Persistence of *Klebsiella pneumoniae* ST258 as the predominant clone of carbapenemase-producing Enterobacteriaceae in post-acute-care hospitals in Israel, 2008–13

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**Objectives:** To study the molecular characteristics of carbapenemase-producing Enterobacteriaceae (CPE) in post-acute-care hospitals (PACHs) in Israel and to analyse the temporal changes between 2008 and 2013.

**Methods:** CPE isolates were obtained during two cross-sectional, point prevalence national surveys of PACHs in Israel performed in 2008 and 2013. Surveillance cultures were collected by streaking rectal swabs onto selective media. Isolates were identified to species level and tested for *bla*KPC, *bla*NDM and *bla*OXA-48 by PCR and by the CarbaNP test. Molecular typing was done by PCR for the *pilv-l* gene, designed for the ST258 KPC-producing *Klebsiella pneumoniae* (KPC-KP) clone, BOX-PCR and MLST.

**Results:** The prevalence of CPE carriage in the first survey was 184/1147 (16%); all of the isolates were KPC-KP. The prevalence of CPE carriage in the second survey was 127/1287 (9.9%); of these isolates, 113 (89%) were KPC-KP, 9 (7%) were other KPC-producing species and 5 (4%) were NDM- and OXA-48-producing CPE (*n* = 1 and 4, respectively). The proportion of the KPC-KP population represented by the ST258 clone increased from 120/184 (65%) in 2008 to 91/113 (80%) in 2013. In 58% (71/122) of the KPC-CPE carriers identified in the 2013 survey, the source of acquisition was determined to be the PACH itself. All four OXA-48 CPE were acquired either directly or indirectly from patients arriving from the Palestinian Authority or Syria.

**Conclusions:** Despite the decreased prevalence of CPE in Israeli PACHs, and the emergence of new types of CPE, the KPC-KP ST258 clone remains the predominant clone represented.

**Keywords:** clonal structure, colonization, KPC

**Introduction**

Since the beginning of the millennium, carbapenemase-producing Enterobacteriaceae (CPE) have become a major problem in healthcare systems worldwide. In Israel, a nationwide outbreak of CPE emerged in 2006, consisting primarily of nosocomial spread of KPC-producing *Klebsiella pneumoniae* (KPC-KP). Both initial reports2 and subsequent studies1 have identified ST258 as the main culprit of this outbreak. CPE spread in Israel has been contained thanks to a nationally coordinated effort. In recent years, several reports have indicated that new types of CPE, including NDM- and OXA-48-producing Enterobacteriaceae, have appeared in Israel.5,6

In addition to CPE spread in acute-care hospitals, an important component of the epidemiology of the Israeli outbreak has been the dissemination into post-acute-care hospitals (PACHs). There is ongoing bidirectional patient movement between these institutions and acute-care hospitals, and as such they have become a reservoir of CPE carriage. As part of the Israeli national intervention to contain the spread of CPE in healthcare facilities, an initiative has been implemented in PACHs, with the goals of improving infection control practices overall and, specifically, reducing the prevalence of CPE. As a result of this programme, the prevalence of CPE in PACHs decreased from 16.8% in 2008 to 12.5% in 2011. In this study, our aim was to examine the molecular epidemiology...
of CPE in PACHs in Israel, and to analyse the temporal changes that have occurred between 2008 and 2013.

Methods

Study design, patients and isolates

This was a retrospective, national, microbiological study of CPE isolated in PACHs in Israel. These facilities provide continued intensive treatment after hospital discharge to patients with conditions that result in reduced ability to perform activities of daily living.10 CPE were isolated in two cross-sectional prevalence surveys performed by the National Center for Infection Control (NCIC), the first from November 2008 to January 2009, involving 12 centres, and the second from June 2013 to January 2014, involving the same 12 centres and an additional 2 PACHs.7,10 In both surveys, screening cultures were obtained from all patients hospitalized in a representative number of wards, determined by selection of at least one ward of each type contained in the facility surveyed. Isolates obtained during the interim additional two surveys done in 2010 and 201110 were not included.

