Diversity of carbapenem-resistant *Klebsiella pneumoniae* ST14 and emergence of a subgroup with KL64 capsular locus in the Arabian Peninsula

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Abstract

To understand the reasons of successful spread of carbapenem-resistant *Klebsiella pneumoniae* ST14 (CRKP-ST14) in countries of the Arabian Peninsula, the resistome, capsular locus, carbapenemase carrying plasmid types, and core genome of isolates from the region were compared to global isolates. Thirty-nine CRKP-ST14 strains isolated from 13 hospitals in the United Arab Emirates, Bahrain, and Saudi Arabia were selected for whole genome sequencing on Illumina MiSeq platform based on the variety of carbapenemase genes carried and plasmids bearing these genes. Their resistome, capsular locus, and core genome MLST were compared to 173 CRKP-ST14 genomes available in public databases. The selected 39 CRKP-ST14 produced either NDM-1, OXA-48, OXA-162, OXA-232, KPC-2, or co-produced NDM-1 and an OXA-48-like carbapenemase. cgMLST revealed three clusters: 16 isolates from five UAE cities (C1), 11 isolates from three UAE cities and Bahrain (C2), and 5 isolates from Saudi Arabia (C3), respectively, and seven singletons. Resistance gene profile, carbapenemase genes, and their plasmid types were variable in both C1 and C2 clusters. The majority of CRKP-ST14 had KL2, but members of the C2 cluster and two further singletons possessed KL64 capsular locus. Based on cgMLST comparison of regional and global isolates, CRKP-ST14 with KL64 from four continents formed a distinct cluster, suggesting a recent emergence and spread of this variant. Our findings confirmed clonal transmission coupled with likely horizontal gene transfer in carbapenem-resistant *Klebsiella pneumoniae* ST14. Dissemination of this genetically flexible, highly resistant clone warrants further monitoring.

Keywords *Klebsiella pneumoniae* · Carbapenem resistance · High-risk clone · Capsular switch

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Introduction

The emergence and spread of carbapenem-resistant *Enterobacteriales* (CRE) is considered to be one of the most serious antibiotic resistance threats [1]. The majority of CRE is *Klebsiella pneumoniae*, which are carbapenem resistant due to the production of carbapenemases (CRKP) [2]. This worldwide trend is also observed in countries of the Arabian Peninsula [3–5]. Spread of CRKP is mainly driven by clonal dissemination of so called high-risk multi-drug resistant (HiR MDR) clones [2]. *K. pneumoniae* ST14 is one of such clones producing various carbapenemases that have been reported from four continents [2, 5, 6] including the Arabian Peninsula [3, 7–9]. Earlier, this clone was shown to have high prevalence among CRE isolated in the United Arab Emirates [8, 10]. To understand the reasons of its successful emergence and spread, we used whole genome sequencing (WGS) to assess the resistome, the capsular locus, the carbapenemase carrying plasmid types, as well as the level of core genome similarity of selected *K. pneumoniae* ST14 isolated in countries of the Arabian Peninsula, which were also compared to whole genomes of *K. pneumoniae* ST14 isolated at other geographical locations.

Materials and methods

Identification of CRKP-ST14 isolates

Altogether 761 independent CRKP strains isolated between June 2011 and June 2016 in 33 hospitals of five countries of the Arabian Peninsula from a collection described earlier [11] were screened by PFGE [12], to select isolates exhibiting $\geq 80\%$ similar patterns to 23 known CRKP-ST14 identified earlier in the UAE [7–9]. These were further divided into sub-clusters based on 90% PFGE pattern similarity threshold (Supplementary Fig. 1). At least one isolate from these PFGE sub-clusters carrying unique carbapenemase genes or their combinations were subjected to multi-locus sequence typing [13] to confirm their sequence type.

Testing of antibiotic susceptibility, carbapenemase production, and detection of carbapenemase genes

Phenotypic confirmation of the isolates' carbapenemase production and methods testing their antibiotic susceptibility was described earlier in [11]. Presence of the common carbapenemase genes (*bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}) were screened by PCR and identified by direct sequencing of the amplicons [9].

