

Discriminating *E. coli* Isolated from Various Human and Nonhuman Sources Based on Analysis of Random Amplified Polymorphic DNA (RAPD) Patterns

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INTRODUCTION

- **High *E. coli* counts in water**
 - indicate fecal pollution
- **Bacterial Source Tracking (BST)**
 - a key step to control fecal pollution in water
- **Random Amplified Polymorphic DNA (RAPD)**
 - a DNA fingerprinting technique to characterize bacteria
 - a useful tool for BST

OBJECTIVE

- To determine the efficacy of using RAPD to differentiate *E. coli* from various human (HM) and nonhuman (NHM) sources.

MATERIALS & METHODS

E. coli Collection (544 Isolates)

Human --	165 Feces	94 Sewage
	30 Urine	30 Blood
Nonhuman --	53 Horse	57 Cow
	10 Pig	20 Chicken
	55 Goose	30 Seagull

* *Except sewage, single isolate/ sample was used.*

RAPD

- DNA extraction
- DNA amplification w/ RCR
(using 3 separate primers)
- Gel electrophoresis
- RAPD pattern documentation
- RAPD pattern analysis –
BioNumerics™

Gel Image of RAPD

primer 1283 primer 1247 primer 2

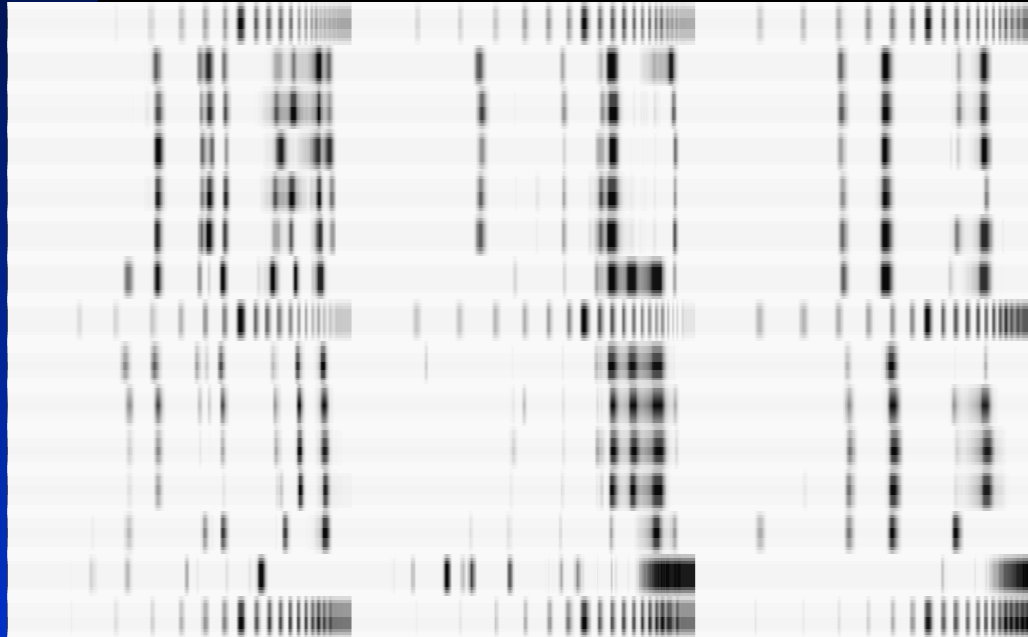


Fig. 1. MANOVA analysis of RAPD patterns of *E. coli* isolated from HM and NHM sources.

HS: ● - Feces, ● - Urine, ● - Blood, ● - Sewage

NHS:

