Testing probiotic strain *Escherichia coli* Nissle 1917 (Mutaflor) for its ability to reduce carriage of multidrug-resistant *E. coli* by elderly residents in long-term care facilities

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A high carriage rate of multidrug-resistant *Escherichia coli* (MDREC) was observed in elderly residents in long-term care facilities. A double-blinded, placebo-controlled trial was carried out to determine whether the probiotic product *E. coli* strain Nissle 1917 (Mutaflor) would compete with MDREC in the bowel and thereby reduce the prevalence of the multiresistant bacteria in faeces and urine. Sixty-nine patients excreting norfloxacin-resistant *E. coli* were randomized to probiotic or placebo groups and administered capsules twice daily. The daily dose of probiotic was 5 x 10⁹ – 5 x 10¹⁰ bacteria. Faecal and urine samples were cultured at baseline and during and after the treatment period. A reduction in baseline carriage was not influenced by probiotic administration. The probiotic strain was detected in faecal specimens collected during the treatment period of only two out of 12 probiotic group subjects that were tested. Genotyping of norfloxacin-resistant *E. coli* isolates showed that 32 strains were prevalent among the patients. Thus, *E. coli* Nissle 1917 does not have the capacity to compete effectively with MDREC in the bowel of elderly patients.

INTRODUCTION

Multidrug-resistant (MDR) strains of *Escherichia coli* (MDREC) were first isolated from mid-stream urine specimens of patients in the Dunedin region in 2002 (Ministry of Health of New Zealand, unpublished data). These isolates were resistant to norfloxacin (MIC >256 μg ml⁻¹) and commonly also to ciprofloxacin, gentamicin, ampicillin, amoxicillin/clavulanate and cephalothin. At least one case of septicemia due to an MDREC occurred at this time. The MDR strains were mostly associated with elderly residents in long-term care facilities (LTCFs). The problem did not abate, despite guidance by local public health officials concerning infection control and appropriate use of antibiotics in LTCFs. A prevalence study was conducted in 2006 to assess carriage of MDREC by residents in four LTCFs. The results of this study showed that up to 50% of residents of the facilities were excreting MDREC in faeces or urine (I. S. Tiong, K. Munro, G. W. Tannock & M. Schultz, unpublished data).

The probiotic strain *E. coli* Nissle 1917 is commercially available as 'Mutaflor' and has been used in prophylaxis and the treatment of acute and chronic inflammatory conditions of the bowel since the 1920s (Schultz, 2008). Probiotic products are part of the self-care health market and have been reported to be efficacious in patients suffering from a wide spectrum of diseases, but most notably in prophylaxis against atopic diseases of children and treatment of antibiotic-associated diarrhoea (Saavedra, 2007). The mechanisms by which probiotics exert beneficial effects are usually unknown (Schultz & Lindström, 2008). We reasoned that *E. coli* Nissle 1917, believed to have the ability to persist in the human bowel following oral administration in capsules and to be ‘antagonistically strong’, might competitively exclude MDREC from elderly patients (Schultz, 2008). The probiotic strain might occupy the same ecological niches in the bowel as those filled by MDREC (Boudeau et al., 2003). Therefore, our hypothesis was that administration of the probiotic would reduce carriage of MDREC in the bowel and excretion of MDREC in the faeces, and hence reduce colonization of the urinary tract by MDREC.

Abbreviations: ESBL, extended-spectrum β-lactamase; LTCF, long-term care facility; MDR, multidrug resistant; MDREC, MDR *Escherichia coli*; UPGMA, unweighted pair group method with arithmetic mean.

Supplementary data and tables are available with the online version of this paper.
METHODS

A double-blinded, placebo-controlled trial was conducted among residents of LTCFs in Dunedin, New Zealand, who were excreting MDREC in faeces and/or urine. The trial was designed for a sample size of 60, which would give 86% power to detect clearance of the MDREC by 40% of the active treatment group with a maximum of 10% clearance in the placebo group.

Participants were recruited by approaching LTCFs in the Dunedin city area and seeking consent from their residents to provide urine and faeces specimens for screening for MDREC. Demographic and health data, including urinary tract infection, co-morbidities, medications and hospitalization details, were also obtained.

