Environmental contamination by carbapenem-resistant Enterobacteriaceae (CRE)

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ABSTRACT

In the last decade, the global emergence of carbapenem resistance in *Enterobacteriaceae* (CRE) has posed great concern to public health. Data concerning the role of environmental contamination in the dissemination of CRE is currently lacking. Here, we aimed to examine the extent of CRE contamination in various sites in the intimate surroundings of the CRE-carriers and to assess the effects of sampling time and cleaning regimens on the recovery rate. We evaluated the performance of two sampling methods, CHROMAgar KPC contact plate and eSwab for the detection of environmental CRE. eSwab was followed by either direct plating or by broth enrichment. First, fourteen sites in the close vicinity of the carrier were evaluated for environmental contamination, and 5 which were found to be contaminated, were further studied. The environmental contamination decreased with distant from the patient; bed area was the most contaminated site. Additionally, we found that the sampling time and the cleaning regimen were critical factors affecting the prevalence of environmental CRE contamination. We found that the CHROMAgar KPC contact plate method was more effective technique for detecting environmental CRE than eSwab-based methods. In summary, our study demonstrated that the vicinity of patients colonized with CRE is often contaminated by these organisms. Using selective contact plates to detect environmental contamination may guide cleaning efficacy and assist with outbreak investigation in an effort to limit the spread of CRE.
INTRODUCTION

Carbapenem resistant Enterobacteriaceae (CRE) have become a major threat to public health worldwide (20, 15, 2). These organisms are spreading globally primarily in the healthcare setting. Physical separation by cohorting carriers and dedicated staff resulted in containing CRE outbreaks (21). Hospital environment, contaminated from infected patients may serve as a source for spread of these bacteria, either directly or indirectly via healthcare personal (6, 4). However, the actual presence of environmental contamination by CRE has not been studied.

Detection of contamination of the healthcare environment requires specialized methods that were mainly studied in various gram-positive organisms, such as Staphylococcus aureus, Enterococci species and Clostridium difficile (9, 22, 14). No standardized methods of CRE environmental culture have been developed. Thus, the aims of our work were to assert the presence of environmental contamination by CRE, to identify the sites that are likely to be contaminated, to evaluate the performance of different environmental culturing methods for recovery of environmental CRE (eCRE) and to evaluate the effects of various parameters on the recovery rate.

MATERIALS AND METHODS

Setting and patient selection

The study was conducted as part of an ongoing surveillance program that had been implemented at the Tel-Aviv Sourasky Medical Center (TASMC), a 1,200-bed tertiary care hospital in Tel-Aviv, Israel. From December 2010 through May 2011, cultures were collected from the environment of 29 KPC-producing CRE carriers, at 2 separate Internal Medicine wards. Five patients were sampled twice at different time points in intervals of approximately 3 months.
Therefore, we referred to a total of 34 patients which were sampled during this study. Environmental samples were collected twice per each patient's sampling: in the morning and at noon, 24 and 4 hours after room cleaning and patient cloths and sheets were changed, respectively.

**Environmental sampling design**

Environmental sampling was coordinated and supervised by the Infection Control Program at TASMC. An initial preliminary study was performed in order to determine the sampling sites for CRE (detailed in the Results section). After the preliminary study, five sampling sites surrounding each CRE-colonized patient were chosen for eCRE sampling: sheets surfaces around the pillow, crotch and legs; personal bed-side table and the infusion pump (20/34 patients). In each ward tested, samples were also taken from an unoccupied bed, to evaluate for non-specific environmental contamination. Environmental samples were immediately (within 30 min) transferred to the laboratory for further work-up.

**Cultivation methods for environmental samples**

Two environmental sampling methods were compared for the recovery of eCRE: (i) Direct application of CHROMAgar KPC contact plates supplemented with 0.7 gr/L lecithin and 4.5 ml/L Tween 80 (CP; HyLabs, Rehovot, Israel); (iia) surface sampling by eSwab (ES; Copan Diagnostics, Italy), followed by either direct streaking on CHROMAgar KPC plates (HyLabs, Israel) or (iib) following enrichment in Brain Heart Infusion (BHI) broth (ESBB).