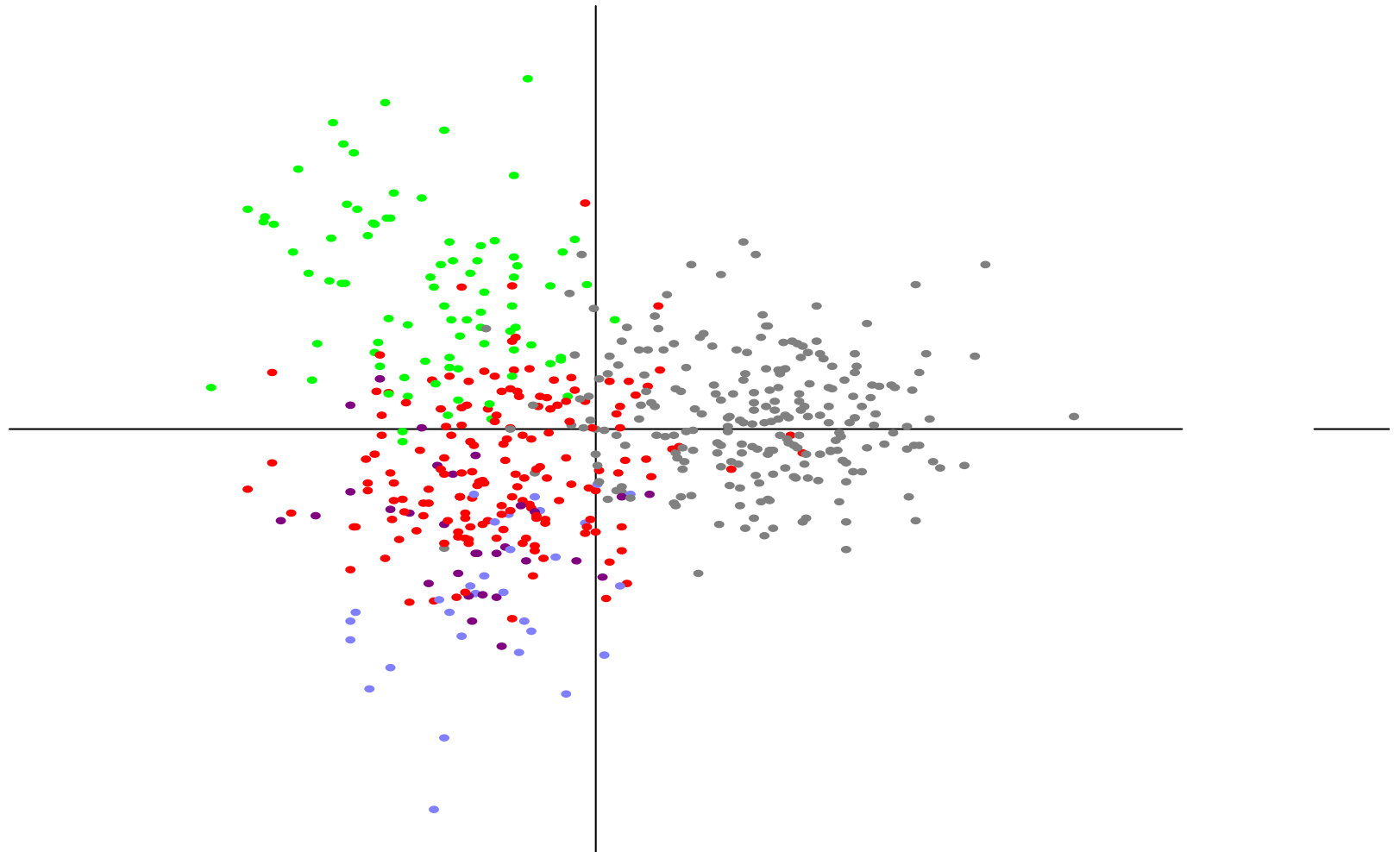


Fig. 2. MANOVA analysis of RAPD patterns of *E. coli* isolated from human sources.

