Eradication of carbapenem-resistant *Enterobacteriaceae* gastrointestinal colonization with nonabsorbable oral antibiotic treatment: A prospective controlled trial

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Key Words:
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SDD
Gentamicin
Colistin

**Background:** Carbapenem-resistant *Enterobacteriaceae* (CRE) are emerging. In attempt to eradicate CRE colonization, we conducted a semirandomized, prospective, controlled trial using oral nonabsorbable antibiotics.

**Methods:** Consecutive hospitalized CRE carriers were studied. Patients whose rectal isolates were gentamicin sensitive but colistin resistant were treated with gentamicin. Patients whose isolates were colistin sensitive but gentamicin resistant were treated with colistin. Patients whose isolates were sensitive to both drugs were randomized to 3 groups of oral antibiotic treatment: gentamicin, colistin, or both. Patients whose isolates were resistant to both drugs, and those who did not consent, were followed for spontaneous eradication.

**Results:** One hundred fifty-two patients were included; 102 were followed for spontaneous eradication for a median duration of 140 days (controls), and 50 received 1 of the 3 drug regimens: gentamicin, 26; colistin, 16; both drugs, 8, followed for a median duration of 33 days. Eradication rates in the 3 treatment groups were 42%, 50%, and 37.5%, respectively, each significantly higher than the 7% spontaneous eradication rate in the control group (\(P < .001\), \(P < .001\), and \(P = .004\), respectively) with no difference between the regimens. No significant adverse effects were observed.

**Conclusion:** Oral antibiotic treatment with nonabsorbable drugs to which CRE is susceptible appears to be an effective and safe for eradication of CRE colonization and, thereby, may reduce patient-to-patient transmission and incidence of clinical infection with this difficult-to-treat organism.

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hypothesized that continuous bacteremia could have been secondary to repeated bloodstream invasion by the CRE from a reservoir in the gastrointestinal (GI) tract through the damaged mucosa during severe mucositis. Several studies have shown that selective digestive decontamination (SDD) treatment may eradicate carriage of multidrug-resistant bacteria, reduce the incidence of nosocomial infections, and control nosocomial outbreaks caused by these organisms.\textsuperscript{10–14} In an attempt to eradicate the GI source of CRE in these patients, to prevent development of associated bacteremia, and to control infection spread to other inpatients, a pilot study of oral treatment with gentamicin, to which CRE was susceptible, was conducted. This study demonstrated a 66% eradication rate of carrier state of CRE among hematology-oncology and BMT recipient patients and resolution of the persistent bacteremia in 62.5% of the patients after eradication of the carrier state.\textsuperscript{15}

These results prompted us to conduct a randomized, prospective, controlled trial throughout the hospital, aimed at eradicating GI CRE colonization, using oral nonabsorbable antibiotic treatment with gentamicin (GM), colistin (COL), or both.

**PATIENTS AND METHODS**

The study was conducted at the Rambam Health Care Campus, a 1,000-bed, tertiary care center in northern Israel. The hospital admits approximately 80,000 patients a year and includes all major departments and services, with 85 intensive care unit (ICU) beds and 25 hematology-BMT beds. All consecutive hospitalized adult patients identified as CRE carriers by rectal surveillance cultures were included in the study, which was approved by the Institutional Review Board (Approval No. 0004-09) and registered in the ClinicalTrials.gov (ID No. NCT00966810).

Rectal surveillance cultures were obtained in the following situations: on admission to the hospital, from high-risk patients (defined as patients who were hospitalized during the previous 6 months in acute care or long-term health care facilities, about 20 patients per day); on admission and routinely once weekly in selected wards such as ICU, hematology-oncology, and BMT; and from contacts of identified carriers.

Data obtained for the study patients included demographics, underlying diagnosis, duration and eradication of CRE colonization, GM blood levels, adverse events, clinical CRE infection, survival status at study completion, and CRE-related mortality.

