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Detection and Differentiation of *Escherichia coli* Populations from Human, Animal and Avian Feces, and Different Water Sources

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Abstract

A total of 138 *E. coli* isolates obtained from fecal samples of various mammals and birds and water samples of different origin were compared. The comparison included multiple antibiotic resistance to a total of 21 antibiotics, the protein profile (PAGE) of the isolates, the DNA fragments of each isolate after treatment with the restriction enzymes, *Hind*III, *Eco*RI, *Eco*RV, *Bam*HI and *Bgl*II and the presence of plasmid(s). Our results indicated that human, horse and Nile water isolates were resistant to 62% of the antibiotics used, while cat isolates were resistant to 47%, mouse isolates 72%, avian to 34%, Ismailia canal 67%, household water reservoir 81% and swimming pool isolates were resistant to 57%. Protein profile (PAGE) analysis is the second method used; the protein with 20.29 kd molecular weight is common in avian, household water reservoir and swimming pool isolates. The third method includes DNA fragments using different restriction enzymes *Hind*III, *Eco*RI, *Eco*RV, *Bam*HI and *Bgl*II.

Keywords: *E. coli*, water, fecal contamination, fingerprinting

Introduction

Fecal pollution of water resources is a problem of increasing worldwide concern [1,2]. Human population growth, inadequate sewage systems, and management of animal waste are some of the issues associated with maintenance of supplies of clean water [3]. Underlying this concern, there are numerous reports of waterborne outbreaks of disease involving fecal organisms such as *Escherichia coli* (which is an indicator of fecal contamination), *Campylobacter jejuni, Salmonella, Vibrio cholerae* and *Shigellae* [4-7]. Identification of the source of the bacterial contamination is an essential first step in seeking to control fecal contamination of water. In particular, it is important to determine whether the source of fecal contamination is of human, livestock, or wildlife origin. Microorganisms of human origin are regarded as having greater potential to cause disease in humans [8]. Farm animals may also harbor human pathogens, including the potentially fatal organism *E. coli* 0157: H7. Poultry are a primary reservoir of *Salmonella* spp. as are swine, which may also carry *Shigella* spp. [9].

A number of analytical methods for differentiating between human and nonhuman sources of fecal pollution have been evaluated, among them the multiple antibiotic resistance tests (MAR). The MAR patterns reflect the selective pressures imposed on the gastrointestinal flora of humans and animals during antibiotic use [10]. The MAR test has been reported to be capable of identifying the source of fecal contamination in water and distinguishing between *E. coli* strains from specific point sources [11,12]. Ribotyping has been compared to multiple antibiotic resistance profiles, and both approaches were re-

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portedly complementary in discriminating between human and nohuman sources of fecal pollution [13]. Ribotyping has since become a popular approach to the problem of differentiating between fecal *E. coli* pollution from humans and that of animals and birds [14]. Ribotyping includes DNA fingerprinting techniques, pulsed field gel electrophoresis [15], PCR of repetitive intergenic sequences [16] and 16s ribosomal DNA length heterogeneity, PCR with terminal restriction fragments length polymorphism [17] have been described as promising for discriminating between fecal origin bacteria from humans and animals.

The objective of this study was to differentiate between *E. coli* isolates of either human origin, animal origin (horse, cat and mouse) or avian origin (pigeon). This study was extended to *E. coli* isolates of different water sources (Nile water, Ismailia canal water, household water reservoir and swimming pool water) to determine which of these isolates was more prevalent in the water samples examined.

Materials and Methods

Bacterial Strains

A total of 138 isolates of *E. coli* were isolated from fecal samples as well as different water sources (three replicate of each). A sum of 15, 13, 17 and 14 isolates from human, horse, cat and mouse feces, respectively, and 23 isolates from avian feces (pigeon). 13 isolates were cultured from Ismailia canal water, 16 isolates from Nile water, 15 isolates from household water reservoir and 12 isolates from swimming pool water.