● - Feces, ● - Urine, ● - Blood, ● - Sewage

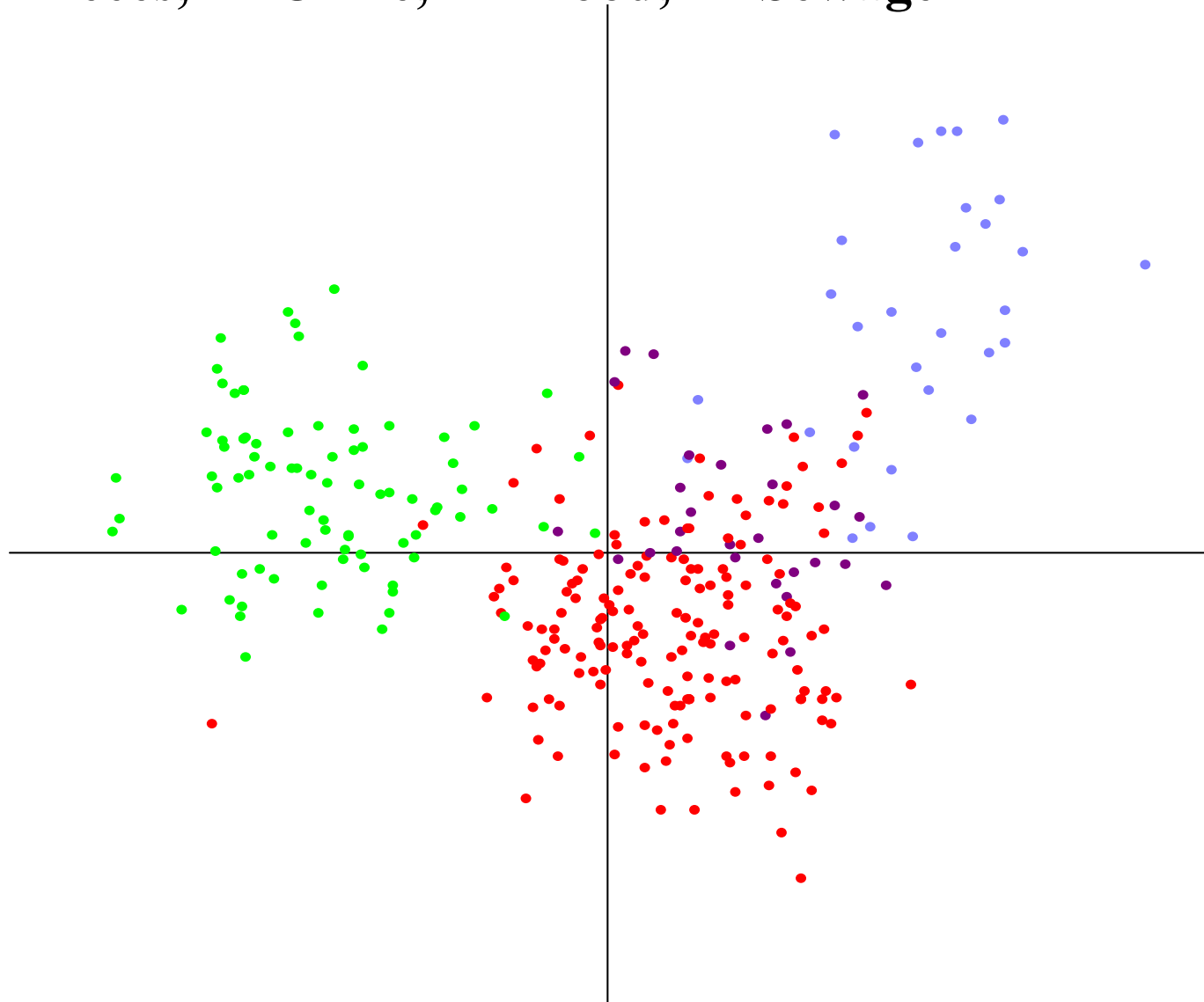


Fig. 3. MANOVA analysis of RAPD patterns of HM and NHM *E. coli* isolated from ● HM sources, ● birds, and ● farm animals (● - 10 Extra Sewage)