Sampling was performed as follows: (i) CP- CHROMAgar KPC contact plates (5 cm diameter, area-19.625 cm²) were pressed to the tested surface for 3-5 seconds and then incubated
at 37°C for 48 h. (iia) ES- the eSwab was moved at right angles up and down within a 10 X 10 cm area defined by a sterile square template frame for approximately one minute. The swab was placed in the eSwab fluid-containing tube and transported to the lab. After 1 min vortex at maximum speed, 200 µl of the suspension were spread onto a CHROMAgar KPC plates and placed for incubation at 37°C for 48 h. (iib) ESBB- environmental sampling was performed as described in section (iia) followed by an enrichment step in which 50 µl of the eSwab medium were inoculated into 3 ml of BHI broth, and incubated at 37°C with shaking at 150 rpm for 48 h. Subsequently, approximately 10 µl of the broth were spread with cotton tipped applicators on a CHROMAgar KPC plate, which was then incubated at 37°C for 48 h.

Characterization of CRE from patients and environmental culture

Detection and identification of CRE in patients were done as previously described (19, 1). Identification of eCRE colonies was performed based on characteristics growth on CHROMAgar KPC according to the manufacturer's instructions (Klebsiella and Enterobacter species, medium-size dark metallic blue colonies; E. coli, medium to large pink/dark rose colonies). Blue and pink colonies were tested by blaKPC PCR (19) and further confirmation using the Vitek 2 system (bioMerieux).

Data analysis

Bivariate analysis of categorical variables was done using the χ² test. Analyses were done using the JMP IN v 3.2.1 software (SAS Institute Inc.).
**RESULTS**

*Identification of sites contaminated with eCRE*

We first sought to identify the environmental sites that were contaminated in the vicinity of the eCRE carriers. Fourteen sites were surveyed 6 times for eCRE using CHROMAgar KPC contact plates: bed linen around the head (pillow), crotch and legs; personal bed-side table, the infusion pump, personal chair, dedicated stethoscope, electrical outlet line, suction machine, respirator, cardiovascular monitor screen, pulse oximeter, manual respirator bag and enteral feeding pump (Fig. 1). eCRE were identified in only 5 of the 14 sites sampled: sheets surfaces around the pillow, crotch and legs; personal bed-side table and the infusion pump. Based on these preliminary data, these sites were further tested in our study.

Five empty beds from the two wards were surveyed for eCRE contamination, to test for non-specific contamination. None of them were found to be contaminated with eCRE.

*The recovery of eCRE using each sampling method*

928 environmental samples were collected in this study from the vicinity of 34 known KPC-producing CRE carriers using the 3 different sampling methods- CP, ES and ESBB. Five sites were sampled from each carrier, except for the infusion pump that was present in the surrounding of 20/34 patients. One patient was not sampled around the legs and two ESBB samples were accidently discarded. A positive eCRE cultures was identified at least once in 30/34 patients (88%).

We evaluated the role of the following variables on the recovery rate of eCRE: the sampling and cultivating method; the sampling site; the time of sampling and the ward. Of the 928 samples, 224 were positive for eCRE using any of the tested method (24%). The recovery
rates of the three sampling methods were 32%, 24% and 16% for CP, ESBB and ES, respectively (Fig. 2A).

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The recovery rate at different sampling sites

The recovery rates of eCRE at the different sites were 68/204 (33%) at the pillow, 63/202 (31%) at the crotch, 46/198 (23%) at the legs, 19/120 (16%) at the infusion pump and 28/204 (14%) at the personal bed-side table (p<0.0001; Fig. 2B). The distribution of these positive eCRE as a function of the sampling cultivation method is shown in Table 1. The CP method was superior at the infusion pump and personal bed-side table sites, but was inferior to the eSwab sampling methods (ES and ESBB) at the pillow site (p>0.05 for all) (Table 1).

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The effect of routine cleaning and ward on the recovery of eCRE

In order to examine the effect of routine cleaning on the endurance of CRE in the environment, we sampled at two different time points during the day: in the morning and at noon, prior to and 4 hours after cloths and sheets replacement, respectively. 465 samples were collected in the morning and 463 at noon. At morning time, 126/465 (27%) of the samples were tested positive to eCRE, whereas only 98/463 (21%) were positive at noon (p<0.05; Fig. 2C).