All media used were prepared according to [18], while the procedures for the detection of *E. coli* were described in the Standard Methods for Examination of Water and WasteWater [19]. Samples of feces (or water samples) were inoculated into peptone water for 24 hours then transferred into lauryl sulfate tryptose broth tubes. The tubes were incubated at 37°C for 24-48 h. A loopful of lauryl sulfate tubes that gave positive presumptive test for *E. coli* (turbidity and gas) were transferred into E. C broth media, incubated at 44.5°C ± 0.5°C for 24 h.

A loopful from positive tubes was streaked onto EMB agar and MacConkey agar to confirm typical *E. coli* in addition to different biochemical tests.

Multiple Antibiotic Resistance Test (MAR): (Disc Diffusion Technique).

The following antibiotics and concentrations (µg/ml) were used in this study: Tobramycin, 10; Gentamycin, 10; Penicillin, 10 Units; Tetramycin, 30; Cefoperazone, 30; Erythromycin, 15; Chloramphenicol, 30; Sulfa (Trimethoprim, 25; Doxycyclin, 30; Rifampicin, 30' Streptomycin, 10; Ofloxacin, 10; Cephradine, 30; Ceftriaxane, 30; Cefotaxime, 75; Clindamycin, 30; Ampicillin, 10; Amoxicllin, 25; Nitrofurantoin, 200; Norfloxacin, 10; and Carbenicillin, 100. The 21 antibiotics were chosen in order to permit comparisons between the different isolates from different feces and water samples. The MAR test was performed on all the isolates using the Muller Hinton Agar according to [20].

Analysis of Protein Pattern by Polyacrylamide Gel Electrophoresis (PAGE)

Selected *E. coli* isolates from the previous samples (Feces and water sources) were subjected to protein analysis using the PAGE technique as described by [21]. Bio-Rad (USA) vertical slab gel with size of 0.75 mm x 14 cm x 14 cm apparatus was used. The total cellular proteins of the selected isolates were fractionated by electrophoresis in one dimentional denatured polyacrylamide gel. The banding patterns were visualized by staining the gel with coomassie brilliant blue and they scored by the computer program “Gel – pro Analyzer v. 3.1 program (windows 95/NT Media Cyberneties 1993-1997 USA)” which was used to estimate the molecular weight of the bands and their quantities.

DNA Extraction and Restriction Digestion

The same fecal *E. coli* isolates which were previously used in protein analysis were grown in Brain Heart Infusion broth (BHI) [18], and DNA were extracted using a genomic DNA purification kit manufactured by Gentra systems, Minneapolis, USA. One µg of each genomic DNA sample was digested at 37°C for 2hrs. with 20 units of restriction endonucleases, namely *Hind*III (which recognizes the sequence A/AGCTT), *Eco*RI (which recognizes the sequence G) AATTC), *Eco*RV (which recognizes the sequence GAT (ATC), *Bgl*II (which recognizes the sequence A/GATCT), and *Bam*HI (which recognizes the sequence G) GATCC). MULTI-CORE™ Buffer Pack (25mM tris acetate, pH7.5 (at 37°C), 100mM potassium acetate, 10mM magnesium acetate, 1mM DTT), was used to enable co-digestion of DNA with more than one enzyme in a single reaction. Modest adjustments in the amount of enzyme used, will ensure complete multiple digestion. The restriction fragments were separated by 2% agarose gel electrophoresis.

Plasmid Extraction

Plasmid DNAs of the selected *E. coli* isolates were obtained by the alkaline lysis method as described by [22]. A mini preparations scale was performed by growing each isolate in 3ml BHI broth at 37°C for 24h.

Results

The results in (Table1) revealed that isolates of household water reservoir displayed the highest percentages of resistance (81%) to most of the antibiotics tested, followed by the isolates of mouse (72%). However, water of Ismailia Canal obtained (67%). Each of human, horse and Nile water isolates gave 62%, while swimming pool isolates, cat isolates and avian isolates showed the lowest percentage of resistance to antibiotics 57%, 47% and 34%, respectively.