General and CPE-targeted infection control policies at Israeli PACHs and assignment of acquisition source

A detailed description of the infection control policies and practices at Israeli PACHs and the national intervention programme to contain the spread of carbapenem-resistant Enterobacteriaceae and CPE are detailed elsewhere.4,7,10 Every CPE isolated from either surveillance or clinical cultures is reported and documented by the NCIC. A positive CPE culture arrived from the Palestinian Authority. This OXA-48-producing isolate was obtained 3 days or more after admission is considered to be acquired in the PACH. Importantly, in 2008 the majority of PACHs were already compliant with the infection control policy to cohort CPE carriers in separate rooms, but only 15% of the institutions performed surveillance cultures on admission versus 100% in 2013. Thus, we were able to account for the source of CPE acquisition in the 2013 survey but not in the 2008 survey. The source of acquisition was determined to be the PACH itself when culture for CPE was positive after 3 days from admission following a negative admission surveillance culture.

Detection and identification of CPE

Rectal swabs were collected and transferred within the same day to the NCIC laboratory. On arrival, swabs were streaked onto CHROMagar KPC (first survey) or MacConkey agar with 1 mg/L imipenem plates (second survey) (HyLabs, Rehovot, Israel)11 and incubated overnight at 37°C. Suspected CPE colonies were subcultured from the respective screening plate onto standard MacConkey plates. Species identification was performed using the ENTEROTEST® kit (second survey) (HyLabs) or, in ambiguous cases, using the VITEK-2 system (both surveys) (bioMérieux, Marcy-l’Étoile, France). Carbenapenemase production was not examined systematically during the first survey and isolates were defined as carbapenem-resistant Enterobacteriaceae based on VITEK-2 susceptibility testing to ertapenem, imipenem and meropenem.7 Isolates from the first survey were stored at −80°C, and were examined, along with all isolates from the second survey, by PCR for blaKPC,12 blaNDM and blaOXA-48.13 Isolates with negative results on these PCR assays were tested for carbenapenemase production by the Carba NP test.16 Isolates were included only if they possessed proven carbenapenemase production by these methods.

Genotyping of KPC-KP isolates

Molecular typing was done for all KPC-KP isolates in a sequential manner. First, isolates were identified as ST258 based on a PCR for the unique pilv-I allele using primers 5′-TGTAGCTGAGGCCGACAC-3′ and 5′-TGATGTCACACCGGTACCA-3′ (product size 320 bp) as previously described.15 Isolates testing negative by pilv-I PCR were analysed by BOX-PCR using primer BOXA1R (5′-CTACGCGG AAAGGGCAACGGTGACG-3′).16 PCR products were resolved using the QIAxcel capillary gel electrophoresis apparatus (Qiagen, Hilden, Germany) and compared visually. Representative isolates of each BOX-PCR type were subjected to MLST for definitive genotyping.17

Results

Molecular epidemiology of CPEs in Israeli PACHs in the two surveys

The prevalence of CPE carriage in the first survey was 184/1147 (16%, 95% CI 13.88%–18.12%); all of the isolates were KPC-KP. Additional isolates defined as carbapenem-resistant K. pneumoniae (n = 11) and Escherichia coli (n = 5) during the time of the survey were identified later as non-carbapenemase producing. The prevalence of CPE carriage in the second survey was substantially lower, 127/1287 (9.9%, 95% CI 8.27%–11.53%; P < 0.001). One-hundred and thirteen (89%) of the isolates were KPC-KP, 9 (7%) were other KPC-producing species and 5 (4%) were other CPE types (Table 1).

The KPC-KP ST258 clone was found in all PACHs in the 2008 survey except for one (H). Its prevalence among all CPE isolates was similar in the 2008 and 2013 surveys—120/184 (65%) and 91/127 (72%), respectively. However, compared with the KPC-KP population alone, the prevalence increased to 91/113 (80.5%) in the 2013 survey. The second most prevalent KPC-KP clone in 2008 (ST340) coexisted with ST258, and was found in four and three PACHs in 2008 and 2013, respectively. Over time its prevalence among all KPC-KP isolates decreased significantly, from 41/184 (22.3%) in 2008 to 10/113 (8.8%) in 2013 (P = 0.001). KPC-KP ST340 was the dominant clone in two centres (designated A and H, Table 1) in 2008, but was superseded in these centres by ST258 in 2013. Other KPC-KP clones were identified only in a single centre and (with the exception of a single isolate of clone ST37) only in one of the surveys.

