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Evaluation of two media and different plating techniques for enumeration of thermotolerant coliforms in river water

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ABSTRACT. Testing for evidence of faecal contamination in river water has been traditionally accomplished by enumeration of thermotolerant coliform bacteria. In this work, deoxycholate lactose and m-Endo Agar LES media were evaluated using different techniques. The colony count methods were pour plate technique on Deoxycholate Agar (DCL pour plate), spread plating on m-Endo Agar LES (ELS spread), and membrane filtration on m-Endo Agar LES (ELF filtration). Typical thermotolerant coliform colonies were analysed by conventional biochemical tests. The three matched pairs showed statistically significant differences ($P < 0.05$) by the Wilcoxon signed rank test. One hundred and twenty three isolates obtained on m-Endo Agar LES (65%) were confirmed as *E. coli.* Likewise, one hundred and twenty isolates obtained on Deoxycholate Agar (71%) were confirmed as *E. coli*. The results of this study showed that the matched DCL-ELF presented the smaller statistically significant difference ($P = 0.042$) so, DCL could be used as alternative to ELF.

Key words: Thermotolerant coliforms in water, culture media, enumeration.

RESUMEN. La determinación de contaminación fecal en aguas se realiza comúnmente por enumeración de coliformes termotolerantes. En el presente trabajo se ensayaron el agar Desoxicolato-lactosa y el m-Endo LES mediante diferentes técnicas de inoculación. Los métodos de recuento fueron: siembra por embebido en agar Desoxicolato, por dispersión sobre la superficie de agar m-Endo LES y por filtración con membrana sobre agar m-Endo LES. Las colonias típicas de coliformes termotolerantes fueron aisladas e identificadas utilizando ensayos bioquímicos convencionales. Los tres pares de datos mostraron diferencias estadísticas significativas ($P < 0.05$) con la prueba de rangos de Wilcoxon. Ciento veintitrés aislados, obtenidos a partir de agar m-Endo LES (65%) fueron confirmados como *E. coli.* Asimismo, ciento veinte aislados de agar Desoxicolato (71%) se identificaron como *E. coli*. Los resultados de este estudio mostraron que el par DCL-ELF tuvo la menor diferencia estadística ($P = 0.042$) por lo que DCL podría ser empleado como una alternativa al método ELF.

Palabras clave: Coliformes termotolerantes en agua, medios de cultivo, recuento.

INTRODUCTION

Testing for evidence of faecal contamination in water has been traditionally accomplished by the enumeration of faecal coliform bacteria. Elevated temperature tests for the separation of coliform into those of faecal and nonfaecal origins have been used in many parts of the world with various modifications.[12] The term "thermotolerant coliforms" is considered to be more appropriated than "faecal coliforms" to describe coliform microorganisms that grown at 44.5 °C. This group includes species that probably are of nonfaecal origin.[1,4] The thermotolerant coliform test is applicable to investigations of stream pollution, raw water sources, sea water and, general water quality monitoring. Membrane filtration procedures for examining drinking water have advantages of increased sensibility and accuracy. When examining samples from sources such as sewage, rivers or lakes, a high sensitive technique, membrane filtration, may be inappropriate and other procedures can be performed, like spread or pour plates. Furthermore, the thermotolerant coliform enumeration varies according to the procedure used and the environmental source. Deoxycholate lactose medium is used in a pour plate technique or as a surface inoculated medium.[5] m-Endo Agar LES was originally described as a confirmatory medium for standard total coliform counts obtained by multiple table techniques, [2] subsequently it was used as a membrane filtration procedure.[3] More recently selective culture media which contain different chromogens and fluorogens have been developed to enumeration total coliforms and *Escherichia coli.*[11,13,15] Dufour *et al.*[8] developed and evaluated a membrane filter procedure for enumerating *E. coli* in freshwater and estuarine coastal water with resuscitation of weakened organisms. They demonstrated that the m-TEC method was suitable for the enumeration *E. coli*. Ciebin *et al.*[7] compared two media to detect coliforms in nontreated waters by membrane filtration, m-FC and m-TEC supplemented with a chromogenic substrate. They demonstrated that either modified basal medium could be used. In Argentina, the most probable number and membrane filtration techniques using deoxycholate agar and m-Endo agar LES are frequently applied for pollution control.[9,10]

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The aim of the present work was to evaluate the selective media deoxycholate lactose and m-Endo Agar LES by using different techniques for enumerating thermotolerant coliform bacteria in river water and compared the effectiveness of two media for detecting *E. coli*.

MATERIAL AND METHODS

Media. The composition laboratory prepared media are shown below (per litre). Deoxycholate Agar: peptone 10 g, lactose 10 g, sodium deoxycholate 0.5 g, sodium chloride 5 g,, sodium citrate 1 g, neutral red 0.03 g, agar 15 g. m-Endo Agar LES: tryptose 10 g, meat peptone 5 g, casein peptone 5 g, yeast extract 1.5 g, sodium chloride 5 g, dipotassium hydrogen phosphate 4.38 g, potassium dihydrogen phosphate 1.38 g, lactose 12.5 g, sodium deoxycholate 0.1 g, sodium lauryl sulphate 0.05 g, sodium sulphite 2.1 g, basic fuchsin 1.05 g, agar 15 g.