Protein Profiles

Table 2 and Fig. 1 present the results obtained for *E. coli* isolates of human, animal and avian origin along with Nile water, Ismailia canal, household water reservoir and swimming pool isolates for their protein patterns. It was found that all of the *E. coli* isolates were identical, except that of the Nile water isolate. This last isolate shows extra bands and could be an interesting one worth further research.

DNA Fingerprints

Data in Table 3 and photo 2 represent the fingerprint patterns for the *E. coli* isolates of different sources. These fingerprints were composed of 1-9 bands ranging in size from 35-1989 bp according to the restriction endonuclease enzymes used *Hind* III, *Eco*RI, *Eco*RV, *Bgl*II and *Bam*HI. Row16 at which the molecular weight ranged between 275-327 present only in avian isolate and the isolate selected from household water reservoir and swimming pool. Although 901 bp band in avian isolates was also present in household water reservoir, 937 bp band in the same raw (r_{10}) was detected in horse isolate as well as

Table 1: Multiple Antibiotic Resistance of *E. coli* isolates.

Antimicrobial agent	Human	Animal feces - isolates				Water isolates			
		Horse	Cat	Mouse	avian	Ismailia Canal water	Nile water	Household reservoir	Swimming Pool
Tobramycin (Tob)	S	S	S	S	S	S	S	R	S
Gentamycin (GM)	S	S	S	S	S	S	S	R	S
Penicillin (P)	R	R	R	R	R	R	R	R	R
Tetramycin (Te)	R	R	R	S	S	R	R	R	R
Cefperazone (CFP)	R	R	S	R	S	R	S	R	S
Erythromycin (E)	R	R	S	R	S	S	R	R	S
Chloramphenicol (C)	R	R	S	S	S	S	R	S	R
Sulfa/trimethoprim (SXT)	R	R	R	R	R	R	R	R	R
Doxycyclin (Do)	R	S	R	R	S	R	R	R	R
Rifampicin (RA)	S	S	S	S	S	R	S	R	S
Streptomycin (S)	R	R	S	R	S	R	S	R	R
Ofloxacin (OFX)	S	S	S	S	S	S	S	S	S
Cephradine (CE)	S	R	R	R	S	R	R	R	R
Ceftriaxone (CRO)	S	S	S	R	S	R	R	R	R
Cefotaxime (CTX)	S	R	R	R	R	R	R	R	S
Clindamycin (DA)	R	R	R	R	S	R	R	R	R
Ampicillin (AM)	R	R	R	R	R	R	R	R	R
Amoxicillin (AMX)	R	R	R	R	R	R	R	R	R
Nitrofurantoin (F)	R	R	R	R	R	R	R	R	R
Norfloxacin (NOR)	S	R	S	R	S	R	S	S	S
Carbenicillin (PY)	S	S	S	S	S	S	S	S	S
% Resistance	62%	62%	47%	72%	34%	67%	62%	81%	57%

S: Sensitive; R: Resistant

Table 2. Protein profiles of *E. coli* isolates.