Characterization of carbapenemase gene-carrying plasmids

Electrophoresis gels of plasmids extracted by the alkaline lysis methods from the wild-type CRKP strains were capillary transferred to Hybond N+ membranes (Roche Diagnostics, Mannheim, Germany) and the membranes were probed with *bla*_{NDM-1} or *bla*_{OXA-48} PCR amplicons labelled and detected with the DIG DNA-labelling and detection kit (Roche Diagnostics, Mannheim, Germany) [14]. Selected carbapenemase bearing plasmids were conjugally transferred into a sodium-azide resistant derivative of rifampicin resistant *Escherichia coli* J53 (J53RAZ) [7]. If conjugation failed, 1 μ g of plasmid DNA purified with Plasmid Maxi Prep Kit (Qiagen, Hilden, Germany) of a wild type isolate was heat-shock transformed into competent cells of *Escherichia coli* DH5 α [7]. Transfer experiments were considered successful if a single-plasmid containing derivative was obtained, confirmed by plasmid electrophoresis and PCR targeting the respective carbapenemase gene [7]. Plasmid incompatibility typing was performed by PCR as described earlier in [3, 7, 15].

Analysis of the whole genomes

Genomic DNA of selected CRKP-ST14 isolates was extracted using the QIAamp DNA Mini Kit (QIAGEN, Crawley, UK), according to the manufacturer's instructions. Bacterial DNA libraries were prepared using the Illumina Nextera®XT DNA Library Preparation Kit (Illumina, USA) and sequenced on Illumina MiSeq platform using 2 $\times$ 250 bp or 2 $\times$ 300 bp paired-end sequencing technology. Reads were quality checked and assembled using the Velvet 1.1.04. algorithm built in the Ridom SeqSphere⁺ software. Core genome MLST (cgMLST) comparison was performed using Ridom SeqSphere⁺ (version 7.2.3). The assembled contigs were analysed by Kleborate for the virulence gene and capsule locus related information [6]. The acquired resistance gene content was assessed using ResFinder 4.0 (<https://cge.cbs.dtu.dk/services/ResFinder/>) [16]. In case the Velvet assembly lacked an expected carbapenemase gene, the respective reads were assembled using SPAdes 3.11 and the contigs produced were re-analyzed in the ResFinder 4.0. The integrity of lipopolysaccharide synthesis regulating genes *mgrB*, *phoP*, *phoQ*, *pmrA*, and *pmrB* [17], and that of the main porin genes (*ompC* and *ompF*) was compared to the respective genes of *K. pneumoniae* MGH78578 (GenBank Acc. No. CP000647). The sequence data generated were uploaded to the European Nucleotide Archives under project number PRJEB44852.

Comparison of local CRKP-ST14 isolates to ones isolated globally

In order to compare *K. pneumoniae* ST14 isolates of the Arabian Peninsula genomes of three carbapenemase gene-carrying *K. pneumoniae* ST14 isolates: PittNDM01, KP617 and NUHL24835 (GenBank accession numbers: CP006798–CP006802, CP012753–CP012755, CP014004–CP014006) and further 170 carbapenemase carrying *K. pneumoniae* ST14 genomes from the Pathogen-Watch (<https://pathogen.watch/genomes/all?genusId=570&mlst=14&speciesId=573> accessed on 02–06–2021) were downloaded. All were compared by cgMLST using Ridom SeqSphere⁺ software. A total of 173 genomes available from the databases were also screened for their respective capsular locus types. One-hundred and nine global isolates from the databases were isolated in the same period as the recovery of our isolates from the Arabian Peninsula. Further 50 isolates used in the comparison were collected between 2017 and 2019, and 14 did not have the collection date specified.

Results

Identification of *Klebsiella pneumoniae* ST14 isolates

Based on PFGE, a total of 172 *K. pneumoniae* strains exhibited patterns $\geq 80\%$ similar to known CRKP-ST14 isolates. The majority of them ($n = 162$) were from hospitals in the UAE, but nine isolates from Riyadh, Saudi Arabia, and one

from Riffa, Bahrain, also exhibited similar PFGE patterns. These 172 isolates could further be divided into 14 PFGE sub-clusters applying the 90% similarity threshold (Supplementary Fig. 1). All but one carried a carbapenemase gene(s), with a remarkable high rate of double carbapenemase producers (42%). The types and distribution of carbapenemase alleles is shown in Supplementary Table 1.

Based on the PFGE subclusters and on the type of carbapenemase genes carried, 70 isolates were subjected to MLST that confirmed sequence type 14 in each case (Supplementary Fig. 1).

