most major hospitals, whereas ST231 strains were absent in the SNE region with uneven distribution in ABD and DUB hospitals (Figure 3). The prevalence of CC14 strains was the highest in the SNE region, with exceptionally high rates in hospitals SNE-A and SNE-B. Hospital ABD-A was the only hospital outside of SNE region with a CC14 rate exceeding the country's average. Nevertheless, even in major hospitals with the lowest prevalence of this clone (hospitals DUB-A and DUB-C), 16.7% of their *K. pneumoniae* belonged to CC14.

Among ST231 strains, all 5 members of cgMLST Cluster 1 were encountered in the same hospital (Hospital ABD-C) (Figure 4 A). On the other hand, the 4 isolates in Cluster 1 of ST147 were distributed among 3 hospitals of 2 regions (Figure 4 B). All 3 major clusters (Clusters 1, 2, and 3) of ST14 contained strains from multiple sources. It was noteworthy that although from the same catchment area, ST14 strains of hospital SNE-A and most from hospital SNE-B belonged to 2 distinct clusters (Clusters 1 and 2) (Figure 4 C). A comparison of CC14 strains from the hospitals with the highest CC14 rates in the country (Figure 3) revealed that although isolates from hospitals SNE-A were mostly OXA-48-like producers, those encountered in hospital SNE-B commonly expressed MBL with concomitantly high rate of ceftazidime-avibactam resistance and with significantly higher meropenem MIC values (Supplementary Table 7).

Association of CC14 strains with Emirati patients

K. pneumoniae was significantly more often isolated from Emirati patients, whereas all other species were more common in the non-Emirati group (Supplementary Table 3). Univariate analysis of age, sex, nationality, sample type, and time of hospitalization revealed that samples from Emirati nationals were 1.896 times more likely to yield *K. pneumoniae* (95% C.I. 1.221–2.945, p = 0.004). A single year increase of age increased the likelihood of isolating this pathogen 1.021 times (95% C.I. 1.012–1.030, p <0.001). Other variables did not affect the recovery rate of the species. However, in a multivariate model, the isolation of *K. pneumoniae* exhibited correlation with the age only; the odds of its recovery rate increased by 1.017 (95% C.I. 1.007–1.027, p = 0.001) by every year. For *E. coli*, the opposite trend was observed: a year increase in age decreased its recovery 0.984-fold (95% C.I. 0.973–0.955, p = 0.004).

It was noteworthy that *K. pneumoniae* from Emirati patients were, beyond trimethoprim-sulfamethoxazole, significantly more resistant to colistin and significantly more often carried both $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM} (19.0% vs 11.0%, p = 0.0292) or a 16S methylase gene (72.3% vs 61.8%, p = 0.0307), particularly *armA* (50.5% vs 36.1%, p = 0.0048) (Supplementary Table 3).

K. pneumoniae CC14 isolates but not members of the other 2 major clones were significantly more common among Emiratis in all collection areas (Supplementary Table 3). In a univariate model, Emiratis had a 2.257-fold higher risk of CC14 infection (95% C.I. 1.455–3.501, p <0.001), whereas one-year increase of age resulted in a 1.011-fold (95% C.I. 1.001–1.022, p = 0.025) increase. In a multivariate model, only Emirati nationality showed similar correlation, increasing the odds 2.088-fold (95% C.I. 1.296–3.364, p = 0.002).

Discussion

In the first nationwide, molecular typing-based surveillance of CRE in countries of the AP, we studied 45.9% of all nonrepeat CRE isolates encountered during a 9-month period in 15 hospitals of the UAE. Findings on the MDR, often XDR nature of CRE isolates that are exhibiting high rates of colistin, tigecycline, and aminoglycoside resistance; the dominance of OXA-48-like carbapenemases with the simultaneously high rate of MBL producers and consequent high rate of nonsusceptibility to ceftazidime-avibactam; and the rarity of KPC-producers (Table 1, Figures 1, 2) confirmed the results of previous, smaller studies (Sonnevend et al., 2020, Sonnevend et al., 2015b). The paucity of *mcr* carriers and the chromosomal mutations detected in a subset of colistin-resistant *K. pneumoniae* imply that chromosomally encoded mechanisms could primarily be responsible for the high rate of colistin resistance among clinical isolates, as anticipated previously (Sonnevend et al., 2016, Sonnevend et al., 2020). It should be noted, however, that in the current study only *mcr-1–5* were targeted by PCR, and the presence of *mcr-6–10* was ruled out only in those 39 colistin-resistant strains that were subjected to WGS (Supplementary Table 4 and 5).