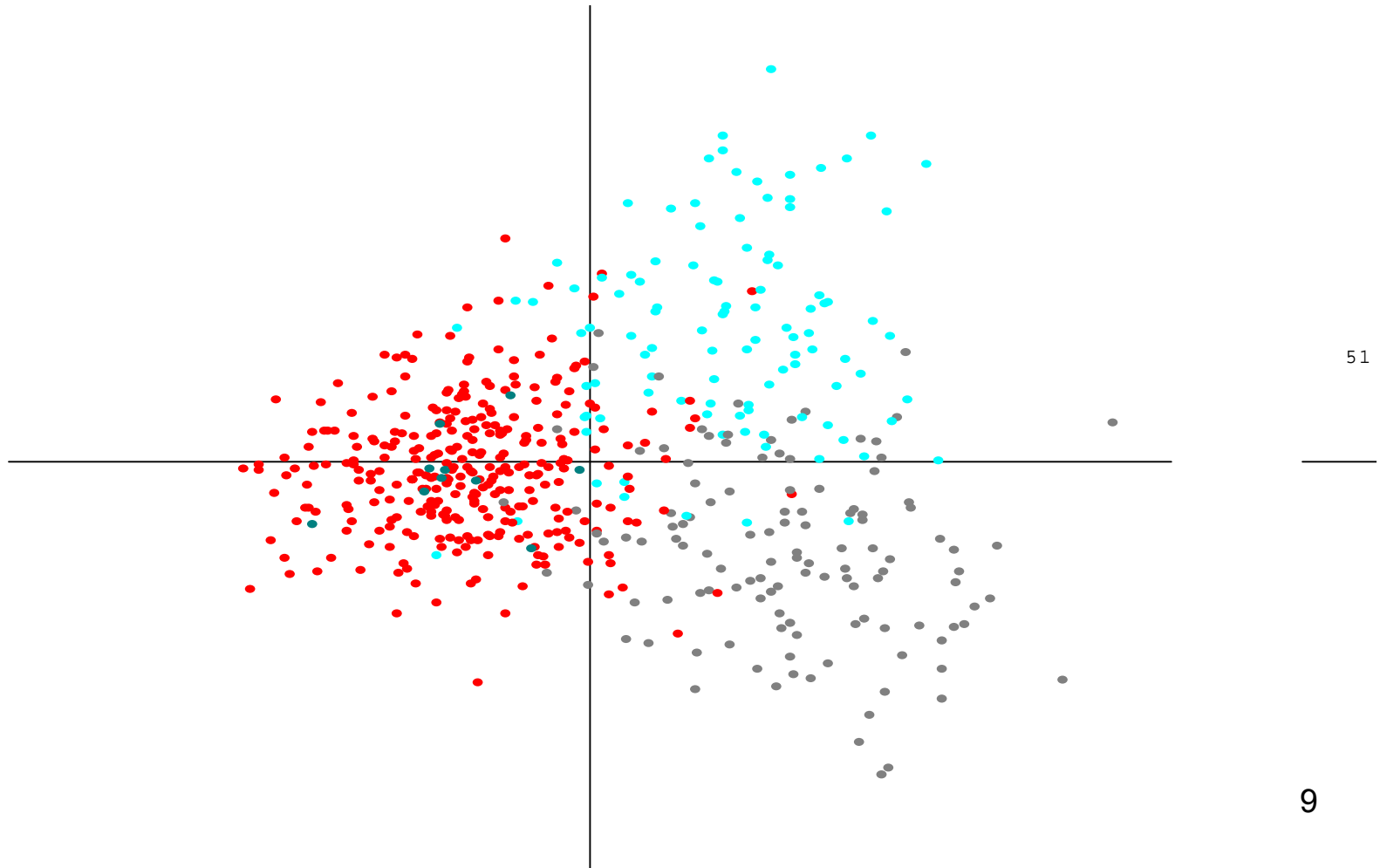












Table 1. Identification of 10 sewage isolates outside the database against library units based on maximum similarity

Isolate	Best Matching Unit	% Similarity	Quality
SW057	Human-Fecal/Urine-Sewage279	82.7%	 ¹
SW058	Human-Fecal/Urine-Sewage279	73.7%	
SW059	Human-Fecal/Urine-Sewage279	86.6%	
SW060	Human-Fecal/Urine-Sewage279	84.3%	
SW061	Human-Fecal/Urine-Sewage279	77.6%	
SW062	Bird-Sg/Gs/Ck105	77.1%	
SW063	Human-Fecal/Urine-Sewage279	91.7%	
SW064	Human-Fecal/Urine-Sewage279	80.8%	
SW065	Human-Fecal/Urine-Sewage279	59.8%	
SW066	Mammal-Pg/Cw/Hs120	77.4%	

¹ Quality factors appear as color dots. They range from red (improbable identification), over orange, yellow (doubtful identification) to green (faithful identification).

- **Table 2. % of *E. coli* isolates correctly and incorrectly assigned to HM and NHM sources**

-
- | Source | # of isolates | % correctly * Identified | % incorrectly Identified |
|---------------|----------------------|---------------------------------|---------------------------------|
| HM | 279 | 79.93 | 20.07 |
| NHM | 225 | 77.33 | 22.67 |

- ** % values were determined by Jackknife Analysis*

RESULTS

- The Jackknife analysis indicated that 80% and 77% of HM and NHM isolates respectively can be correctly identified using RAPD analysis.
- The Discriminant analysis (MANOVA) showed that HM and NHM isolates can be separated from each other on the 2-D plot.

(Cont.)

- *E. coli* isolated from different HM sources (sewage, feces, blood and urine) can be further separated into 3 clusters on the MANOVA plot.
- Ten sewage isolates not in data base were grouped in HM cluster on MONOVA plot.
- Seven of the 10 sewage isolates were identified as human isolates based on maximum similarity.

CONCLUSIONS

- Both Jackknife and MANOVA discriminant analyses showed that RAPD is a useful tool for discriminating *E. coli* from HM and NHM sources.
- *E. coli* isolated from sewage are different from those isolated from human feces. To track the source of human contamination in natural water, it is important to include RAPD patterns of sewage isolates in the library.

ACKNOWLEDGEMENT

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