Analysis of the carbapenemase genes and their localizations

The 70 CRKP-ST14 isolates were further investigated by testing the plasmid localization of their respective carbapenemase genes. Based on the alleles of *bla*_{NDM} and *bla*_{OXA} carbapenemase genes and on their association with different plasmids, strains were divided into 13 subgroups (SGs) (Table 1). In one isolate (ABC225) no carbapenemase genes or the production of such enzymes could be detected. Two strains (ABC220 and ABC224, already described in [9]) harboured *bla*_{KPC-2} on IncX3 plasmids, while all the others carried *bla*_{NDM-1} alone or in combination with various alleles of *bla*_{OXA-48-like}. The *bla*_{OXA-232} gene detected by PCR could not be localized by hybridization, neither was the carbapenemase gene transferable by conjugation nor by transformation from one isolate (ABC334). On the other hand, although the plasmid electrophoresis gel of isolate ABC643 did not hybridize with the NDM-probe, from this strain the

Table 1 Subgroups of CRKP-ST14 with various alleles of carbapenemases located on different plasmids

Subgroup	N	N selected for WGS	Prototype strains*	NDM-1 plasmid			OXA plasmid			
				Size	Inc type	C	OXA allele	Size	Inc type	C
A	17	4	ABC252	240 Kb	IncHI1B	C	OXA-232	6 Kb	IncColKP3	NC
B	4	4	SA121	240 Kb	IncHI1B	C	OXA-48	60 Kb	IncL	C
C	4	4	ABC261	240 Kb	IncHI1B	C	OXA-162	60 Kb	IncL	C
D	9	6	ABC131 ABC643	240 Kb	IncHI1B	C	none	NA	NA	NA
E	1	1	ABC240	120 Kb	IncFII	C	OXA-232	60 Kb	IncColKP3, IncR	NC
F	7	4	RH60	120 Kb	IncFII	C	OXA-232	6 Kb	IncColKP3	NC
G	3	3	DHKp6	120 Kb	IncFII	C	none	NA	NA	NA
H	5	5	MCH15	NA	NA	NA	OXA-162	60 Kb	IncL	NC
I	1	1	ABC232	NA	NA	NA	OXA-232	10 Kb	IncColKP3	NC
J	15	4	ABC270	NA	NA	NA	OXA-232	6 Kb	IncColKP3	NC
K	1	1	ABC334	NA	NA	NA	OXA-232	not localized by hybridization		NC
L	2	1	ABC220	KPC-2 on IncX3 plasmid						
M	1	1	ABC225	Carbapenemase negative						

*Strain from which the plasmid transfer and incompatibility typing was performed

C conjugative, NC non-conjugative, NA not applicable

*bla*_{NDM-1} was successfully transferred by conjugation into *E. coli* J53_{RAZ} and the carbapenemase gene was identified on an approx. 240 kb IncHI1B plasmid from the transconjugant. Consequently, ABC643 was considered being member of subgroup D (Table 1). From each of the 13 subgroups identified, up to five isolates, overall 39 isolates were selected for whole genome sequencing (Table 2).

Clonal relatedness and resistome of the carbapenem-resistant *K. pneumoniae* ST14

The assembly statistics of the 39 isolates subjected to WGS is summarized in Supplementary Table 2. The cgST types are listed in Table 2. Core genome MLST revealed seven singletons and three clusters that differed by up to 14 out of 2358 alleles (Table 2 and Fig. 1).

As it is shown in Table 2, Cluster 1 and Cluster 2 contained isolates from various hospitals in different cities carrying different carbapenemases, whereas Cluster 3 consisted of isolates exclusively from one hospital in Saudi Arabia.

Beyond the carbapenemase and other beta-lactamase genes, all isolates carried acquired resistance genes coding for aminoglycoside, trimethoprim and sulfamethoxazole resistance, and they variably possessed macrolide, tetracycline, rifampicin and plasmid mediated quinolone resistance genes. Importantly, the majority of isolates in the three clusters carried the *armA* 16S methylase gene. The array of acquired antibiotic resistance genes matched the antimicrobial susceptibility profiles of the isolates (Supplementary Table 3), e.g., high-level tetracycline resistance

was observed with isolates possessing the *tet(D)* gene. Similarly, the few isolates susceptible to chloramphenicol were the ones lacking any phenicol resistance genes. However, the lack of acquired phenicol resistance genes did not translate always to susceptibility to chloramphenicol, suggesting alternative resistance mechanisms, possibly efflux. The colistin resistance observed was not due to the presence of mobile colistin resistance genes (*mcr1-9*) and did not correlate with certain clusters. Colistin resistance in 7 of the 13 colistin resistant isolates was due to insertional, frameshift or non-synonymous point mutations of the *mgrB* gene. In further two isolates point mutations in *phoQ*, and in another three point mutations in *pmrB* were responsible for this phenotype. In one isolate the regulatory genes examined were all wild-type (Table 2), but further investigation of the colistin resistance mechanism in this single isolate was beyond the scope of our current study.