Beyond these general findings, the current study, for the first time, revealed important regional and interhospital differences and similarities (Figure 1, 2). Strains expressing OXA-48-like and NDM carbapenemases, either as single or multiple enzymes, and those carrying 16S methylase genes *armA* or *rmtF* were present in all major hospitals (Figure 2). We could identify 3 major, highly resistant *K. pneumoniae* clones, CC14, ST231, and CC147, representing 48.6% of all CRE isolates (Table 3, Supplementary Table 6). The presence CC14 strains, which is the largest and most resistant clone in all major hospitals (Figure 3), was particularly noteworthy. Although ST/CC14 strains have been reported earlier locally and globally (Al-Baloushi et al., 2018, Moubareck et al., 2018, Navon-Venezia et al., 2017), we are not aware of such dominance of this clone elsewhere. Its widespread presence and particularly the fact that often isolates of the same cgMLST were recovered from multiple hospitals (Figure 4) suggest the possibility of interhospital transfer.

Interhospital transfer of clones, however, cannot completely explain the scenario observed. The 2 hospitals of the same region (SNE) with the highest prevalence of CC14 in the entire country (Figure 3) exhibited considerably different resistance-related figures (Figure 1, 2). cgMLST revealed that at least some of the ST14 strains from these 2 hospitals belonged to 2 different clusters (Figure 4). Indeed, members of this clone that were encountered in these 2 institutions were considerably different regarding their resistance profiles and the major resistance genes carried (Table 5), implying the presence of distinct subclones. Such variation among ST14 strains has been described in countries of the broader region (Mouftah et al., 2021).

It was noteworthy that in the current study, 90.6% of the ST14 strains subjected to WGS carried the genetic makeup of capsule type KL64 (Supplementary Table 5), whereas among strains collected between 2011 and 2016, it was 36.4% only, with the KL2 type dominating in the UAE (Mouftah et al., 2021). Further investigations should clarify the reasons and the epidemiological and clinical significance of this change.

In each region, the hospital with the highest rates of most resistance-related parameters (ABU-C, DUB-D, and SNE-B) were those with the highest or the second highest local prevalence of the CC14 clone, further emphasizing the impact of this clone on local CRE burden (Figure 1 and 3). Two of these hospitals, ABD-C and DUB-D, were large, tertiary care hospitals (Supplementary Table 1), whereas SNE-B provides secondary care. This hospital had the highest resistance-related figures in the country in all but one parameter (resistance to ceftazidime-avibactam) (Figure 1). The previous observation highlights that studies like ours can help in identifying hospitals for focused and increased vigilance of antibiotic stewardship practices.

An important finding of the current study is the significant association of CC14 strains with Emirati patients in all collection ar-