Water samples. Forty five water samples were collected from different points along Suquía river, Córdoba, Argentina, in order to obtain different levels and strains of thermotolerant coliform bacteria. The sampling points were: (a) immediately upstream of Córdoba, (b) 2 locations within the city and (c) downstream of Córdoba. The water samples were collected at a point in the centre of the river, held at 4°C and tested within 6 h of collection.

Enumeration of thermotolerant coliforms. The volume of sample examined varied according to the source in order to obtain countable numbers of colonies. The colony count methods were pour plate technique on Deoxycholate Agar (DLC), spread plating on m-Endo Agar LES (ELS), and membrane filtration on m-Endo Agar LES (ELF). The membrane filters used were Millipore filter, type HAWG047S3. Duplicate sets of plates were prepared for each of the techniques used. The plates were incubated for 24 h at 44.5 ± 0.2°C. The typical colonies developing on the surface of m-Endo Agar LES are larger than those on the filters surface.

Identification of *E. coli*. Typical thermotolerant coliform colonies were selected from plates of Deoxycholate Agar and m-Endo Agar LES from 45 water samples. The purity of isolates was checked after 1 day of incubation on nutrient agar slants. The strains were examined by Gram stain, cytochrome oxidase production and oxidation/fermentation glucose test. All the strains that were identified as members of the family Enterobacteriaceae were tested for arabinose fermentation, use of citrate, hydrogen sulfide production, indole production, potassium cyanide tolerance, lysine decarboxylase production, mucate oxydation, tryptophane deaminase production, and acetoin production.

Statistical analysis. Water samples were examined by DCL, ELF and ELS. Colony counts were converted to log counts/ml and three matched pairs statistically analysed. Because the sample pairs may not be normally distributed, a non parametric Wilcoxon signed rank test was used to detect statistically significant differences. The signed rank test was used to test the null hypothesis that the median differences between each matched pairs from a series of water samples were zero. The Spearman rank coefficient of correlation was also calculated.

RESULTS

The thermotolerant coliform counts, the comparison between the three methods, and the Spearman coefficient are shown in Fig. 1, 2 and 3. Results from the statistical analysis are shown in Table 1.

To compare the effectiveness of Deoxycholate Agar and m-Endo Agar LES for detecting *E. coli*, 188 typical thermotolerant coliform colonies from m-Endo agar and 168 typical thermotolerant coliform colonies from Deoxycholate Agar were purified and the strains were identified. One hundred and twenty three isolates (65 %) were identified as *E. coli* on m-Endo Agar LES and one hundred and twenty isolates (71 %) were identified as *E. coli* on Deoxycholate Agar.

DISCUSSION

When comparing DCL vs. ELF, the majority of the points of DCL fall bellow the line of equivalence (Fig. 1), indicating consistently greater recovery by DCL procedure.

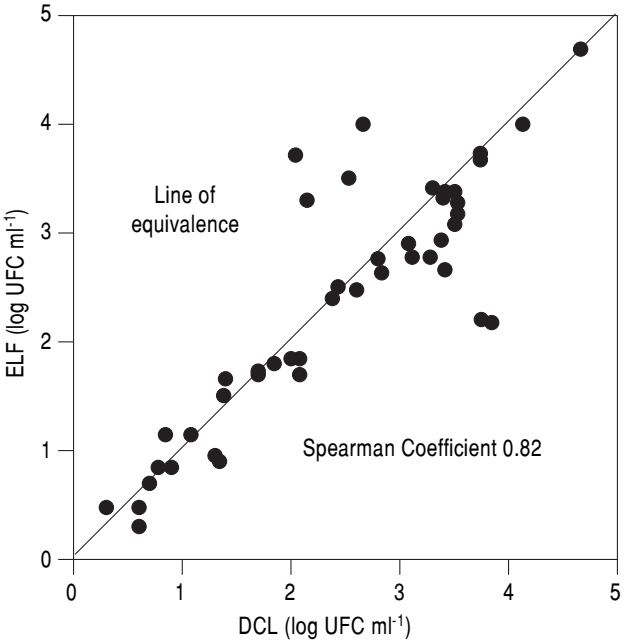

Figure 1. *Comparison of deoxycholate pour plate (DCL) with Endo LES filtration (ELF).*