We assume that the only carbapenemase non-producing strain (ABC225) was resistant to carbapenems due to the production of multiple beta-lactamases, including ones with extended-spectrum (*bla*_{TEM-1A}, *bla*_{OXA-1}, *bla*_{OXA-9}, *bla*_{CTX-M-15}) combined with deletions in the *ompF* gene: a 19 base-long one starting at nucleotide 676 and a single nucleotide one at position 872, while the *ompC* gene was wild-type (data not shown).

All isolates, but ABC681 possessed the yersiniabactin siderophore genes encoded on ICEKp5 mobile element, but none of them had genes characteristic to hypervirulent *K. pneumoniae*, i.e., *rmpA*, *rmpA2*, and *iutA*. Interestingly, isolates in Cluster 2 and further two singletons (RH60 and

Table 2 Characteristics, cgMLST clustering, and resistome of strains subjected to whole genome sequencing

Strain ID	Year of isolation	Specimen	Hospital	cgMLST Complex type	cgMLST cluster	Subgroup by carbapenemase gene and plasmids	blaNDM-1	blaOXA-48	blaOXA-162	blaOXA-232	blaSHV-2	blaTEM-1A	blaTEM-1B	blaOXA-9	blaCTX-M-15	ermA	catA1	catA2	catA3	catA4	catA5	catA6	catA7	catA8	catA9	catA10	catA11	catA12	catA13	catA14	catA15	catA16	catA17	catA18	catA19	catA20	catA21	catA22	catA23	catA24	catA25	catA26	catA27	catA28	catA29	catA30	catA31	catA32	catA33	catA34	catA35	catA36	catA37	catA38	catA39	catA40	catA41	catA42	catA43	catA44	catA45	catA46	catA47	catA48	catA49	catA50	catA51	catA52	catA53	catA54	catA55	catA56	catA57	catA58	catA59	catA60	catA61	catA62	catA63	catA64	catA65	catA66	catA67	catA68	catA69	catA70	catA71	catA72	catA73	catA74	catA75	catA76	catA77	catA78	catA79	catA80	catA81	catA82	catA83	catA84	catA85	catA86	catA87	catA88	catA89	catA90	catA91	catA92	catA93	catA94	catA95	catA96	catA97	catA98	catA99	catA100	catA101	catA102	catA103	catA104	catA105	catA106	catA107	catA108	catA109	catA110	catA111	catA112	catA113	catA114	catA115	catA116	catA117	catA118	catA119	catA120	catA121	catA122	catA123	catA124	catA125	catA126	catA127	catA128	catA129	catA130	catA131	catA132	catA133	catA134	catA135	catA136	catA137	catA138	catA139	catA140	catA141	catA142	catA143	catA144	catA145	catA146	catA147	catA148	catA149	catA150	catA151	catA152	catA153	catA154	catA155	catA156	catA157	catA158	catA159	catA160	catA161	catA162	catA163	catA164	catA165	catA166	catA167	catA168	catA169	catA170	catA171	catA172	catA173	catA174	catA175	catA176	catA177	catA178	catA179	catA180	catA181	catA182	catA183	catA184	catA185	catA186	catA187	catA188	catA189	catA190	catA191	catA192	catA193	catA194	catA195	catA196	catA197	catA198	catA199	catA200	catA201	catA202	catA203	catA204	catA205	catA206	catA207	catA208	catA209	catA210	catA211	catA212	catA213	catA214	catA215	catA216	catA217	catA218	catA219	catA220	catA221	catA222	catA223	catA224	catA225	catA226	catA227	catA228	catA229	catA230	catA231	catA232	catA233	catA234	catA235	catA236	catA237	catA238	catA239	catA240	catA241	catA242	catA243	catA244	catA245	catA246	catA247	catA248	catA249	catA250	catA251	catA252	catA253	catA254	catA255	catA256	catA257	catA258	catA259	catA260	catA261	catA262	catA263	catA264	catA265	catA266	catA267	catA268	catA269	catA270	catA271	catA272	catA273	catA274	catA275	catA276	catA277	catA278	catA279	catA280	catA281	catA282	catA283	catA284	catA285	catA286	catA287	catA288	catA289	catA290	catA291	catA292	catA293	catA294	catA295	catA296	catA297	catA298	catA299	catA300	catA301	catA302	catA303	catA304	catA305	catA306	catA307	catA308	catA309	catA310	catA311	catA312	catA313	catA314	catA315	catA316	catA317	catA318	catA319	catA320	catA321	catA322	catA323	catA324	catA325	catA326	catA327	catA328	catA329	catA330	catA331	catA332	catA333	catA334	catA335	catA336	catA337	catA338	catA339	catA340	catA341	catA342	catA343	catA344	catA345	