504 environmental samples were collected from ward A and 424 from ward B, from the vicinity of 18 and 16 patients, respectively. The recovery rates differed significantly - 146/504 (29%) at ward A and 78/424 (18%) at ward B (p=0.0002; Fig. 2D). We have examined the recovery rates data of eCRE at the different sampling sites in each ward ward. In only one site, infusion pump, the recovery rate of eCRE was lower in ward A compared to B (3% vs. 18%,
respectively, p=0.0002), while at the legs site the recovery rate in ward A was higher than in B (25% vs. 13%, respectively, p=0.0367).

DISCUSSION

In the present study we documented the contamination of the hospital environment, in the vicinity of KPC-producing CRE carriers. eCRE were detected in the surroundings of 88% of these patients. This finding has ominous implications regarding the ability of the environment to serve as vector for transmission of CRE in the healthcare settings.

We identified several factors, both methodological and environmental, that significantly affect the retrieval rate of eCRE. First, we found that the sampling-cultivating method has great implication on the sensitivity of the sampling. We compared the performances of CHROMagar contact plates (CP) vs. eSwabs (ES) as sampling tools. The CHROMagar KPC medium was chosen based on previous study of ours that showed their high performance for detecting KPC-producing CRE (1). The additive surface-active components (Lecithin and Tween 80) were added to eliminate the effect of disinfectants present in the environment that may inhibit growth of microorganisms (7, 10). The eSwab was chosen thanks to its increased sensitivity that could be ascribe both to the flocculated characteristics and to the transport Amies solution, which acts as a non-selective fluid and facilitates sampling of bacteria (5). In addition to the 2 sampling methods, we also added an enrichment step that was compared with direct plating from the swab, in order to improve the recovery of slow-growing bacteria (12, 8).

All sampling methods, CP, ES with and without enrichment were able to recover CRE from the environment. Overall, the CP method was superior compared to ES despite the fact that a greater surface area was sampled by the swab (100 cm²) compared to the contact plate (19.625 cm²).
Our findings are in accordance with other studies, which observed a better recovery of environmental infectious bacteria with contact plates than with the swab method followed by direct plating or enrichment step (11, 16), although this difference may vary according to the organism sought. Obee et al. (16) showed higher recovery rate of methicillin-resistant *Staphylococcus aureus* (MRSA) from a stainless steel table using meticillin contact plates compared with a swab method. In contrast, Lemman et al. (11) showed that Rodac plates were superior to the swab technique in detecting Gram-positive cocci, whereas the swab method exhibited higher performance in detecting Gram-negative rods. The authors also obtained improvement in the detection rate for Gram negative bacteria by using enrichment step after swab sampling.

Previous studies suggested several explanations for the shortcomings of the swab method in sampling the hospital surroundings for infectious bacteria. These include the following: damage to the bacteria cells during swabbing (16); adhesion of bacterial cells to the swab fabrics, which can then be trapped within the swab bud (18, 13, 8, 5); the amount of pressure being applied to the swab handle during swabbing can limit the number of bacteria collected from the surface (13); and the transport media which can affect bacteria survival (17, 18). Thus, it is possible that the lower recovery rates obtained by the swab method in our study might result from one or several of these factors.

We were able to improve significantly the recovery rate of the swab method (Fig. 2A) by applying an enrichment step prior to plating. This observation is in accordance with previous studies on various bacteria. Hallgren et al. (9) were able to obtain a significant increase in the detection sensitivity of VRE from the environment using selective broth enrichment step compared to direct plating.
Contamination by drug-resistant bacteria may be found on several surfaces, including the floor, the bed-frame, the furniture, the patients’ clothes and the bed sheets (23). In our first part of the study, we identified 5 locations that are most likely to be contaminated- the bed surfaces, the infusion pump and the personal table. We found that the detection rate of eCRE is reduced with increased distance from the carrier, with the bed surfaces being the most contaminated sites. This reduction is probably due to the fact that medical equipments and items in distance from the patients are less exposed to hand-touched or body secretion of CRE-carriers. Similar findings were previously observed with different organisms. Dancer (3) reported that the bed linen, patients’ gowns, and the over bed table were the most contaminated area with MRSA compared with other items such as the bed rails, bedside lockers and infusion pumps. Similarly, Lemmen et al. (12) observed reduction in the detection rate of multi-resistant Gram-positive bacteria with distance from patients harboring these organisms. However, this trend was not observed for the Gram-negative bacteria.