**Microbiologic studies**

Rectal swab screening samples were cultured on PD420 CHROMagar Klebsiella pneumoniae carbapenemase (KPC) plates (Hy Laboratories Ltd, Rehovot, Israel). DNA was extracted from suspected KPC–possessing blue colonies using QIamp DNA mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s instructions. KPC was detected using polymerase chain reaction (PCR)–based assays specific for blaKPC gene, as described.\textsuperscript{16} Other CRE were detected using the Hodge test according to the Clinical Laboratory Standards Institute (CLSI) methods\textsuperscript{17} whenever PCR was negative. Susceptibility to GM was determined by the disk diffusion test. The minimal inhibitory concentration (MIC) for COL was determined by the E-test (AB Biodisk, Salone, Sweden). Isolates were considered COL-susceptible if the MIC was less than or equal to 2 mg/L in accordance with the European Committee on Antimicrobial Susceptibility Testing breakpoints.\textsuperscript{18}

**Preparation of study drugs**

GM 80-mg capsules were prepared using GM sulphate powder (Lot No. 08A24-N07) mixed with lactose diluents filled into gel capsules size No. 3. COL 100 mg (2,000,000 units) capsules were prepared using COL sulphate powder (Lot No. 20090101) mixed with lactose diluents filled into gel capsules size No. 1.\textsuperscript{19} COL sulphate was used to achieve acid and soluble preparation. The capsules were filled using an extemporaneous filling method by a manual filling machine (Feton automatic capsule filling machine).

**Study design**

Patients who did not consent or whose isolates were resistant to both drugs were followed with repeated rectal swabs to assess spontaneous eradication rate (control group). Patients whose rectal isolates were GM susceptible but COL resistant were treated with oral capsules of GM sulphate 80 mg 4 times daily. Patients whose isolates were COL susceptible but GM resistant were treated with oral capsules of COL sulphate 100 mg 4 times daily. Patients whose isolates were sensitive to both drugs were randomized by balanced randomization of total set of subjects to 3 groups of oral antibiotic treatment: GM, COL, or both (Fig 1).

Oral drug treatment was given until eradication, or for a maximum of 60 days, whichever came first. Patients were followed by repeated rectal swabs to determine eradication (minimum interval between 2 samples was 3 days). Blood and other relevant clinical cultures were obtained as clinically indicated. Patients with CRE-associated clinical infection were additionally treated with intravenous GM or COL or tigecyclin (based on in vitro susceptibility) or a combination of those drugs.

**Definitions**

“Colonization (carrier state)” was defined as the presence of at least 1 positive rectal swab for CRE. “Eradication” was defined when 3 consecutive rectal swabs were negative for CRE, including PCR testing of the third negative sample. “Failure of eradication for the treatment group” was defined when (1) CRE carriage persisted after 60 days of oral antibiotic treatment, (2) CRE relapsed after presumed eradication, (3) isolate turned resistant to the administered drug, and (4) premature discontinuation of drug treatment (death, loss to follow-up, unwilling to continue participation in the study). “Failure of eradication for the control group” was defined when CRE colonization persisted through the end of the study. Patients were excluded from the control arm if the interval between first and last rectal swabs was < 20 days (when duration of follow-up was shorter than 20 days. Overall, 34 patients died or were lost for follow-up).
Table 1: Demographics and underlying conditions in all groups of patients

<table>
<thead>
<tr>
<th>Age: average (range), y</th>
<th>Gentamicin treatment (n = 26)</th>
<th>Colistin treatment (n = 16)</th>
<th>Gentamicin + colistin treatment (n = 8)</th>
<th>All treatment regimens (N = 50)</th>
<th>Control group follow-up only (N = 102)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male/female (% male)</td>
<td>16/10 (62)</td>
<td>7/9 (44)</td>
<td>6/2 (75)</td>
<td>29/21 (58)</td>
<td>61/41 (60)</td>
<td>.832</td>
</tr>
<tr>
<td>Underlying diseases, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematologic malignancies + SCT</td>
<td>15</td>
<td>15</td>
<td>4</td>
<td>34 (68)</td>
<td>12 (12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Solid tumor</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>15 (15)</td>
<td>.004</td>
</tr>
<tr>
<td>Medical illness</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>11 (22)</td>
<td>15 (15)</td>
<td>.262</td>
</tr>
<tr>
<td>Surgical illness</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5 (10)</td>
<td>16 (16)</td>
<td>.340</td>
</tr>
<tr>
<td>Bedridden</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>44 (43)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

P value, statistical significance of the difference between all treated patients and control patients; SCT, stem cell transplantation.