Recruitment stopped when 75 MDREC-positive residents had been identified (to allow for dropouts during the trial).

Capsules containing probiotic or placebo were provided by Ardeypharm. The participants were randomized into probiotic or placebo groups and administered one capsule twice daily for 5 weeks by nurses. The daily dose was 5 x 10^5–5 x 10^6 cfu. The placebo consisted of a similar gelatin capsule filled with starch powder.

The trial was approved by the Lower South Regional Ethics Committee (approval number LRS/06/09/041).

**Isolation of norfloxacin-resistant E. coli from faeces and urine.**

Faecal and urine samples were collected from participants during an initial screen up to 3 months before the probiotic trial commenced (baseline), then 1 week before the 5 week probiotic/placebo period was completed (during) and again 5 weeks after treatment had ceased (follow-up). The specimens were delivered to the laboratory on the day that they were collected. Primary (direct inoculation of an agar plate with faeces or urine) and enrichment cultures (~500 mg faeces per day that they were collected). Primary (direct inoculation of an agar plate with faeces or urine) and enrichment cultures (~500 mg faeces or 1 ml urine per 10 ml medium) were prepared and incubated aerobically at 37 °C for 24 h. EC medium (Difco) containing 64 μg norfloxacin ml⁻¹ was used to select norfloxacin-resistant E. coli. Primary cultures were also prepared on EC agar plates without the addition of norfloxacin. Enrichment broths were subcultured onto EC agar plates containing 64 μg norfloxacin ml⁻¹. Colonies of norfloxacin-resistant E. coli of different morphologies from each specimen that yielded growth were stored at 4 °C on EC agar slants containing 64 μg norfloxacin ml⁻¹.

**Antibiotic susceptibility tests.** One hundred and three norfloxacin-resistant isolates were selected from storage after the trial had been completed and the cloning code had been broken. These isolates were from 20 probiotic patients and represented cultures obtained during the various periods of the study. Therefore, it was possible to compare the antibiotic susceptibilities of E. coli strains carried by the subjects throughout the study. Antibiotic sensitivity and extended-spectrum β-lactamase (ESBL) tests were carried out using the Clinical and Laboratory Standards Institute (CLSI, 2006) using KB sensitivity plates (Fort Richard Laboratories). BBL Sensi-Disc antimicrobial susceptibility test disks (BD Diagnostics) were used for antibiotic sensitivity testing and testing for ESBLs. The following disks were used: ampicillin 10 μg, norfloxacin 10 μg, gentamicin 10 μg, cephalothin 30 μg, amoxicillin/clavulanate 20/10 μg, nitrofurantoin 300 μg, trimethoprim 5 μg, sulfamethoxazole/trimethoprim 1.25/23.75 μg and ciprofloxacin 5 μg. Screening of isolates for ESBLs was carried out using cefotaxime 30 μg and ceftazidime 30 μg disks. All plates were incubated for 16–18 h at 37 °C before interpretation. The antibiotic susceptibilities were interpreted using a BBL Sensi-Disc zone diameter interpretive chart (BD Diagnostics), and the ESBL tests were interpreted using the criteria described by CLSI (2007). The ESBL-positive control strain Klebsiella pneumoniae ATCC 700603 (Fort Richard Laboratories) was tested alongside each group of isolates. Norfloxacin MICs were determined using E-test strips (AB Biodisk).

**Genotyping (PFGE) of norfloxacin-resistant isolates.** The 103 isolates selected from storage for antibiotic susceptibility testing were genotyped using PFGE of digests of genomic DNA. Genotyping was carried out to determine whether a single strain of norfloxacin-resistant bacteria had colonized all of the patients. The USA Centers for Disease Control and Prevention PulseNet procedure was used to prepare agarose plugs and to lyse the cells of each isolate (http://www.cdc.gov/pulsenet/). The plugs were cut into pieces of approximately 4 x 5 mm and the DNA digested in 8 μl H buffer containing 2 μl restriction enzyme Xbal (Roche Diagnostics) for 4 h at 37 °C. The Salmo nella serotype Braenderup H9812 standard was used for comparisons between gels. Electrophoresis was performed in a Bio-Rad CHEF-DR-III instrument in Tris/borate buffer (Bio-Rad Laboratories) for 19 h at 6 V cm⁻¹ and 14 °C with an angle of 120° and switch time of 2.2–63.8 s. The gels were stained in ethidium bromide solution (5 μg ml⁻¹) for 30 min. DNA profiles were recorded using a Gel Logic 200 imaging system. The profiles were compared using GelCompar II version 4.6 (Applied Maths). Profiles were compared using the Dice coefficient, dendrogram-type unweighted pair group method with arithmetic mean (UPGMA) and a position tolerance of 2%. Isolates that had genotypes of 95% or greater similarity were considered to be the same strain (Swaminathan et al., 2001).