All pilv-I-negative KPC-KP isolates were from clones other than ST258, except for eight isolates that were positive on prp-PCR testing and were identified in PACH F in the 2008 survey. Similar isolates (pilv-l-negative, prp-positive ST258 KPC-KP) were also identified at the same PACH in a previous study.16

Sources of CPE acquisition, 2013 survey

CPE other than KPC-KP, including NDM- and OXA-48-producing Enterobacteriaceae, were identified only in the 2013 survey (Table 1). The single NDM-producing K. pneumoniae isolate was presumably acquired at PACH C, where NDM-producing CPE have been previously reported. The acquisition of the OXA-48-producing Klebsiella oxytoca carrier in PACH M was via previous contact with a Syrian patient in an acute care centre. In PACH H, the index patient arrived from the Palestinian Authority. This OXA-48-producing K. pneumoniae isolate had a BOX-PCR pattern similar to those of the isolates of the other two OXA-48-producing K. pneumoniae carriers in that centre (data not shown), suggesting intra-institutional transmission.

In the 2013 survey, study of carriage of KPC-KP (n = 122) showed that 71 patients (58%) acquired KPC-KP in the PACH while 39 patients (32%) were colonized prior to PACH admission; for 12 patients (10%), the source of acquisition could not be determined. Acquisition in the PACH was common for carriers of both ST258 (52/91, 57%) and other KPC-CPE types (19/31, 61%).
Discussion

In this study we outline the first systematic national survey of the clonal structure of CPE, and a unique overview of the changes that have occurred in the PACH system between 2008 and 2013. The study provides further evidence for the predominance of ST258 KPC-KP among CPE,18 and illustrates the similarity in clonal structure of KPC-KP in acute-care centres3 and PACHs in Israel.

Analysis of the molecular features and the clonal structure of the CPE isolates reveals two types of temporal change between the surveys. The second survey is characterized by an increase in the rate of ST258 in the KPC-KP population from 65% to 80% and by an increase in the prevalence of non-KPC-KP CPE from 0% to 11%, reflecting the increased diversity of CPE types in Israel.5,6,12 Both of these changes occurred while the overall prevalence of CPE declined from 16.1% to 9.9%. This observation further demonstrates the superior fitness of the ST258 clone and its ability to spread efficiently in the hospital environment. Of note, the second most common clone, ST340, is a single-locus variant of ST258 and was already identified in early reports from Israel.19 The superior fitness of ST258 and its closely related clones, such as ST340 and ST11, is further demonstrated by their global dissemination through Europe,20,21 the USA22 and South America.23

The increased diversity in the non-KPC-KP CPE population may have implications regarding infection-prevention practices, due to differing microbiological features and epidemiology. Indeed, one notable difference was exemplified by the OXA-48-producing CPE isolates, which were all acquired either directly or indirectly from patients arriving from the Palestinian Authority or Syria. This phenomenon highlights the importance of timely identification, screening and preemptive contact isolation of patients admitted from high-risk areas. Another important difference relates to the lower carbapenem MIC values for some of the non-KPC-KP CPE (e.g. OXA-48-producing CPE) compared with the typically high MIC values for ST258 KPC-KP,2,11 which may result in lower sensitivity of surveillance testing.11 Of note, due to the differences in the microbiological-screening methodology applied in the two surveys, it is difficult to exclude the possible presence of non-KPC-KP CPE in the first survey. However, as the first cases of non-imported OXA-48 CPE were detected in Israel only after 2011,5 it is unlikely that OXA-48 CPE had a significant presence in Israeli PACHs in 2008.

As evident from the 2013 survey, most CPE isolates, regardless of their type, were acquired at the PACH itself. This highlights the ongoing effort required at both the local and the national levels in order to contain the spread of CPE and adjust to new changes and challenges posed by the ever-changing epidemiology of these organisms.

Table 1. Clonal structure of KPC-KP and other CPE isolates in Israeli PACHs, 2008–13

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<th>KPC-KP</th>
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<tr>
<td>PACH</td>
<td>no. of ST258</td>
<td>no. of other STs</td>
</tr>
<tr>
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<tr>
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</tr>
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</table>

аIsolates were not available.
бNot included in the 2008 survey.
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Transparency declarations

None to declare.

References