Table 2: Microbiologic data of rectal isolates, rectal sampling, and duration of treatment/follow-up

<table>
<thead>
<tr>
<th>Rectal isolates, n (%)</th>
<th>Gentamicin treatment (n = 26)</th>
<th>Colistin treatment (n = 16)</th>
<th>Gentamicin + colistin treatment (n = 8)</th>
<th>All treatment regimens (N = 50)</th>
<th>Control group follow-up only (N = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>25 (96)</td>
<td>10 (62.5)</td>
<td>8 (100)</td>
<td>43 (86)</td>
<td>97 (95)</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>2 (12.5)</td>
<td>3 (19)</td>
<td>4 (8)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1 (4)</td>
<td>3 (19)</td>
<td>4 (8)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Citrobacter</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumonia + Enterobacter spp</td>
<td>1 (1)</td>
<td></td>
<td></td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumonia + Enterobacter spp + Escherichia coli</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis

Categorical variables were analyzed by the $\chi^2$ test and Fisher exact test, as appropriate. Continuous variables were compared between groups by Student t test or the Mann-Whitney test, as indicated. All reported P values are 2-sided, and a value of less than .05 was considered significant. All statistical analyses were done with SPSS 17.0 software (SPSS Inc, Chicago, IL). Cumulative eradication curves were prepared by means of the Kaplan-Meier method, and univariate eradication distributions were compared with use of the log-rank test.

RESULTS

The study was conducted over a 24-month period, starting on June 1, 2009: 152 patients were included in the final analysis. Demographics and underlying conditions are presented in Table 1.

Rectal isolates and duration of treatment/follow-up

Fifty patients received 1 of the 3 drug regimens: GM, 26 patients; COL, 16 patients; GM + COL, 8 patients. One hundred two patients were evaluated for spontaneous eradication (control group), and, of them, 3 had their rectal isolate resistant to both drugs.

Rectal isolates included Klebsiella sp (KLB) in 141 patients; Escherichia coli (COL) in 4 patients; Enterobacter sp (EBR) in 3 patients; Citrobacter in 1 patient; KLB + EBR in 1 patient; KLB + EBR + COL in 2 patients (Table 2).

GM susceptibility rate of rectal isolates was 67% (83/124), and that of COL was 90% (96/106). Duration of the follow-up in all drug treatment groups was much shorter than that of the control group (33 vs 140 days, respectively) because the duration of treatment was limited to a maximum of 60 days. The control group was followed for as long as possible within the study period.

Eradication of rectal carriage

The spontaneous eradication rate observed in the control group was 7% (7/102) after a median follow-up period of 140 days (range, 20-737). The eradication rate with GM was 42% (11/26), after a median time of treatment of 31 (range, 12-60) days. Failure of eradication was noted in 58% (15/26) of patients treated with oral GM. Reasons for failure were persistence of CRE colonization in 4 patients; 3 patients stopped treatment prematurely (Two did not want to continue to swallow the pills, and 1 did not want to provide a rectal swab); 2 patients relapsed after apparent eradication, and, in 6 patients, the isolate became resistant to GM during therapy after an average of 18 treatment days. The eradication rate with COL treatment was 50% (8/16), after a median time of treatment of 54 (range, 22-62) days. Failure of eradication was noted in 50% (8/16) of patients receiving COL. Reasons for failure were premature discontinuation of treatment in 3 patients (Two died during treatment, and 1 patient’s primary physician advised against.); relapse in 4 patients; and development of resistance during treatment in 1 patient. The eradication rate with the combination treatment was 37.5% (3/8) after a median time of treatment of 45 (range, 28-50) days. Failure of eradication was noted in 62.5% (5/8) of the patients treated with a combination of GM and COL. Reasons for failure were premature discontinuation of treatment in 3 patients (One did not want to continue, 1 died during treatment, and 1 was advised against by his primary physician) and relapse in 2 patients. The eradication rate of each treatment group (42%, 50%, 37.5%, respectively) was significantly higher than the 7% spontaneous eradication rate in the control group ($P < .001$, $P < .001$, $P = .004$, respectively), with no difference among the 3 treatment groups. In addition, the eradication rate of 44% observed among the...
50 patients on any treatment was significantly higher than the 7% spontaneous eradication rate \((P < .001)\) (Table 3). Cumulative eradication curves for patients who received treatment and controls \((P < .001)\) are compared in Figure 2.

**Adverse events**

No significant adverse effects were observed in any of the treatment regimens. No GI absorption of GM was found. Blood GM levels measured on several occasions in 10 patients receiving oral GM amounted to 0 to 0.4 mg/L.