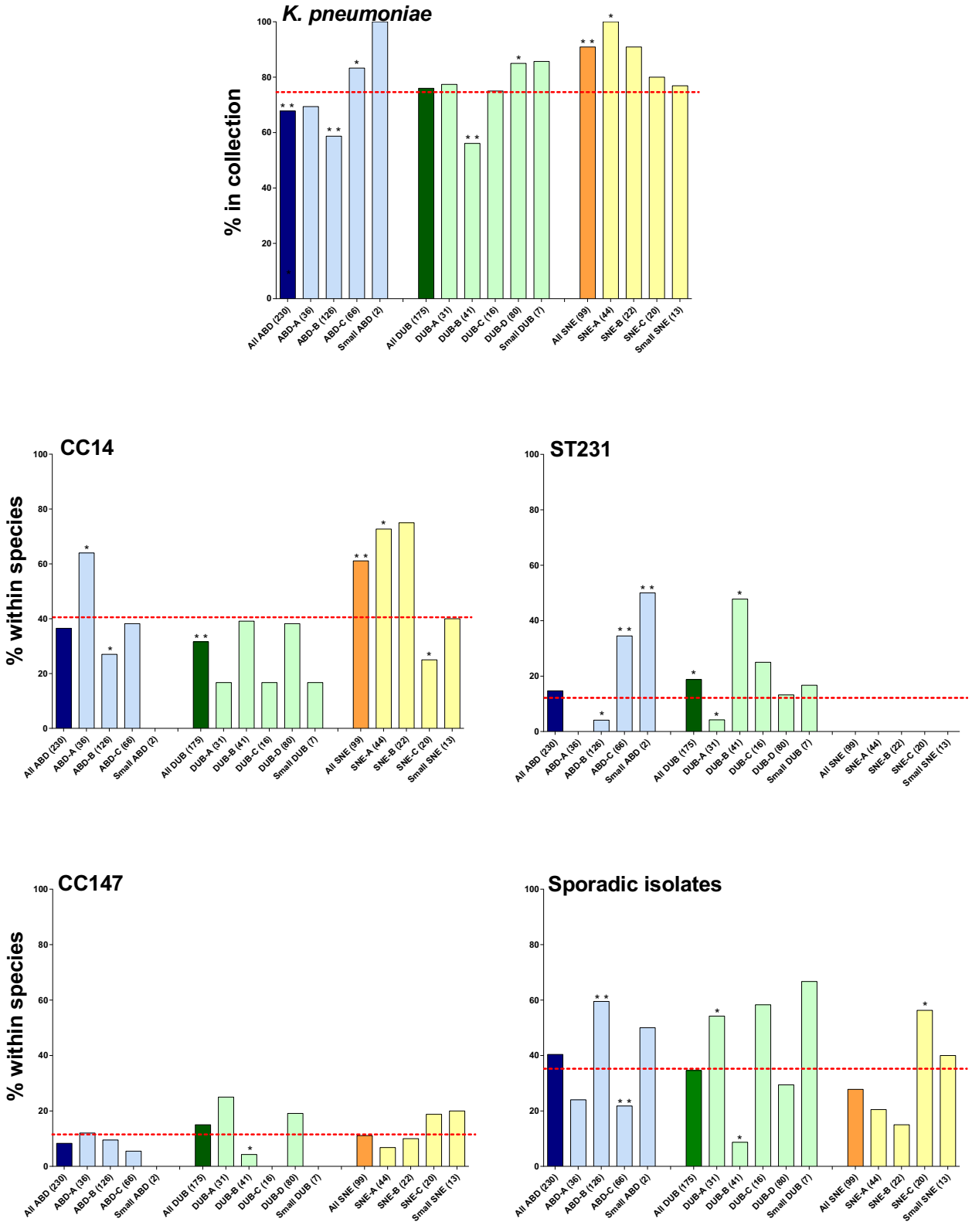

Figure 3. Distribution of *Klebsiella pneumoniae* clones and sporadic isolates among strains collected in different regions and hospitals.
Legend: * indicates significance level $p=0.05\text{-}0.001$, ** indicates significance level $p<0.001$. If above columns representing catchment areas (darker colors) they indicate differences from the other 2 regions combined. If above a column of any particular hospital (lighter colors), they indicate that the value is significantly different from those of the other hospitals combined from the same region. Dotted line indicates the average of all hospitals in the UAE.