catA346	catA347	catA348	catA349	catA350	catA351	catA352	catA353	catA354	catA355	catA356	catA357	catA358	catA359	catA360	catA361	catA362	catA363	catA364	catA365	catA366	catA367	catA368	catA369	catA370	catA371	catA372	catA373	catA374	catA375	catA376	catA377	catA378	catA379	catA380	catA381	catA382	catA383	catA384	catA385	catA386	catA387	catA388	catA389	catA390	catA391	catA392	catA393	catA394	catA395	catA396	catA397	catA398	catA399	catA400	catA401	catA402	catA403	catA404	catA405	catA406	catA407	catA408	catA409	catA410	catA411	catA412	catA413	catA414	catA415	catA416	catA417	catA418	catA419	catA420	catA421	catA422	catA423	catA424	catA425	catA426	catA427	catA428	catA429	catA430	catA431	catA432	catA433	catA434	catA435	catA436	catA437	catA438	catA439	catA440	catA441	catA442	catA443	catA444	catA445	catA446	catA447	catA448	catA449	catA450	catA451	catA452	catA453	catA454	catA455	catA456	catA457	catA458	catA459	catA460	catA461	catA462	catA463	catA464	catA465	catA466	catA467	catA468	catA469	catA470	catA471	catA472	catA473	catA474	catA475	catA476	catA477	catA478	catA479	catA480	catA481	catA482	catA483	catA484	catA485	catA486	catA487	catA488	catA489	catA490	catA491	catA492	catA493	catA494	catA495	catA496	catA497	catA498	catA499	catA500	catA501	catA502	catA503	catA504	catA505	catA506	catA507	catA508	catA509	catA510	catA511	catA512	catA513	catA514	catA515	catA516	catA517	catA518	catA519	catA520	catA521	catA522	catA523	catA524	catA525	catA526	catA527	catA528	catA529	catA530	catA531	catA532	catA533	catA534	catA535	catA536	catA537	catA538	catA539	catA540	catA541	catA542	catA543	catA544	catA545	catA546	catA547	catA548	catA549	catA550	catA551	catA552	catA553	catA554	catA555	catA556	catA557	catA558	catA559	catA560	catA561	catA562	catA563	catA564	catA565	catA566	catA567	catA568	catA569	catA570	catA571	catA572	catA573	catA574	catA575	catA576	catA577	catA578	catA579	catA580	catA581	catA582	catA583	catA584	catA585	catA586	catA587	catA588	catA589	catA590	catA591	catA592	catA593	catA594	catA595	catA596	catA597	catA598	catA599	catA600	catA601	catA602	catA603	catA604	catA605	catA606	catA607	catA608	catA609	catA610	catA611	catA612	catA613	catA614	catA615	catA616	catA617	catA618	catA619	catA620	catA621	catA622	catA623	catA624	catA625	catA626	catA627	catA628	catA629	catA630	catA631	catA632	catA633	catA634	catA635	catA636	catA637	catA638	catA639	catA640	catA641	catA642	catA643	catA644	catA645	catA646	catA647	catA648	catA649	catA650	catA651	catA652	catA653	catA654	catA655	catA656	catA657	catA658	catA659	catA660	catA661	catA662	catA663	catA664	catA665	catA666	catA667	catA668	catA669	catA670	catA671	catA672	catA673	catA674	catA675	catA676	catA677	catA678	catA679	catA680	catA681	catA682	catA683	catA684	catA685	catA686	catA687	catA688	catA689	catA690	catA691	catA692	catA693	catA694	catA695	catA696	catA697	catA698	catA699	catA700	catA701	catA702	catA703	catA704	catA705	catA706	catA707	catA708	catA709	catA710	catA711	catA712	catA713	catA714	catA715	catA716	catA717	catA718	catA719	catA720	catA721	catA722	catA723	catA724	catA725	catA726	catA727	catA728	catA729	catA730	catA731	catA732	catA733	catA734	catA735	catA736	catA737	catA738	catA739	catA740	catA741	catA742	catA743	catA744	catA745	catA746	catA747	catA748	catA749	catA750	catA751	catA752	catA753	catA754	catA755	catA756	catA757	catA758	catA759	catA760	catA761	catA762	catA763	catA764	catA765	catA766	catA767	catA768	catA769	catA770	catA771	catA772	catA773	catA774	catA775	catA776	catA777	catA778	catA779	catA780	catA781	catA782	catA783	catA784	catA785	catA786	catA787	catA788	catA789	catA790	catA791	catA792	catA793	catA794	catA795	catA796	catA797	catA798	catA799	catA800	catA801	catA802	catA803	catA804	catA805	catA806	catA807	catA808	