Mol. wt \ Lanes	Marker	Human	Animal			avian	Ismailia Canal water	Nile water	Household reservoir	Swimming pool
			Horse	Cat	Mouse					
155.73(r_1)	-	-	-	+	-	-	+	+	-	-
140.76(r_2)	-	-	+	+	+	+	-	+	-	-
111.00(r_3)	-	-	-	-	-	-	+	+	-	-
89.11(r_4)	+	-	+	-	+	-	-	+	-	-
74.79(r_5)	-	-	-	+	-	-	+	+	+	+
68.33(r_6)	+	-	+	+	+	+	-	+	-	-
60.50(r_7)	-	-	-	-	-	+	+	+	+	+
49.75(r_8)	-	-	-	-	-	-	-	+	-	-
45.82(r_9)	-	+	+	+	+	+	+	+	+	+
41.19(r_{10})	+	+	+	+	+	+	+	+	+	+
38.60(r_{11})	-	-	-	-	-	-	-	+	-	-
36.18(r_{12})	-	+	+	+	+	+	+	+	+	+
34.90 (r_{13})	-	+	+	+	+	+	+	+	+	+
32.59(r_{14})	-	-	-	-	-	-	-	+	-	-
30.66(r_{15})	+	+	+	+	+	+	+	+	+	+
28.76(r_{16})	-	+	+	+	+	+	+	+	+	+
28.28(r_{17})	-	+	+	+	+	+	+	+	+	+
26.58(r_{18})	-	+	+	+	+	+	+	+	+	+
24.35(r_{19})	-	+	+	+	+	+	+	+	+	+
23.74(r_{20})	-	+	+	+	+	+	+	+	+	+
22.52(r_{21})	-	+	-	-	-	-	-	+	-	-
21.52(r_{22})	-	-	+	-	-	-	-	+	+	-
19.98(r_{23})	+	-	-	-	-	-	-	+	-	-
19.08(r_{24})	-	+	+	+	+	+	+	+	+	+
18.33(r_{25})	-	+	+	+	+	+	+	+	+	+
17.53(r_{26})	-	+	+	+	+	+	+	-	+	+
17.13(r_{27})	-	-	+	-	+	-	+	-	+	+
16.68(r_{28})	-	+	+	+	+	+	+	+	+	+
15.71(r_{29})	-	-	-	-	-	-	-	+	+	-
15.12(r_{30})	-	-	-	+	-	+	-	+	-	-
14.30(r_{31})	+	+	+	+	+	+	+	+	+	+
13.94(r_{32})	-	-	-	-	-	-	-	+	-	-
13.54(r_{33})	-	+	+	-	-	-	-	+	-	-
12.94(r_{34})	-	+	+	+	+	+	+	+	+	+
12.18(r_{35})	-	+	+	+	+	+	-	+	-	-
11.31(r_{36})	-	+	+	+	+	+	+	+	+	+
9.99(r_{37})	-	+	+	+	+	+	+	+	+	+

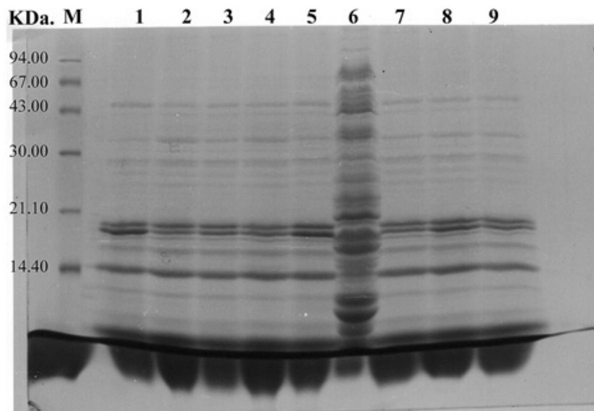

Fig. 1. Protein profile of *E. coli* isolates show: Lane (M): molecular weight marker, Lane (1): Human isolate, Lane (2): Horse isolate, Lane (3): Mouse isolate, Lane (4): Cat isolate, Lane (5): Bird isolate, Lane (6): Nile isolate, Lane (7): Ismailia canal isolate, Lane (8): Water reservoir isolate, and Lane (9): Swimming pool isolate.