**Detection of the probiotic strain in specimens.** Ten colonies were picked randomly from each primary E. coli (without norfloxacin) plate culture prepared from urine or faeces during the treatment and follow-up periods of the trial. The subcultures were stored in 96-well microtitre plates at −20 °C as described by Zimmer et al. (1997). To identify E. coli Nissle 1917 among the stored isolates, bacterial cells were transferred from a storage well by toothpick to PCR tubes. Multiplex PCR utilizing primers Muta 5–10 were used as described by Blum-Oehler et al. (2003). The amplified sequences obtained by this method have been reported to be specific for E. coli Nissle 1917 (Blum-Oehler et al., 2003). Isolates from patients administered probiotic in two LTCFs were examined, comprising 240 faecal isolates obtained from 12 patients during treatment and follow-up periods, and 200 urine isolates obtained from 10 patients during treatment and follow-up periods.

RESULTS

A total of 192 residents of 14 LTCFs agreed to provide urine and faeces specimens for screening, and 191 did so (see supplementary data and Supplementary Table S1, available in JMM Online).

Seventy-five MDREC-positive residents were identified; five died prior to randomization, one died after randomization but before taking any treatment, and 69 were randomized and commenced treatment, 36 with probiotic treatment and 33 with placebo. Table 1 shows the characteristics of the treatment and placebo groups. Participants in the placebo group were more likely to have had at least one course of antibiotics in the previous year, but this difference was not statistically significant. One participant in each arm of the study died during the study, and one from the treatment arm changed their mind and withdrew 2 days after commencing treatment. Sixty-six completed the study and provided post-study specimens (34 treatment, 32 placebo).
Primary plate cultures were used to obtain an indication that moderate to large numbers of MDREC were present, whereas enrichment cultures increased the sensitivity of detection by permitting the amplification of small numbers of MDREC to detectable levels.

After 5 weeks of treatment, there was no significant difference between the probiotic and placebo groups with respect to changes in carriage of norfloxacin-resistant E. coli (Tables 2 and 3). At the end of the treatment period, enrichment cultures of faeces (Table 2) showed that 58% of the placebo group versus 77% of the treatment group remained positive (not significant) (enrichment cultures of urine, 76 vs 80%; not significant; Table 3).

Detection of the probiotic strain in patient faeces

The probiotic strain was detected by PCR in the faeces of two of the 12 patients tested during the treatment period and four of the 12 during the follow-up period. The probiotic strain was detected in the urine (but not in the faeces) of three of the 10 patients tested during the treatment period but was not detected during follow-up. Therefore, E. coli Nissle 1917 had only a limited ability to establish in detectable numbers in the bowel or urinary tract of elderly people harbouring norfloxacin-resistant E. coli.

Antibiotic susceptibility

In order to characterize further the MDREC with which the rest-home residents were infected, 103 isolates of norfloxacin-resistant E. coli were tested for susceptibility to other commonly used antibiotics (see Supplementary Tables S2–S4, available in JMM Online). The majority of the isolates were susceptible to nitrofurantoin (95/103), trimethoprim (72/103) and sulfamethoxazole/trimethoprim (73/103). All of the isolates were resistant to norfloxacin (MIC $\geq 256$ mg ml$^{-1}$) and ciprofloxacin, and the majority were resistant to ampicillin (82/103), gentamicin (57/103) and cephalothin (70/103). Forty-seven of the 103 isolates were resistant to amoxicillin/clavulanate. The combination of multiresistances differed among strains. None of the isolates were ESBL producers. Therefore, the majority of norfloxacin-resistant isolates were MDREC, but combinations of drug resistances varied with strain.