catA809	catA810	catA811	catA812	catA813	catA814	catA815	catA816	catA817	catA818	catA819	catA820	catA821	catA822	catA823	catA824	catA825	catA826	catA827	catA828	catA829	catA830	catA831	catA832	catA833	catA834	catA835	catA836	catA837	catA838	catA839	catA840	catA841	catA842	catA843	catA844	catA845	catA846	catA847	catA848	catA849	catA850	catA851	catA852	catA853	catA854	catA855	catA856	catA857	catA858	catA859	catA860	catA861	catA862	catA863	catA864	catA865	catA866	catA867	catA868	catA869	catA870	catA871	catA872	catA873	catA874	catA875	catA876	catA877	catA878	catA879	catA880	catA881	catA882	catA883	catA884	catA885	catA886	catA887	catA888	catA889	catA890	catA891	catA892	catA893	catA894	catA895	catA896	catA897	catA898	catA899	catA900	catA901	catA902	catA903	catA904	catA905	catA906	catA907	catA908	catA909	catA910	catA911	catA912	catA913	catA914	catA915	catA916	catA917	catA918	catA919	catA920	catA921	catA922	catA923	catA924	catA925	catA926	catA927	catA928	catA929	catA930	catA931	catA932	catA933	catA934	catA935	catA936	catA937	catA938	catA939	catA940	catA941	catA942	catA943	catA944	catA945	catA946	catA947	catA948	catA949	catA950	catA951	catA952	catA953	catA954	catA955	catA956	catA957	catA958	catA959	catA960	catA961	catA962	catA963	catA964	catA965	catA966	catA967	catA968	catA969	catA970	catA971	catA972	catA973	catA974	catA975	catA976	catA977	catA978	catA979	catA980	catA981	catA982	catA983	catA984	catA985	catA986	catA987	catA988	catA989	catA990	catA991	catA992	catA993	catA994	catA995	catA996	catA997	catA998	catA999	catA1000	catA1001	catA1002	catA1003	catA1004	catA1005	catA1006	catA1007	catA1008	catA1009	catA1010	catA1011	catA1012	catA1013	catA1014	catA1015	catA1016	catA1017	catA1018	catA1019	catA1020	catA1021	catA1022	catA1023	catA1024	catA1025	catA1026	catA1027	catA1028	catA1029	catA1030	catA1031	catA1032	catA1033	catA1034	catA1035	catA1036	catA1037	catA1038	catA1039	catA1040	catA1041	catA1042	catA1043	catA1044	catA1045	catA1046	catA1047	catA1048	catA1049	catA1050	catA1051	catA1052	catA1053	catA1054	catA1055	catA1056	catA1057	catA1058	catA1059	catA1060	catA1061	catA1062	catA1063	catA1064	catA1065	catA1066	catA1067	catA1068	catA1069	catA1070	catA1071	catA1072	catA1073	catA1074	catA1075	catA1076	catA1077	catA1078	catA1079	catA1080	catA1081	catA1082	catA1083	catA1084	catA1085	catA1086	catA1087	catA1088	catA1089	catA1090	catA1091	catA1092	catA1093	catA1094	catA1095	catA1096	catA1097	catA1098	catA1099	catA1100	catA1101	catA1102	catA1103	catA1104	catA1105	catA1106	catA1107	catA1108	catA1109	catA1110	catA1111	catA1112	catA1113	catA1114	catA1115	catA1116	catA1117	catA1118	catA1119	catA1120	catA1121	catA1122	catA1123	catA1124	catA1125	catA1126	catA1127	catA1128	catA1129	catA1130	catA1131	catA1132	catA1133	catA1134	catA1135	catA1136	catA1137	catA1138	catA1139	catA1140	catA1141	catA1142	catA1143	catA1144	catA1145	catA1146	catA1147	catA1148	catA1149	catA1150	catA1151	catA1152	catA1153	catA1154	catA1155	catA1156	catA1157	catA1158	catA1159	catA1160	catA1161	catA1162	catA1163	catA1164	catA1165	catA1166	catA1167	catA1168	catA1169	catA1170	catA1171	catA1172	catA1173	catA1174	catA1175	catA1176	catA1177	catA1178	catA1179	catA1180	catA1181	catA1182	catA1183	catA1184	catA1185	catA1186	catA1187	catA1188	catA1189	catA1190	catA1191	catA1192	catA1193	catA1194	catA1195	catA1196	catA1197	catA1198	catA1199	catA1200	catA1201	catA1202	catA1203	catA1204	catA1205	catA1206	catA1207	catA1208	catA1209	catA1210	catA1211
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Fig. 1 Minimum spanning tree based on cgMLST of the 39 *K. pneumoniae* ST14. Distance is based on number of differences of the 2358 alleles in *K. pneumoniae* sensu lato cgMLST scheme with cluster alert distance set at 15