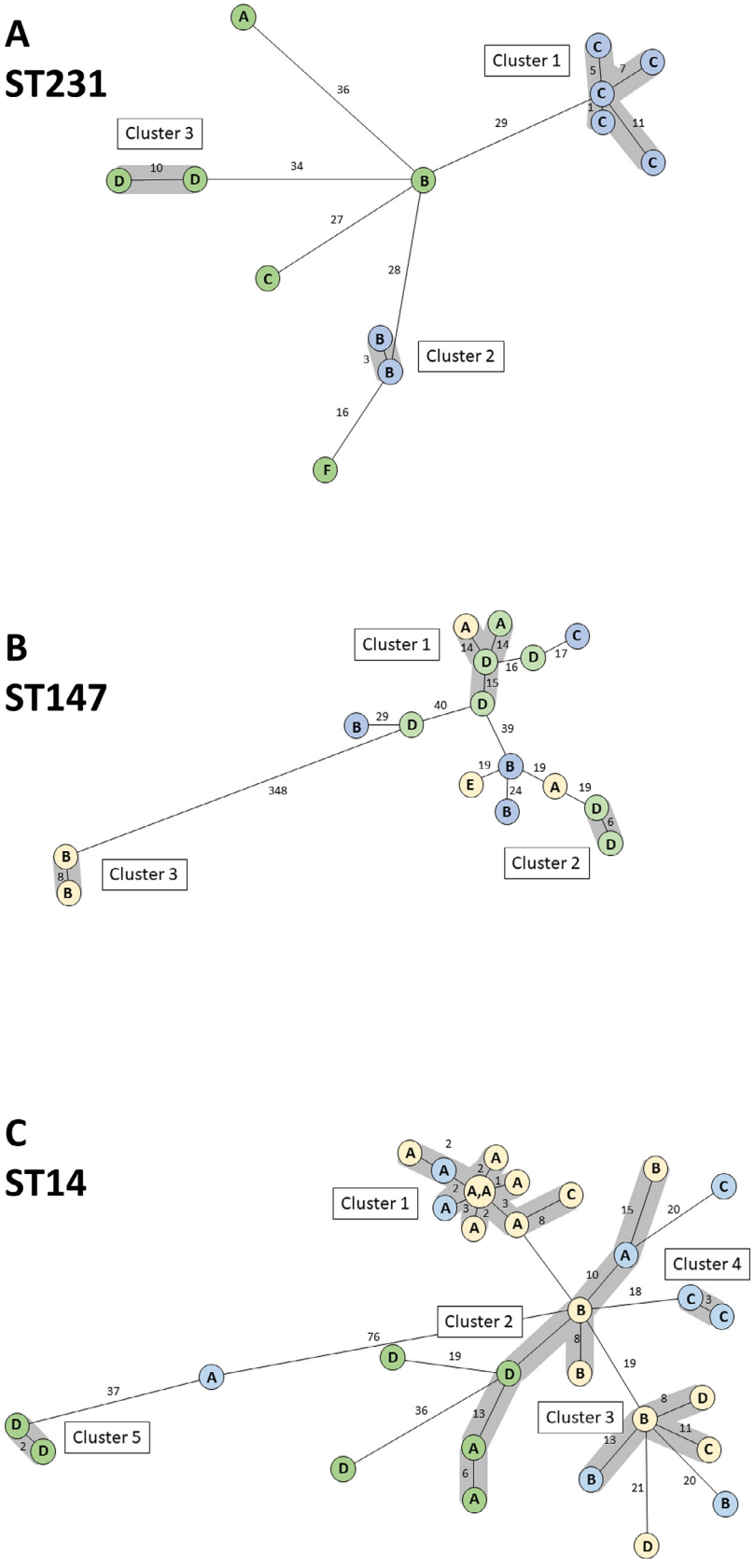

Figure 4. Minimum spanning trees based on cgMLST of *Klebsiella pneumoniae.* clones.
Legend: Blue circles – ABD region, green circles – DUB region, yellow circles – SNE region. Capital letters indicate within the circles indicate hospitals in the region. Numbers on branches represent numbers of differing alleles.

eas (Supplementary Table 3). This supports our previous observation in a few Dubai hospitals (Moubareck et al., 2018). The scale of the current study, however, allowed us to show that this association with the other prevalent clones was absent (Supplementary Table 3). However, as a weakness of the study, we could not identify the reasons behind this association. Data regarding previous exposure to health care, travel history, diagnosis, comorbidities, the details of care, antibiotic treatment or sample taking strategies, and the time of the first isolation of any particular clone were not available to us; therefore, we could not relate those to the isolation of CC14 strains. In the current study, a subset of strains was subjected to WGS only. Future, larger scale, whole genome-based typing studies with access to more clinical data could help to reveal further details of this apparent association.

To the best of our knowledge, this is the first study to provide molecular typing-based data that could serve as baseline values for subsequent investigations to assess the future dynamics of the CRE epidemic in the UAE.

Ethical Approval

The study was approved by the Dubai Scientific Research Committee (DSREC-06/2018_08) and by the Ministry of Health and Prevention Research Ethics Committee (MOHP/REC-27/2018). Exemption from ethics approval was granted by the UAE University Human Research Ethics Committee (ERH_20185790 FAZ/fa/1807E).

Declaration of Competing Interest

TP and TAR received a grant supporting this study from Pfizer (Grant ID 40900593) and ÁS received a grant from University of Pécs, Medical School, Hungary (Kispál Gyula Grant No 300852). Drs. NA and members of the CRE Study group declare that they are employees of different hospitals from which the strains had been collected from for the study.

Funding

This work was supported by Pfizer Inc. [grant number 40900593] to TP and TAR and by University of Pécs Medical School [Kispál Gyula Grant 300852] to ÁS.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.04.034.

References

Abd El Ghany M, Sharaf H, Al-Agamy MH, Shibl A, Hill-Cawthorne GA, Hong PY. Genomic characterization of NDM-1 and 5, and OXA-181 carbapenemases in uropathogenic Escherichia coli isolates from Riyadh, Saudi Arabia. PLOS ONE 2018;13.

Al-Abdely H, AlHababi R, Dada HM, Roushdy H, Alanazi MM, Alessa AA, et al. Molecular characterization of carbapenem-resistant Enterobacterales in thirteen tertiary care hospitals in Saudi Arabia. Ann Saudi Med 2021;41:63–70.

Al-Agamy MH, Aljallal A, Radwan HH, Shibl AM. Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive Escherichia coli and Klebsiella pneumoniae in Riyadh hospitals. J Infect Public Health 2018;11:64–8.

Al-Baloushi AE, Pál T, Ghazawi A, Sonnevend A. Genetic support of carbapenemases in double carbapenemase producer Klebsiella pneumoniae isolated in the Arabian Peninsula. Acta Microbiol Immunol Hung 2018;65:135–50.