Genotyping (PFGE profiles)

The mode of infection with MDREC is likely to vary from person to person, and therefore LTCFs might harbour distinctive strains, different from those of other LTCFs. To test this hypothesis, 103 norfloxacin-resistant isolates were typed using PFGE. Examples of the PFGE profiles are

Table 1. Participant characteristics (n=69)

<table>
<thead>
<tr>
<th>Characteristics of MDREC-positive specimens</th>
<th>Probiotic (N=36)*</th>
<th>Placebo (N=33)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces only, n (%)</td>
<td>17 (47)</td>
<td>19 (58)</td>
</tr>
<tr>
<td>Urine only, n (%)</td>
<td>4 (11)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Faeces and urine, n (%)</td>
<td>15 (42)</td>
<td>11 (33)</td>
</tr>
<tr>
<td>Age mean (sd)</td>
<td>82 (8.8)</td>
<td>81 (10.2)</td>
</tr>
<tr>
<td>Lived in current institution for &gt;1 year, n (%)</td>
<td>23/33 (70)</td>
<td>25/32 (76)</td>
</tr>
<tr>
<td>At least one diagnosis of urinary tract infection in the past year, n (%)</td>
<td>12/33 (36)</td>
<td>14/31 (45)</td>
</tr>
<tr>
<td>Hospitalization in the past year, n (%)</td>
<td>14/33 (42)</td>
<td>16/31 (52)</td>
</tr>
<tr>
<td>Antibiotic use in the past year, n (%)</td>
<td>24/33 (73)</td>
<td>28/31 (90)</td>
</tr>
</tbody>
</table>

*Where some data are missing, the denominator is shown in the cell (n/N).

Table 2. Detection of norfloxacin-resistant E. coli in faeces by primary culture and enrichment culture

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>Change relative to baseline</th>
<th>Primary culture (n=53*)</th>
<th>Enrichment culture (n=60†)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Placebo group (n=23)</td>
<td>Probiotic group (n=30)</td>
</tr>
<tr>
<td>During</td>
<td>Remained positive</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Became negative</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Data not available</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Remained positive</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Became negative</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Data not available</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*Fifty-three subjects were positive at baseline (beginning of trial).
†Sixty subjects were positive at baseline.
shown in Fig. 1. The profiles were compared using GelCompar II version 4.6 (Applied Maths) using the Dice coefficient, dendrogram-type UPGMA and a position tolerance of 2%. Isolates that had genotypes of 95% or greater similarity were considered to be the same strain (Swaminathan et al., 2001; Tenover et al., 1995). On this basis, 32 strains were detected among the 103 isolates. Comparison of strains isolated from particular patients during the progression of the trial showed that two of 20 patients harboured the same strain throughout the study, three patients retained one strain throughout the study but were also colonized with other norfloxacin-resistant strains that changed over time, and the remaining patients carried a variety of strains that differed during the progression of the study. One strain was detected in residents of five of the LTCFs, but, in general, different strains were detected in different facilities. Therefore, overall, a large number of genotypically distinctive strains were carried by the elderly patients. One strain was detected in residents of five of the LTCFs, but, in general, different strains were detected in different facilities. Therefore, overall, a large number of genotypically distinctive strains were carried by the elderly patients. One strain was detected in five of the LTCFs, suggesting that there might have been transfer of MDREC between facilities. The \( E. coli \) Nissle 1917 PFGE profile (genotype) was dissimilar from that of the norfloxacin-resistant isolates (<80% similarity). Therefore, drug resistance had not been transferred to the probiotic strain, which is an important safety feature of probiotic therapy.

**DISCUSSION**

The emergence of MDREC and other MDR microorganisms is a global problem and is the unavoidable consequence of unselective antibiotic use. In 2003, Public Health South in southern New Zealand became aware of a spreading infection with an MDREC among residents of LTCFs. Whilst the potential of this strain to cause disease was unclear, most patients remained asymptomatic carriers, so significant clinical problems with untreatable infections in the elderly and immunocompromised population had to be anticipated. As colonization with these MDR bacteria is of questionable clinical significance in otherwise healthy individuals, treatment with antimicrobials is difficult to defend, so other strategies need to be developed.