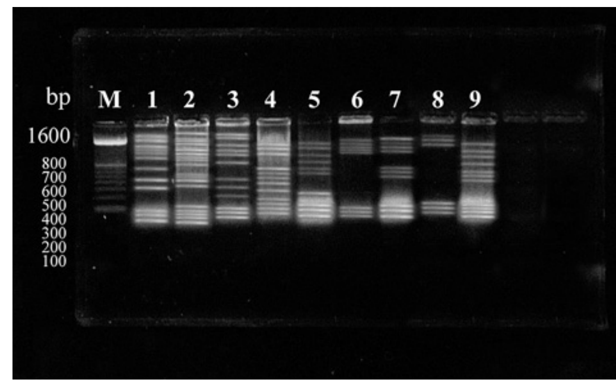

Fig. 2. Comparison of restriction endonucleases HindIII, EcoRI, EcoRV, Bam HI and BglII cleavage patterns of DNA from 9 *E. coli* isolates. Lane (M): DNA marker (100 bp ladder – Pharmacia). Lane (1): Swimming pool isolate, Lane (2): Horse isolate, Lane (3): Mouse isolate, Lane (4): Bird isolate, Lane (5): Water reservoir isolate, Lane (6): Ismailia canal isolate, Lane (7): Nil.

Table 3. Classification analysis of DNA of *E. coli* isolates. Using restriction enzymes.

Lanes	Marker	Human	Animal			avian	Ismailia Canal water	Nile water	Household reservoir	Swimming pool
			Horse	Cat	Mouse					
Rows	Mol.wt (bp)	Mol.wt (bp)	Mol.w (bp)	Mol.wt (bp)	Mol.wt (bp)	Mol.wt (bp)	Mol.wt (bp)	Mol.wt (bp)	Mol.wt (bp)	Mol.wt (bp)
r_1	-	-	+	+	+	-	-	-	-	+
r_2	+	-	+	+	+	-	+	+	-	+
r_3	-	-	+	-	-	-	-	-	-	-
r_4	-	+	+	+	+	+	+	+	+	+
r_5	-	-	-	-	-	+	+	+	+	-
r_6	-	+	+	-	+	-	+	+	-	+
r_7	-	+	+	-	+	+	+	-	+	-
r_8	-	-	+	-	-	-	-	-	-	+
r_9	-	+	+	-	+	-	-	-	+	+
r_{10}	-	-	+	-	-	+	-	+	+	-
r_{11}	+	+	-	-	-	+	-	+	+	+
r_{12}	+	-	+	-	-	-	-	-	-	-
r_{13}	+	+	+	-	-	+	-	+	-	-
r_{14}	+	+	+	-	+	+	-	-	-	+
r_{15}	+	-	+	-	+	-	-	-	-	-
r_{16}	+	-	-	-	-	+	-	-	+	+
r_{17}	+	-	-	-	+	+	-	-	-	-
r_{18}	-	-	+	+	-	+	-	-	+	-
r_{19}	+	+	-	-	+	+	+	+	-	-
r_{20}	-	+	+	+	+	-	-	+	+	+
r_{21}	-	+	+	+	-	+	+	-	-	+
r_{22}	-	+	+	-	+	-	+	+	+	+
r_{23}	-	-	+	-	-	-	-	-	-	-

Nile water isolate. In row 11, the molecular weight (ranging from 1056-1098 bp), was found to be present in isolates from human, horse, mouse, household water reservoir, and swimming pool. The results indicated in Table 3 showed that there is a band which was present in all the isolates of the present study, had 1366 pb molecular weight, suggesting that it is a common sequence in *E. coli.*

Table 4 represents the amount of DNA with reference to the total amount per lane, and it is found that in human isolate the amount of DNA ranged between 4.3064 –12.714 bp had a total of 68.02%. In animal isolates (horse, cat and mouse), the amount of DNA ranged between 1.8261 – 19.071 bp, with a percentage of 89.095%, 87.562% and 74.216%, respectively, were higher than the amount of DNA in human isolates. Avian protein amount ranged between 2.643 and 14.367 bp, which is found to be approximately similar to that of the mouse isolate. The isolates of Ismailia canal water, Nile water, household water reservoir and swimming pool showed a DNA amount of 99.576%, 51.396%, 61.591% and 90.352%, respectively.

Plasmid Profiles

All of the *E. coli,* isolates studied, contained a large plasmid of 2737bp, were the amount of DNA per lane in

Table 4. The amount of DNA (band of *E. coli* isolates. Using restriction enzymes.