Alotaibi FE, Bukhari EE, Al-Mohizea MM, Hafiz T, Essa EB, AlTokhais YI. Emergence of carbapenem-resistant Enterobacteriaceae isolated from patients in a university hospital in Saudi Arabia. Epidemiology, clinical profiles and outcomes. J Infect Public Health 2017;10:667–73.

CIA. United Arab Emirates; 2022, https://www.cia.gov/the-world-factbook/countries/united-arab-emirates/#people-and-society; (accessed 15th of January 2022).

Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol 2005;43:4178–82.

Gautom RK. Rapid pulsed-field gel electrophoresis protocol for typing of Escherichia coli O157:H7 and other gram-negative organisms in 1 day. J Clin Microbiol 1997;35:2977–80.

Hansen GT. Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infect Dis Ther 2021;10:75–92.

Jamal WY, Albert MJ, VO Rotimi. High prevalence of New Delhi metallo-beta-Lactamase-1 (NDM-1) producers among carbapenem-resistant Enterobacteriaceae in Kuwait. PLOS ONE 2016;11.

Memish ZA, Assiri A, Almasri M, Roshdy H, Hathout H, Kaase M, et al. Molecular characterization of carbapenemase production among gram-negative bacteria in Saudi arabia. Microb Drug Resist 2015;21:307–14.

. United Arab Emirates surveillance of antimicrobial resistance [annual report], 2019. Abu Dhabi, United Arab Emirates: Abu Dhabi Public Health Center; 2021.

Moubareck CA, Mouftah SF, Pál T, Ghazawi A, Halat DH, Nabi A, et al. Clonal emergence of Klebsiella pneumoniae ST14 co-producing OXA-48-type and NDM carbapenemases with high rate of colistin resistance in Dubai, United Arab Emirates. Int J Antimicrob Agents 2018;52:90–5.

Mouftah SF, Pál T, Higgins PG, Ghazawi A, Idaghdour Y, Alqahtani M, et al. Diversity of carbapenem-resistant Klebsiella pneumoniae ST14 and emergence of a subgroup with KL64 capsular locus in the Arabian Peninsula. Eur J Clin Microbiol Infect Dis 2021.

Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 2017;41:252–75.

Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 2014;5:643.

Pál T, Ghazawi A, Darwish D, Villa L, Carattoli A, Hashmey R, et al. Characterization of NDM-7 carbapenemase-producing Escherichia coli isolates in the Arabian Peninsula. Microb Drug Resist 2017;23:871–8.

Sonnevend Á, Ghazawi A, Alqahtani M, Shibl A, Jamal W, Hashmey R, et al. Plasmid-mediated colistin resistance in Escherichia coli from the Arabian Peninsula. Int J Infect Dis 2016;50:85–90.

Sonnevend Á, Ghazawi A, Darwish D, AlDeesi Z, Kadhum AF, Pál T. Characterization of KPC-type carbapenemase-producing Klebsiella pneumoniae strains isolated in the Arabian Peninsula. J Antimicrob Chemother 2015a;70:1592–3.

Sonnevend Á, Ghazawi A, Darwish D, Barathan G, Hashmey R, Ashraf T, et al. In vitro efficacy of ceftazidime-avibactam, aztreonam-avibactam and other rescue antibiotics against carbapenem-resistant Enterobacterales from the Arabian Peninsula. Int J Infect Dis 2020;99:253–9.

Sonnevend Á, Ghazawi A, Yahfoufi N, Al-Baloushi A, Hashmey R, Mathew M, et al. VIM-4 carbapenemase-producing Enterobacter cloacae in the United Arab Emirates. Clin Microbiol Infect 2012;18:E494–6.

Sonnevend Á, Ghazawi AA, Hashmey R, Jamal W, Rotimi VO, Shibl AM, et al. Characterization of carbapenem-resistant Enterobacteriaceae with high rate of autochthonous transmission in the Arabian Peninsula. PLOS ONE 2015b;10.

Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 2006;60:1136–51.

Zaman TU, Alrodayyan M, Albladi M, Aldrees M, Siddique MI, Aljohani S, et al. Clonal diversity and genetic profiling of antibiotic resistance among multidrug/carbapenem-resistant Klebsiella pneumoniae isolates from a tertiary care hospital in Saudi Arabia. BMC Infect Dis 2018;18:205.

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4.

Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, et al. Molecular characterization of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in the countries of the Gulf Cooperation Council: dominance of OXA-48 and NDM producers. Antimicrob Agents Chemother 2014;58:3085–90.