Probiotic bacteria are defined as ‘living microorganisms that when administered in adequate amounts confer benefits to the host’ (Reid et al., 2006). Mechanisms of probiotic therapy remain unclear but might involve bacterial interactions in the bowel and/or immunological phenomena (Schultz & Lindström, 2008). \( E. coli \) strain Nissle 1917 is a well-known probiotic strain that has been used in the maintenance of remission in patients with ulcerative colitis and irritable bowel syndrome (Schultz, 2008). The aim of this study was to evaluate whether probiotic \( E. coli \) Nissle 1917 was safe and could competitively exclude MDREC from the intestine and urine of LTCF residents.

**Table 3. Detection of norfloxacin-resistant \( E. coli \) in urine by primary culture and enrichment culture**

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>Change relative to baseline</th>
<th>Primary culture ( n=24^* )</th>
<th>Enrichment culture ( n=33^{†} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Placebo group ( n=10 )</td>
<td>Probiotic group ( n=14 )</td>
</tr>
<tr>
<td>During</td>
<td>Remained positive</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Became negative</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Data not available</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Remained positive</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Became negative</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Data not available</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Twenty-four subjects were positive at baseline.
†Thirty-three subjects were positive at baseline.

**Fig. 1.** Examples of PFGE profiles of \( E. coli \) strains. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, \( S. typhimurium \) serotype Braenderup H9812 standard; lanes 2–5 and 7–10, norfloxacin-resistant isolates; lane 13, \( E. coli \) Nissle 1917.
The initial screen of residents of LTCFs confirmed previous observations concerning the high prevalence of norfloxacin-resistant *E. coli* among residents. Sufficient subjects were recruited from this pool of patients to perform a probiotic trial of appropriate statistical power. However, daily administration of the probiotic for 5 weeks did not influence the carriage of norfloxacin-resistant *E. coli* in faeces or urine relative to the placebo group. Side effects were not reported.

*E. coli* Nissle 1917 is described as a probiotic that is ‘a successful colonizer of the human gut’ and as an ‘antagonistically strong *E. coli* strain’ (Prilassnig et al., 2007; Schultz, 2008). It should be noted, however, that with regard to adult humans, published bacteriological data relate only to administration of the probiotic to humans who had previously been dosed with antibiotic prior to probiotic administration. Under these circumstances, the probiotic strain was able to occupy ecological niches vacated by autochthonous *E. coli* (Blum-Oehler et al., 2003; Prilassnig et al., 2007). In a more realistic setting, our study shows that *E. coli* Nissle 1917 had limited ability to persist in the bowel of elderly humans who were already harbouring antibiotic-resistant *E. coli*.

The failure of the probiotic to compete effectively with the norfloxacin-resistant *E. coli* might have been due to the multiplicity of strains that were prevalent among residents of the LTCFs. There was evidence of a dynamic situation in terms of colonization of the bowel as the study progressed: strains were gained or lost as time passed. Whilst a few strains persisted in some subjects, temporal changes in the composition of the antibiotic-resistant population occurred. MDREC has been detected in humans in the Otago region since 2002. Therefore, a considerable period of time has elapsed for well-adapted strains, perhaps aided by horizontal gene transfer, to be enriched in LTCF residents. Clearly, they have superior competitive power to *E. coli* Nissle 1917. Antibiotic treatment during the trial might have compromised the effectiveness of the probiotic strain, which does not possess acquired antibiotic resistances. Probiotic treatment was for a prolonged period, however, and sporadic, short-term therapy should not have affected the efficacy of the probiotic. At the very least, it was reassuring to observe that the probiotic strain did not acquire resistance to norfloxacin during the trial, making probiotic therapy safe for the general public.

Prevention of the selection and spread of antibiotic-resistant strains in the community must continue to rely on sound training of staff in care facilities with regard to infection control measures and the judicious use of antibiotics.

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**REFERENCES**