Lanes	Marker	Human	Animal			avian	Ismailia Canal water	Nile water	Household reservoir	Swimming pool
			Horse	Cat	Mouse					
Rows	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)	amount (ng)
r_1			7.1217	7.0781	4.4893					8.3404
r_2	66.458		4.4768	18.104	6.0493		14.799	4.0956		11.255
r_3			4.7295							
r_4		4.3064	4.6741	19.071	3.6617	14.367	12.892	0.40714	2.6849	4.9463
r_5						8.3496	11.431	1.2723	3.5235	
r_6		5.311	5.183		5.0845		7.3561	1.1584		2.583
r_7		4.9109	3.6758		5.8421	7.9891	7.7333		2.4289	
r_8			1.8261							1.6073
r_9		5.9504	3.3786		4.7623				2.2489	6.2964
r_{10}			3.4135			8.77		1.611	1.6139	
r_{11}	5.8011	4.3989				2.6432		1.7709	1.3689	7.593
r_{12}			3.1417							
r_{13}	1.0852	4.632	3.4164			4.4655		2.057		
r_{14}	1.7814	4.3708	6.1258		3.5287	6.9291				13.366
r_{15}	3.0585		5.5137		3.6229					
r_{16}	4.1795					5.8805			5.7185	11.251
r_{17}	4.0907				3.2465	3.5324				
r_{18}			6.375	18.532		3.3987			13.839	
r_{19}	13.529	12.714			13.181	4.6473	13.593	12.545		
r_{20}		7.5611	5.7582	17.066	8.1751			14.436	13.906	9.6051
r_{21}		7.157	7.3748	7.71		4.4096	11.666			7.8869
r_{22}		6.9054	5.0311		12.573		20.106	12.044	14.258	
r_{23}			7.8792							5.6209
Sum In Lane	99.984 100	68.018 100	89.095 100	87.562 100	74.216	75.382. 100	99.576 100	51.396 100	61.591 100	90.352 100

human and swimming pool isolates were 22.88% and 22.64%, respectively. Obvious similarities appeared between the amount of DNA per lane in avian (4.4249%) and household water reservoirs (4.8634%), as well as a similarity between the amount of DNA in cat, mouse and Ismailia canal water isolates, equal 0.9616%, 0.9247% and 0.5977%, respectively.

Discussion

The goal of this study was to identify a procedure that could be used to differentiate *E. coli* isolates from various host origins, by allowing the development of a system for determining the sources of fecal pollution of water. A total of 138 *E. coli* isolates were collected from nine known host sources in different water locations in Cairo and from its origin (feces) of human, animal and avian. The results of the present study indicated that protein analysis (PAGE), DNA fingerprinting using five endonuclease enzymes and (MAR), provided the highest level of discriminatory capacity for differentiating various *E. coli* isolates and for identifying the fecal contamination sources.

Multiple antibiotic resistance (MAR) is one of the three methods investigated in this work that represent the simplest and the least expensive approach. Several researchers have previously used the MAR profiles of streptococci to identify fecal pollution sources. [23] reported that the MAR was 84%, when streptococcal isolates were pooled into four possible categories (cattle, human, poultry and wildlife). [24] demonstrated that the average for the correct identification rate for fecal streptococcus source was 87%. These results are better than the 64.58% that was obtained in the study of [10], where all the *E. coli* isolates were pooled into three possible categories (human, livestock, and wildlife). The difference in these results might be attributed to the different bacterial strains and the antibiotics used and the difference in the diversities of the bacterial collections is also a factor which must be taken into consideration. In the present study a more diverse representative collection of *E. coli* isolates was used. This sampling protocol could produce a more heterogeneous collection of bacterial isolates than other protocols. The average MAR indices for human, horse and Nile water isolates were higher (62%) than those for the swimming pool (57%), cat (47%) and avian isolates (34%). These results are consistent with other reports that the MAR indices of fecal *E. coli* from human and livestock isolates were higher indices [9, 25].

