Analysis of Laura’s listeria experiment

“Which treatment was more effective in keeping listeria down?”

We consider a linear model for log-listeria count \( (\mu_{ij}(t)) \) under treatment \( i \) at temperature \( j \) and time \( t \) (in days):

\[
\log(\mu_{ij}(t)) = (\alpha \beta)_{ij} + [(\tau \alpha)_i + (\tau \beta)_j + (\tau \alpha \beta)_{ij}].t.
\]

For four of the treatments, 0 listeria counts were observed at each day. Since these treatments clearly eliminate listeria altogether, they need not be included in the model. The remaining treatments are “50,1000,listeria” and “listnisi.” There is no evidence of any effect on the death rates among these remaining treatments, though they do seem to have different counts at day 0 (manifested by significantly different intercepts in the model.) So that the model is not overparameterized, SAS imposes the constraints

\[
(\tau \beta)_2 = 0 \quad \text{and} \quad (\tau \alpha \beta)_{12} = (\tau \alpha \beta)_{22} = (\tau \alpha \beta)_{32} = (\tau \alpha \beta)_{41} = (\tau \alpha \beta)_{42} = 0.
\]

The 8 prediction equations for log listeria count are then

\[
\begin{align*}
\mu_{11}(t) &= \hat{\alpha \beta}_{11} + [(\hat{\tau \alpha})_1 + (\hat{\tau \beta})_1 + (\hat{\tau \alpha \beta})_{11}]t \\
\mu_{12}(t) &= \hat{\alpha \beta}_{12} + [(\hat{\tau \alpha})_1]t \\
\mu_{21}(t) &= \hat{\alpha \beta}_{21} + [(\hat{\tau \alpha})_2 + (\hat{\tau \beta})_1 + (\hat{\tau \alpha \beta})_{21}]t \\
\mu_{22}(t) &= \hat{\alpha \beta}_{22} + [(\hat{\tau \alpha})_2]t \\
\mu_{31}(t) &= \hat{\alpha \beta}_{31} + [(\hat{\tau \alpha})_3 + (\hat{\tau \beta})_1 + (\hat{\tau \alpha \beta})_{31}]t \\
\mu_{32}(t) &= \hat{\alpha \beta}_{32} + [(\hat{\tau \alpha})_3]t \\
\mu_{41}(t) &= \hat{\alpha \beta}_{41} + [(\hat{\tau \alpha})_4 + (\hat{\tau \beta})_1]t \\
\mu_{42}(t) &= \hat{\alpha \beta}_{42} + [(\hat{\tau \alpha})_4]t
\end{align*}
\]

(This is analogous to the model fit by Dan for the previous dataset. The goodness-of-fit statistics don’t indicate any problems.)

Substitution of the parameter estimates (see SAS output, next page) gives

\[
\begin{align*}
\mu_{11}(t) &= 5.58 - 0.03 \times t \\
\mu_{12}(t) &= 24.61 - 0.94 \times t \\
\mu_{21}(t) &= 4.87 - 0.02 \times t \\
\mu_{22}(t) &= 22.31 - 0.85 \times t \\
\mu_{31}(t) &= 5.68 - 0.01 \times t \\
\mu_{32}(t) &= 27.09 - 1.08 \times t \\
\mu_{41}(t) &= 6.08 - 0.06 \times t \\
\mu_{42}(t) &= 23.89 - 0.94 \times t
\end{align*}
\]
### Solution for Fixed Effects