The environmental surface being sampled may play a role in the detection efficiency of the different sampling methods. Several surface characteristics such as surface charge, topography and hydrophobicity can affect the retrieval efficiency of the collection method. According to Obee et al. (16) contact plates are effective in observing bacteria on flat and usual shape surfaces, while swabbing is sufficient for dry surfaces. Accordingly, in our study, the recovery rates by the contact plate method was inferior to eSwab in detecting bacteria at the irregular pillow site which considered to be non flat, and less accessible for sampling, but, was superior at the personal bed-side table and infusion pump sites, which are flat and regular.

Two environmental factors were found to affect the recovery rate of eCRE. First, the time from cleaning to sampling was a significant factor. Although hardly surprising, it highlights the
importance of frequent cleaning, especially in the vicinity of carriers of resistant bacteria, in order to reduce the potential of environmental-related transmission. But also that shortly after cleaning the patient close vicinity is re-contaminated. Furthermore, we were able to observe differences in the cleaning quality between ward A and B, as ward A was significantly more contaminated than ward B. This may be explained by factors such as the ratio of degree of crowdedness, the staff/patients ratio and also by differences in the infrastructure. The difference was especially pronounced in the recovery of eCRE from the bed side equipments (personal bed-side table and infusion pump). As the two wards are both at the same institution sharing similar resources, it indicates the importance of attention by the ward management to meticulous cleaning routines. Also, it demonstrates the potential value of environmental cultures as a quality indicator tool in the healthcare setting.

In conclusion, the study preformed in our hospital had shown the existence of CRE contamination at the patients' surroundings in different wards and the utility of different sampling-cultivation methods. It highlights the importance of standard cleaning regimens of surfaces and items at the patients' intimate surrounding and awareness of its role in CRE dissemination and transmission to other patients.
REFERENCES


<table>
<thead>
<tr>
<th>eCRE sampling method</th>
<th>P value (^a)</th>
<th>Distribution of positive eCRE/total positive recovered at the respective sampling site (% recovery)</th>
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<tbody>
<tr>
<td>Pillow</td>
<td>p=0.1619</td>
<td>24/100 (24%) 29/100 (29%) 20/100 (20%) 16/100 (16%) 11/100 (11%)</td>
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<td>Crotch</td>
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<td>Legs</td>
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<td>Personal bed-side</td>
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<td>table</td>
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<tr>
<td>Infusion pump</td>
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<tr>
<td>CP</td>
<td>p=0.0011</td>
<td>19/50 (38%) 15/50 (30%) 10/50 (20%) 5/50 (10%) 1/50 (2%)</td>
</tr>
<tr>
<td>ES</td>
<td>p=0.0051</td>
<td>25/74 (34%) 19/74 (26%) 16/74 (22%) 7/74 (9%) 7/74 (9%)</td>
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<tr>
<td>ESBB</td>
<td></td>
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\(^a\) The p value relates to the differences between sites for a particular sampling method.

CP = CHROMAgar KPC contact plates; ES- eSwab sampling, direct plating onto CHROMAgar KPC plates; ESBB- eSwab sampling followed by broth enrichment prior to plating.
Figure legend

**FIG 1** Locations of testing for environmental CRE (eCRE). 1. Personal bed-side table; 2-4. bed linen around the pillow (2), crotch (3) and legs (4); 5. pulse oximeter; 6. personal bed side chair; 7. electrical outlet line; 8. manual respirator bag; 9. infusion pump; 10. dedicated stethoscope; 11. ventilator; 12. suction machine; 13. cardiovascular monitor screen and 14. enteral feeding pump.

**FIG 2** Recovery rates (% positive samples) of environmental CRE (eCRE) from the patients’ surrounding. A. The effect of the 3 sampling-cultivation methods on the recovery rate of eCRE. CP- CHROMAgar KPC Contact plates; ES- eSwab sampling, direct plating onto CHROMAgar KPC plates; ESBB- eSwab sampling, broth enrichment prior to plating; B. The recovery rates of eCRE from 5 different sites in the vicinity of the carriers: pillow; crotch; legs; personal bed-side table; and infusion pump; C. The effect of sampling time on the recovery rate of eCRE. Morning and noon samples were done before and 4 hours after cloths and sheets replacement, respectively; and D. The recovery rate of eCRE from two wards at TASMC.