**Clinical isolates and outcome**

Positive CRE clinical cultures (from any source other than rectal swab) were recovered from 81 of 152 (53%) patients included in the study. CRE clinical culture was positive in 58 of 102 (57%) patients in the control group, as compared with 23 of 50 (46%) patients on antibiotic treatment. The difference was not statistically significant. Positive blood cultures were observed in 13 of 102 (13%) patients in the control group, as compared with 8 of 50 (16%) patients on treatment, also a nonsignificant difference (Table 4).

Intravenous antibiotic treatment was administered to 20 patients in the treatment groups. It was given to 12 patients out of 26 (46%) who were on oral GM, to 5 patients out of 16 (31%) who were on oral COL, and to 3 patients out of 8 (37.5%) who were on both oral drugs. Three patients did not receive systemic antibiotics despite having a clinical isolate, which was considered a colonizer. In the control group, 42 patients out of 102 (41%) received intravenous antibiotics, and 16 patients with clinical isolates were considered colonized. There was no statistically significant difference in the frequency of intravenous antibiotic administration between the control group and the treatment groups, as well as among the 3 different treatment groups. Also, there was no correlation between the successful eradication and the administration of intravenous antibiotics in any of the study groups.

Overall mortality during the follow-up period in the control group was 54 of 102 (53%), significantly higher than the 11 of 50 (22%) in the treatment group \((P < .001)\). However, CRE-related mortality was similar in the control group (6/102, 6%) and the treatment group (3/50, 6%). Nevertheless, there was a trend toward lower mortality \((P = .051)\) among patients who succeeded eradication on treatment (2/22, 9%), compared with those who failed eradication on treatment (9/28, 32%). Moreover, patients who had successful eradication of their carrier state, whether spontaneous or on treatment, had significantly lower mortality rates compared with those who remained persistent carriers, with or without treatment \((17\% vs 49\%, \text{respectively}, \quad P = .002)\) (Table 5).

**DISCUSSION**

This prospective, semirandomized, controlled trial has demonstrated 44% eradication of the CRE carrier state, using treatment with oral nonabsorbable antibiotics to which CRE was susceptible, as compared with the 7% spontaneous eradication rate.

Infections caused by CRE are reported to have crude mortality rates as high as 47% to 70%8,20,21 and attributable mortality of up to 33%9,8,22-25 probably related to the panresistance of these organisms and the lack of availability of effective antibiotic therapy. Strict infection control measures have been recommended by the CDC and by health authorities around the world9 to prevent transmission of CRE from patient to patient, reduce the reservoir of colonized patients, and thereby reduce the rates of nosocomial infections caused by this difficult-to-treat organism. In March 2007,
A large outbreak of CRE colonization and bloodstream infection among our BMT inpatients during the autumn of 2008 caused us to conduct a pilot study of oral GM treatment for the eradication of CRE carrier state. The favorable results of this trial15 motivated us to start this wide-scale, prospective, randomized study.

The design of the study was such that the control group included carriers who could not or would not consent and carriers whose isolate was resistant to both study drugs. This study design was chosen because the majority of carriers, as previously reported,27 were either severely ill (ICU type of patients) or bedridden patients with cognitive impairment, unable to give consent. Exclusion of these patients from the study would ultimately eliminate most of the carriers from participation. Therefore, we received Institutional Review Board approval to include these patients for rectal sampling as the only study procedure, which is the standard of care in our medical center (sampling patients at risk) anyway.

The oral antibiotic decolonization treatment with GM and COL was used because the vast majority of the CRE strains in our institution were susceptible only to these 2 antimicrobials28 and because these drugs are well-known components of SDD regimens based on their oral nonabsorbance.29

Because of the fact that the study was not completely randomized, there were statistically significant differences in the demographics and underlying conditions between the control group and the treatment group as a whole. The average age of the control group was higher, and more patients in this group were bedridden and had more significant comorbidities, as compared with the treatment group. However, there were no statistically significant differences in the demographics and underlying conditions among the 3 different oral treatment arms.

We used a prolonged treatment period of up to 60 days, unlike most traditional SDD regimens, based on our pilot study experience, which showed eradication after a median of 27 treatment days.15 We used a definition of eradication as 3 consecutive negative rectal swabs for CRE as well as a negative PCR of the third negative specimen. This is a much more strict definition than was used in a recently published study,28 which used 1 negative rectal sample to define eradication. In many patients, the rectal samples were intermittently positive, which pointed toward sampling error or a lower burden of organisms and not a real eradication. Thus, it is more likely that eradication defined as 3 consecutive negative samples is a real eradication. Nevertheless, even with the use of this strict definition of eradication, oral antibiotic treatment with nonabsorbable drugs to which CRE is sensitive effectively eradicated the CRE carrier state.