The fingerprinting test was used for identification of fecal *E. coli* from specific sources, and it has been shown to be a powerful tool for molecular characterization of various bacterial species, and it is proved to be quite accurate for discriminating between fingerprint patterns of isolates of human and nonhuman origin [26,27].

The choice of the restriction enzymes is crucial for the effectiveness of fingerprint analysis. In this study five restriction enzymes (*Hind*III, *Eco*RI, *Eco*RV, *Bam*HI and *Bgl*II) were selected. A total of 23 bands were generated and their size ranged from 35- 1989 bp and 99 fragments. The present results were consistent with those of [10] who demonstrated that two restriction enzymes (*Eco*RI and *Mse*I) and two sets of primers (*Eco*RI-A plus *Mse*I–G and *Eco*RI –C plus *Mse*I–CA) were selected, since the genomes of three *E. coli* strains have been sequenced, the number of bands and fragments generated by using fingerprint analysis. Selective primers *Eco*RI–A and *Mse*I-G should produce more bands (50-500bp) and hence should permit a more accurate discrimination.

The difference in the number of bands and/or fragments detected, between the present results and those of [10] could be explained by the genetic diversity of *E. coli* isolates, inefficient amplification of some of the fragments and inability of the software to detect some weak bands.

In conclusion, from the four different methods that were evaluated to differentiate the *E. coli* isolates from various sources and the origin responsible for water pollution, the method of fingerprint analysis provided the greatest discriminatory power and the highest rate of correct classification, while the three other methods (MAR, protein profile and plasmid profile), provided from moderate to high degrees of correct classification.

References

1. FLEISHER J. M., KAY D., SALMEN R. I., JONES F., WYER M. D., GODFREE A. F. Marine waters, contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. Am. J. Public Health. **86**, 1228, **1996.**
2. SAUER T. J., DANIEL T., C. NICHOLS D. J., WEST, C. P., MOORE JR. P. A., WHEELER G. L. Runoff water quality from poultry litter –treated pasture and forest sites. J. Environ. Qual. **29**, 515, **2000**.
3. U. S. ENVIRONMENTAL PROTECTION AGENCY**.** TMDL tracking system data, version 1.0. Total maximum daily load program. U. S. Environmental Protection Agency Office of Water, Washington, D. C.[Online] http://www.epa.gov/owow/tmd/trcksys.html, **1998.**
4. JONES I. G., ROWORTH M**.** An outbreak of *Escherichia coli* 0157 and campylobacteriosis associated with contamination of a drinking water supply. Public Health (London) **110**, 277, **1996**.
5. GUGNANI H. C**.** Some emerging food and water borne pathogens, J. Commun. Dis. **31**, 65, **1999.**
6. BERROUANE Y. F, MCNUTT I. A., BUSCHELMAN B. J., RHOMBERG P. R., SANFORD M. D., HOLLIS R. J., PFALLER M. A., HERWALDT L. A. Outbreak of severe *Pseudomonas aeruginosa* infections caused by a contaminated drain in a whirlpool bathtub. Clin. Infect. Dis. **31**, 1331, **2000.**
7. LICENCE K., OATES K. R., SYNGE B. A., REID T. M. An outbreak of *E. coli* 0157 infection with evidence of spread from animals to man through contamination of a private water supply. Epidemiol. Infect. **126**, 135, **2001.**