ABC334) possessed capsular locus KL64, not the KL2, i.e., one typically associated with *K. pneumoniae* ST14 and also present in the remaining isolates (Fig. 1). Although isolates carrying the KL64 locus clustered by their core genome, the carbapenemase genes carried by them were variable: some of them possessed *bla*_{NDM-1}, others *bla*_{OXA-232}, or both of these carbapenemases (Table 2 and Fig. 2).

Comparison of *K. pneumoniae* ST14 isolated in the Arabian Peninsula to international *K. pneumoniae* ST14 genomes

The neighbour-joining tree generated by comparing the cgMLST of local as well as global, altogether 212 *K. pneumoniae* ST14 isolates is presented in Fig. 2. *K. pneumoniae*

Fig. 2 Neighbour-joining tree based on the cgMLST of 212 *K. pneumoniae* ST14. Color code is based on the capsular locus type. The strains marked with red dotted line are isolated in 2017–2019, i.e., after the strains investigated in the present study

ST14 genomes with KL2 capsular locus formed several clusters, whereas isolates of the ST14 clone having other capsular locus types (KL16, KL64, KL107, and KL133) clearly separated from the previous groups. The core genomes of *K. pneumoniae* ST14 harbouring KL64 from the UAE and Bahrain formed a distinct cluster with NDM-1 or NDM-5 and/or OXA-232 producing *K. pneumoniae* ST14 isolates from the Netherlands, Australia, India and the USA and with one isolate from India with capsular locus KL107, which also contained the *wzi64* allele.

Discussion

Numerous high-risk *K. pneumoniae* clones, among them ST14, have now been identified [2]. ST14 strains have been reported to produce a wide range of carbapenemases: apart from being the sequence type from which the *bla*_{NDM-1} gene was first identified [18], various *bla*_{KPC} [9, 19, 20], *bla*_{NDM} [21, 22], and *bla*_{OXA-48-like} [6, 23–26] alleles or double carbapenemase producers have been reported from different parts of the world, including the Middle East [7, 8, 27–31]. Furthermore, ST14 strains appear to be one of the most wide-spread, highly resistant CRE clones in Dubai [8].

Even in the light of this variety of CRKP-ST14 strains, the diversity of carbapenem-resistant mechanisms encountered in the relatively small pool of ST14 strains isolated in countries of the Arabian Peninsula was remarkable (Table 1). Beyond strains carrying *bla*_{NDM-1}, *bla*_{OXA-232}, *bla*_{KPC-2}, and combinations of *bla*_{NDM-1} either with *bla*_{OXA-232}, or with *bla*_{OXA-48}, we identified isolates with *bla*_{OXA-162}. To the best of our knowledge, this is the first observation of this allele alone or in combination with *bla*_{NDM-1} in this high-risk clone of *K. pneumoniae*. This variety of carbapenemase gene content further confirms the earlier observations describing this high-risk clone as one which is particularly fit to acquire resistance plasmids and spread the resistance traits [2, 6]. Furthermore, we also encountered a strain, which did not produce a dedicated carbapenemase, yet was resistant to carbapenems due to ESBL production and *ompF* mutation, a phenomenon otherwise not uncommon among CRKP [10, 11].

The diversity of ST14 strains was further accentuated by the various types of, mostly conjugative, plasmids carrying the carbapenemase genes. Even the same allele of a carbapenemase gene (*bla*_{NDM-1}) could be localised on two different plasmids, IncHI1b and IncFII, in our isolates. On the other hand, all *bla*_{OXA-48} and *bla*_{OXA-162} were located on

IncL type plasmids, while *bla*_{OXA-232} was associated with IncColKP3 type plasmids and in a single isolate (ABC240) with an IncColKP3–IncR composite plasmid (Table 1).