The eradication rate achieved on treatment was 44%. There may be several explanations as to why this rate was not higher. The drug doses used in the current study were based on SDD regimens.10-14 We did not measure drug concentration in feces, and it could be that they were below the MIC of some of the isolates, and higher doses could provide better results. Some of the failures were due to relapse of the carrier state (positive culture after 3 negatives). However, one could not exclude the possibility that, in some cases, it was actually re-infection and that the real eradication rate was higher. Another explanation could be the development of resistance to the administered drug. This is always a concern with prolonged treatment with possible suboptimal doses. Indeed, some of the isolates developed resistance to the administered drug (1/16 in COL and 6/26 in GM). It is possible, especially with GM, that doses used were too low. However, resistance occurred only on mono-therapy and never on combination therapy. One of the advantages of combination antibiotic therapy is reduction of resistance emergence rate, and this could well be a reason to prefer the combination treatment arm. It is noteworthy that we did not observe any significant adverse events, not even renal function impairment, despite including patients with renal function abnormalities and those receiving concomitant nephrotoxic drugs.

As previously mentioned, clinical infection with CRE yields a high crude mortality rate and has a significant attributable mortality as well.8,20-25 Eradication of colonization with these highly resistant organisms in hospitalized patients definitely causes decrease in transmission of CRE to other hospitalized patients. However, does it lead also to reduction in clinical infection or mortality? In the current study, overall mortality in the control group was significantly higher than that of the treatment group (53% vs 22%, respectively, P < .001), probably because of underlying comorbidities. The patients in the control group were significantly older, and 43% of them were bedridden because of significant physical and mental impairment as compared with the patients in the treatment groups, where none of them was bedridden. CRE-related mortality however, among patients who had CRE bloodstream infection, was similar in both groups (3/8 vs 6/13 in patients and controls, respectively). However, when mortality rate in patients who had successful eradication of the carrier state (either spontaneous or on treatment) was compared with that of patients failing eradication, significantly lower mortality (17% vs 49%, respectively, P = .002) was found in the former group. This could point toward a real reduction of mortality attributed to the eradication of CRE carrier state.

There are 2 recently published studies dealing with decontamination of GI colonization of CRE with oral GM and polymixin E. Perez et al30 used a mouse model to examine the effect of oral antibiotic treatment on the elimination of intestinal colonization with KPC-producing K pneumoniae. They found that orogastric treatment with GM and polymixin E suppressed KPC-producing K pneumoniae to undetectable levels in the majority of mice. In the second study, which was a randomized, double-blind, placebo-controlled trial conducted by Saidel-Odes et al,30 oral GM and polymixin E were used in humans for the eradication of carbapenem-resistant K pneumoniae carriage. The latter authors reported a significant reduction in the carriage rate in the treatment arm as compared with the placebo arm, after 7 days of therapy, a difference that lost significance after 6 weeks of follow-up. There are some major differences between their study design and ours. Saidel-Odes et al treated their patients for 7 days compared with 60 days in our trial. A longer duration of treatment is probably preferable because eradication occurred after 31 to 54 days of therapy in the current trial. The second major difference is the
“liberal” definition of eradication used in the study by Saidel-Odes et al, which is likely to have caused false over-estimation of the eradication rate. Indeed, in 6 weeks of follow-up, colonization reappeared. Nevertheless, this study is an important addition to the “proof of concept.”

On the other hand, a very recent study by Zimmerman et al. studying the duration of CRE carriage, supports our findings of very low spontaneous eradication rate. Zimmerman et al. followed CRE carriers and found mean time to culture negativity to be 387 days. Culture negativity in their study was defined as 1 negative rectal swab for CRE, a definition that probably overestimates the “true negatives” compared with our definition, which required 3 consecutive negative rectal swabs.

In summary, treatment with oral nonabsorbable antibiotics, to which CRE is susceptible, appears to be safe and effective for eradication of the CRE carrier state. Reducing the reservoir of CRE carriers in health care facilities may thereby reduce patient-to-patient transmission and the incidence of clinical infection with this difficult-to-treat organism.

References