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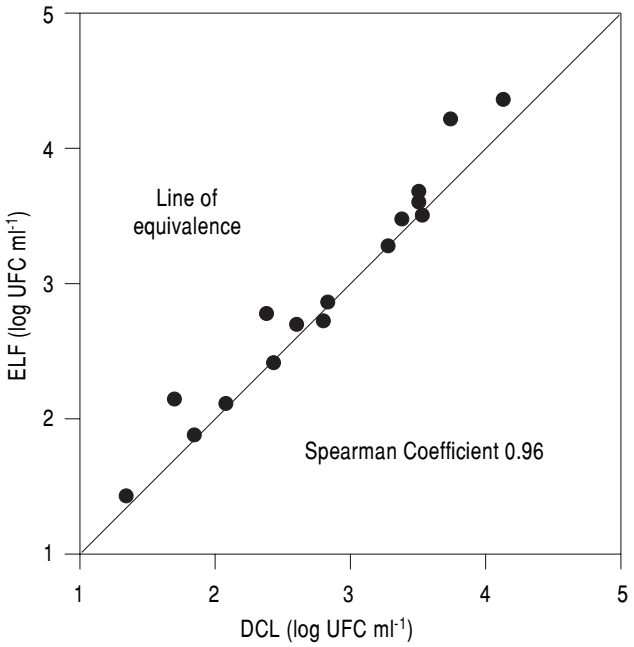

Figure 2. *Comparison of deoxycholate pour plate (DCL) with Endo LES spread (ELS).*

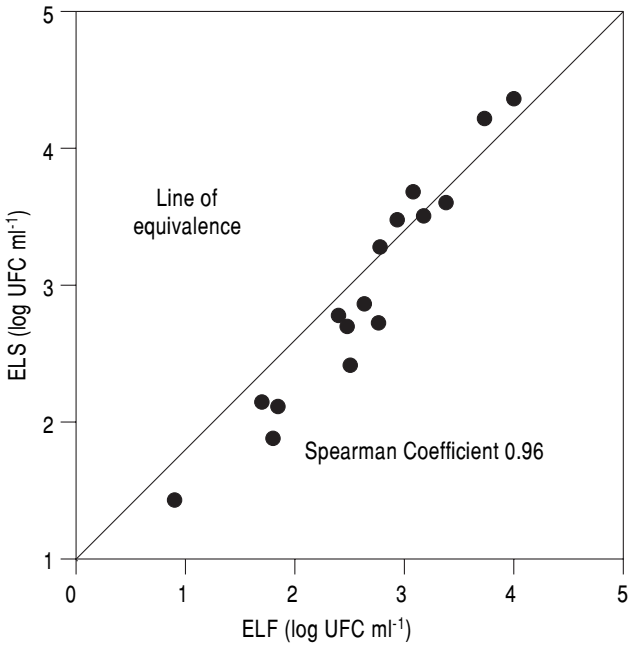

Figure 3. *Comparison of Endo LES filtration (ELF) with Endo LES spread (ELS).*

Table 1. Comparison of thermotolerant coliform counts by three methods.

Method	*No. samples*	*Median* [a]	*Percentiles* *25%*	*75%*
DCL	45	2.531	1.393	3.414
ELF	45	2.505	1.621	3.306
ELS	16	2.821	2.280	3.553

[a] Log UFC/ml.
Matched pairs comparisons (Wilcoxon Signed Rank Test): DCL-ELF: W = -337.0, P = 0.042; DCL-ELS: W = 102.0, P = 0.002; ELF-ELS: W = 128.0, P < 0.001.

The signed rank test demonstrated that there is a statistically significant difference between the methods (P=0.042). For ELS vs. DCL, ELS gives high counts, the sign rank test demonstrates that there is a significant statistical difference between the methods (P=0.002) (Fig. 2, Table 1). The comparison of ELF and ELS showed a superior level of count by the ELF method when the thermotolerant coliform count was less of 3 log CFU/ml. For counts in excess of 3 log CFU/ml, the ELS procedure show a superior count (Fig. 3). The Wilcoxon sign rank test indicates a statistically significant difference between the two methods too (P<0.001). Spearman rank order correlation indicates that the three matched pairs showed a strong positive linear correlation (Fig. 1, 2 and 3). Furthermore, the matched pair DCL-ELF showed the smaller statistically significant difference: P = 0.042 (Table 1); also DCL could be an alternative to ELF standard method. Spearman rank order correlation showed a low value for DCL-ELF probably due to the different number of pairs samples analysed. The thermotolerant coliform counts, and other indicators are significantly influenced by the climatic conditions, the pluvial rainfall and the spatial variability; the technique to choice would depend of the level of water contamination.[9,10]

Emiliani et. al.[10] found that the percentage of *E. coli* among the thermotolerant coliform in fluvial recreational water was very variable, but the average was low (26 %) compared with to the levels in temperate regions of other countries (> 90 %). In this work, the percentage of strains identified as *E. coli* from two media (65 % and 71 %) were similar to the results founded by Ramteke *et al* in different sources of water in India.[14] This values are low compared with the reported in environmental sources by other authors that used chromogenic substrates (95.7%, 98.6% and 97.3%, respectively).[6,7]

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