| Effect       | trt | Temp | Estimate | Error | DF | t Value | Pr > |t| |
|--------------|-----|------|----------|-------|----|---------|-------|---|
| trt*Temp     | 50  | 5    | 5.5835   | 0.2183| 8  | 25.58   | <.0001|
| trt*Temp     | 50  | 18   | 24.6137  | 2.3576| 8  | 10.44   | <.0001|
| trt*Temp     | 1000| 5    | 4.8717   | 0.2183| 8  | 22.32   | <.0001|
| trt*Temp     | 1000| 18   | 22.3059  | 2.3576| 8  | 9.46    | <.0001|
| trt*Temp     | listeria | 5 | 5.6836   | 0.2183| 8  | 26.04   | <.0001|
| trt*Temp     | listeria | 18 | 28.0941  | 2.3576| 8  | 11.92   | <.0001|
| trt*Temp     | listnisi | 5 | 6.0849   | 0.2183| 8  | 27.87   | <.0001|
| trt*Temp     | listnisi | 18 | 23.8878  | 2.3576| 8  | 10.13   | <.0001|
| Time*trt     | 50  |      | -0.9422  | 0.09974| 80 | -9.45   | <.0001|
| Time*trt     | 1000|      | -0.8520  | 0.09974| 80 | -8.54   | <.0001|
| Time*trt     | listeria |      | -1.0780  | 0.09974| 80 | -10.81  | <.0001|
| Time*trt     | listnisi |      | -0.9377  | 0.09974| 80 | -9.40   | <.0001|
| Time*Temp    | 50  |      | 0.8774   | 0.09963| 80 | 8.81    | <.0001|
| Time*Temp    | 18  |      |          |       |    |         |       |
| Time*trt*Temp| 50  | 5    | 0.03254  | 0.1409| 80 | 0.23    | 0.8179|
| Time*trt*Temp| 50  | 18   | 0        |       |    |         |       |
| Time*trt*Temp| 1000| 5    | -0.05463 | 0.1409| 80 | -0.39   | 0.6992|
| Time*trt*Temp| 1000| 18   | 0        |       |    |         |       |
| Time*trt*Temp| listeria | 5 | 0.1882   | 0.1409| 80 | 1.34    | 0.1855|
| Time*trt*Temp| listnisi | 18 | 0        |       |    |         |       |
| Time*trt*Temp| listnisi | 18 | 0        |       |    |         |       |

### Type 3 Tests of Fixed Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num</th>
<th>Den</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<td>80</td>
<td>1.09</td>
<td>0.3669</td>
</tr>
</tbody>
</table>

Inspection of the tests for fixed effects reveals that there is evidence of different initial conditions for the four treatments (the intercepts differ significantly.) There is a significant ($p < 0.0001$) shift in log listeria count when the temperature is increased from 5° to 18°, but there is no evidence of any treatment effects in the time period before ($p = 0.55$) or after ($p = 0.37$) the temperature shift.

(See SAS code after graphs.)
trt=1000

Pred

0 1 2 3 4 5

Time

trt=50

Pred

0 1 2 3 4 5 6

Time

trt=listeria

Pred

0 1 2 3 4 5 6

Time

trt=listnisi

Pred

0 1 2 3 4 5 6 7

Time

3
data one;
  infile "listeria2.txt" dlm='09'x firstobs=3;
  do j=1 to 3;
    input trt $ Rep Time Temp pH Species $ logcfu @;
    count=round(10**logcfu,1);
    if (count=1) then ypos=0; else ypos=1;
    output;
  end;
run;

/* The tables generated by PROC FREQ below provide an indication of where all the 0 counts were. The 0's make analyses invalid if included.*/

data listeria;
  set one;
  if species="listeria";
run;

/*proc sort data=one; by trt;run;*/
/*proc sort data=listeria; by trt;run;*/

proc freq;
  title "ypos=1 means positive counts, ypos=0 means 0 counts";
  by trt;
  *tables ypos*species*time;
  tables ypos*time;
run;
endsas;*/
data listeria;
    set one;
    if species="listeria";
    if (trt="c" or trt="lac" or trt="lacnisi" or trt="nisin") then delete;
        day=time;
run;

proc mixed data=listeria order=data;
    title "normal linear model for logcfu w/ random jar effects";
    *by species;
    class day trt temp rep;
    /* no ph */
    /*model count=trt*temp trt*time temp*time ph/noint dist=gamma */
    model logcfu=trt*temp trt*time temp*time trt*temp*time pH/noint solution outp=two;
    repeated day/type=ar(1) subject=rep(trt);
run;

/*proc corr data=listeria;
   var ph logcfu;
run;*/

proc print data=two;run;
proc sort; by trt;run;
proc print;run;
goptions device=pslepsf;
symbol1 i=join value="dot" c=black;
symbol2 i=none value="circle" c=black;

proc gplot;
    by trt;
    plot pred*time logcfu*time/overlay;
    *plot lpred*time;
    *plot lcfu*time;
run;

symbol1 i=join value="dot" c=black;
symbol2 i=join value="circle" c=black;
symbol3 i=join value="plus" c=black;
symbol4 i=join value="diamong" c=black;

proc gplot;
    plot pred*time=trt;
run;