The cgMLST results did not completely overlap with these carbapenemase gene-, and plasmid-based groups (Table 1 and 2). Considering only the carbapenemase genes, members of cgMLST cluster 3 were the most homogenous, as all such strains produced NDM and OXA-48 with the exception of a single isolate, presumably having lost the plasmid coding for the latter carbapenemase. This lack of heterogeneity was not surprising as all these strains were isolated in the same hospital in Saudi Arabia. Interestingly, they clustered with Saudi isolates of 2018, and German isolates of 2016–2019 deposited in the PathogenWatch database (Fig. 2). On the other hand, members of the other two cgMLST clusters were more heterogeneous: despite being highly similar at the genome level: they carried a variety of plasmids coding for a variety of carbapenemase alleles coded on different plasmids. In some cases, such differences were even seen among isolates from the same hospital (e.g., see Cluster C1 isolates from Hospital G in Abu Dhabi, Table 2). This considerable heterogeneity within the same ST, even within the same cgMLST cluster, suggests that horizontal transfer of carbapenemase gene-coding plasmids considerably contributes to the epidemiology of ST14 even in a relatively small region. Nevertheless, the fact that same cgMLST group strains possessing the same carbapenemase bearing plasmid could be isolated from multiple hospitals (e.g., Cluster C1 strains of plasmid group A from hospitals A in Sharjah, C in Al Ain, and B in Abu Dhabi) indicates the importance of clonal, inter-hospital spread, as well.

The variety and wide array of genes coding for resistance to numerous antibiotic classes within clusters were also notable findings supporting the previous notion. It also explains how members of the clone develop to being extremely drug resistant, or even pan-drug resistant, which was already observed in the region [8, 30].

Recently, and concerning the convergent evolution of antimicrobial resistance and virulence was observed in *K. pneumoniae*, mostly, but not exclusively, in South-East Asia [6, 32, 33]. Some of these hypervirulent isolates expressed non-K1 capsular types, i.e., K2, K54, or K64 [33, 34]. Although virulence genes typically associated with hypervirulent *K. pneumoniae* (*rmpA/A2*, *iutA*) were not identified in our strains (data not shown), the two capsular types (KL2 and KL64) detected in carbapenem-resistant *K. pneumoniae* ST14 have commonly been linked to hypervirulence and invasive diseases [6, 33, 35]. Further studies are needed to investigate whether this capsular switch in CRKP-ST14 from the well-known K2 type to K64 increases or changes virulence, or simply helps the pathogen to avoid immune recognition, and helps the dissemination of this clone, as was hypothesized in case of

KPC-producing *K. pneumoniae* CC258 with capsular variations [35, 36]. Our data does not allow us to speculate on the molecular mechanisms involved in the emergence of K64 strains. Nevertheless, as the K2 and K64 loci are considerably different [37], the role of recombination rather than local, mutation-based evolution seems to be more likely. However, irrespective of the mechanism behind, the clustering of cgMLST of ST14-K64 isolates collected in 2013–2016 from the UAE and Bahrain and those of other three continents, i.e., Australia from 2016, USA and India from 2017, and the Netherlands (Fig. 2) suggests a potentially emerging subgroup of the *K. pneumoniae* ST14 clone. The dissemination and virulence potential of this subgroup needs further monitoring.

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Author contribution SFM carried out molecular typing, plasmid analysis, detection of carbapenemase genes, participated in whole genome sequencing, in data analysis and in drafting the manuscript, TP participated in designing the study, in the collection of the isolates, in data interpretation and in drafting the manuscript, PGH participated in whole genome sequencing, in data analysis, and in drafting the manuscript, AG participated in molecular typing by PFGE and MLST, and in drafting the manuscript, YI participated in whole genome sequencing and in data analysis, MA and ASO participated in collecting the isolates and in drafting the manuscript, TAR participated in molecular testing and in drafting the manuscript, and AS initiated the study, participated in its design, in the analysis of all data, in drafting the manuscript.

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Data availability The sequence data generated were uploaded to the European Nucleotide Archives under project number PRJEB44852.

Code availability Not applicable.

Declarations

Ethics approval Ethics approval was not necessary as no samples have been collected for the sake of this particular study, the information was recorded in a manner that subjects could not be identified and there were no personal identifiers linked to the specimens and the project did not involve identifiable living humans.

Consent to participate Not applicable.

Consent to publication All co-authors agreed to the submission of the current form of the manuscript.

Conflict of interest TP has received grants independently of this project, from Merck, Pfizer and Becton–Dickinson. The other authors have no conflict of interest to declare.

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