8. PUCCH M. C., MCANULTY J. M., LESJAK M., SHAW N., HERON L., WATSON J. M. A statewide outbreak of cryptosporidiosis in new south Wales associated with swimming at public pools. Epidemiol. Infect. **126**, 389, **2001.**
9. KRUMPERMAN P. H**.** Multiple antibiotic resistamce indexing of *Escherichia coli* to identify high risk sources of fecal contamination of foods. Appl. Envirom. Microbiol. **46**, 165, **1983.**
10. GUAN S., XU R., CHEN S., ODUMERU J., GYLES C**.** Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. Appl. Environ. Microbiol. **68** (6), 2690, **2002.**
11. PARVEEN S., MURPHREE R. L., EDMISTON L., KASPAR C. W., PORTIER K. M., TAMPLIN M. L. Association of multiple- antibiotic – resistance profiles with point and non point sources of *Escherichia coli* in Apalachicola Bay. Appl. Environ. Microbiol. **63**, 2607, **1997.**
12. HARWOOD V. J., WHITLOCK J., WITHINGTON V**.** Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical water. Appl. Environ. Microbiol. **66**, 3698, **2000.**
13. PARVEEN S., PORTIER K. M., ROBINSON K., EDMISTON L., TAMPLIN M. L**.** Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. Appl. Environ. Microbiol. **65**, 3142, **1999.**
14. CARSON A. C., SHEAR B. L., ELLERSIECK M. R., ASFAW A. Identification of fecal *Escherichia coli* from humans and animals by ribotyping. Appl. Environ. Microbiol**. 6-7** (4), 1503, **2001.**
15. KARIUKI S., GILKS C., KIMARI J., OBANDA A., MUYODI J., WAIYAKI P., HART C. A. Genotype analysis to *Escherichia coli* strains isolated from children and chickens living in close contact. Appl. Envirom. Microbiol. **65**, 472, **1999.**
16. DOMBEK P. F., JOHNSON I. K., ZIMMERLEY S. J., SADOWSKY M. J**.** Use of repetitive DNA Sequences and PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. **66**, 2572, **2000.**
17. BERNHARD A. E., FIELD K. G**.** A PCR assay to discriminate human and animal feces on the basis of host differences in Bacteroides –Prevotella genes encoding 16s-rRNA. Appl. Environ. Microbiol. **66**, 4571, **2000.**
18. DIFCO MANUAL (10th Ed.) Dehydrated culture media reagents for microbiol, DIFCO Laboratories. Detroit Michigan 48232 USA, **1994.**
19. AMERICAN PUBLIC HEALTH ASSOCIATION (APHA**).** Standard methods for the examination of water and waste water. American Public Health Association, Washington, D. C. **1995.**
20. GREENWOOD D**.** Antibiotics and chemotherapeutic agents used in the therapy of bacterial infection. In: Toply and Wilson's Microbiology and Microbial Infections. Balows A. and Duerden B. I.(eds). volume (2), Systematic Bacteriology Ninth edn. Oxford University press, Inc. New York. pp. 195-229, **1998.**
21. GALLAGHER S., SMITH J. A**.** One dimentional gel electrophoresis of protein In current protocol in molecular biology (Ausubel *et al*., eds.) unit 10.2. Greene Publishing and Wiley – Interscience, New York. **1995.**
22. MANIATIS T., FRITSCH E. F., SAMBROOK J. Molecular cloning. A laboratory manual. Publisher: Coldspring Harbor Laboratory. **1982.**
23. WIGGINS B. A. Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters. Appl. Envirom. Microbiol. **62**, 3997, **1996.**
24. HAGEDORN C., ROBINSON S. L., FILTZ J. R., GRUBBS S. M., ANGIER T. A., RENCAU, R. B. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Micorbiol. **65**, 5522, **1999.**
25. KASPAR C. W., BURGESS J. L., KNIGHT I. T., COLWELL R. R. Antibiotic resistance indexing of *Escherichia coli* to identify sources of fecal contamination in water. Can. J. Microbiol. **36**, 891, **1990.**
26. VALSANGIACOMA C., BAGGI F., GAIA V., BALMELLI T., PEDUZZI R., PIFFARETTI J. C. Use of amplified fragment length polymorphism in molecular typing of *Legionella pneumophila* and application to epidemiological studies. J. Clin. Microbiol. **33**, 1716, **1995.**
27. DESAI M., THRELFALL E. J., STANLEY J. Fluorescent amplified Fragment length polymorphism subtyping of the *Salmonella enterica* serovar Enteritidis phage type 4 clone complex. J. Clin. Microbiol. **39,** 201, **2001.**