Isolation and biochemical identification of *Escherichia coli* from wastewater effluents of food and beverage industry

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Abstract

The aim of this study was the isolation and biochemical identification of *E. coli* from industrial wastewater effluents. Sixty samples were collected from different sources in Lahore. The results revealed that *E. coli* was found in higher concentration in wastewater of food and beverage industries. Wastewater is an important reservoir for *E. coli* and presented significant acute toxicity if released into the receiving water body without being adequately treated. Results revealed the presence of both gram negative and positive bacteria. There was nonsignificant variation among all the samples of wastewater. The highest concentration of *E. coli* was observed in wastewater of food industry Site A (Rettigon road) and beverage industry Site F (Wahdat road). Biochemical and serological tests confirmed the presence of *E. coli*.

Keywords: *E. coli*, wastewater, food industry, beverage industry, effluent.

Yiyecek ve içecek endüstrisi atıksu deşarlıklarından *Escherichia coli* eldesi ve biyokimyasal tanımlanması

Özet

Bu çalışmanın amacı endüstriyel atıksu deşarlıklarından *E. coli* eldesi ve biyokimyasal tanımlanmasıdır. Lahor'da farklı kaynaklardan 60 örnek toplandı. Sonuçlar yiyecek ve içecek endüstrisi atıksularında daha fazla konsantrasyonda *E. coli* bulunduğunu gösterdi. Atıksu önemli bir *E. coli* deposudur ve yeterli olarak muamele edilmenden alcı su kaynağına salınırsa ileri derecede toksik olabilir. Sonuçlar hem gram pozitif, hem de gram negatif bakteri varlığı gösterdi. En yüksek *E. coli* konsantrasyonu A bölgesi (Rettigon yolu) yiyecek endüstrisinin ve F bölgesi (Wahdat yolu) içecek endüstrisinin atıksularında gözlemendi. Biyolojik ve serolojik testler *E. coli* varlığını doğruladı.

Anahtar kelimeler: *E. coli*, atıksu, yiyecek endüstrisi, içecek endüstrisi, atıksu deşarjı.

Introduction

Industrial waste is the most common source of water pollution in the present day (Ogedengbe and Akinbile, 2004) and it increases yearly due to the fact that industries are increasing as most countries are getting industrialized. Industries produce wastes which are peculiar in terms of type, volume and frequency depending on the type of industry and population that uses the product (Odumosu, 1992). Water and wastewater management constitutes a practical problem for the food and beverage industry. In spite of significant improvement over the last 20 years, water consumption and disposal remain critical from environmental and economic standpoint (Fillaudeau *et al*., 2005).

A food processing industry is involved with the total environment from the farm to the customer. Water is absolutely necessary for many steps in the food processing industry. At present, there is no economical substitute of water. Consequently water conservation and water reuse are necessary. By
practicing conservation and reuse, the amount of liquid waste and pollution potential from the food processing is reduced (Mercer, 1964).

On a global scale, contamination of drinking water by pathogenic bacteria causes the most significant health risk to humans, and there have been countless numbers of disease outbreaks and poisonings resulting from exposure to untreated or poorly treated drinking water. However, significant risks to human health may also result from exposure to toxic contaminants that are often globally ubiquitous in waters from which drinking water is derived. The presence of *E. coli* is a definite indication of fecal contamination (WHO, 2004). Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhea, urinary tract infections, septicemia, and neonatal meningitis (Orskov and Orskov, 1992). The magnitude of the problem of bacterial contamination deserves more elaborate studies from the point of production of waste effluents to the point of consumption at all intermediary levels. The aim of the present research was isolation and biochemical identification of *E. coli* from industrial effluents of food and beverage industries in Lahore.

**Material and methods**

**Sample collection**

Sampling was completed in two successive months from March to April for the microbial assessment of waste effluents from food and beverage industries. Total of sixty samples were collected. In March, effluents of food industries were collected from industries near Rettigon road, Township industrial area, Township industrial estate. In April, effluents of beverage industries were collected from industries near Multan road and Wahdat road. Data from each sample was collected and recorded in the data book. Samples were collected in hermetically sealed, sterilized falcon tubes and were kept at 4ºC until analysis.

**Sample processing**

The technique described by Theodor Escherich, 1885 was used for isolation of *E. coli* (Escherich, 1885). To prevent contamination, the area was swabbed with 70% ethanol prior to opening any sample container. Samples (0.5 ml) were taken in 10 ml LB (Luria Bertani) broth medium in test tube, and vortexed for one minute and left for thirty minutes at room temperature. Then supernatant (1ml) was taken from this test tube and a 2-fold serial dilution was prepared (Reddy, 2007). After this, 500 ml from the final dilution tube was spreaded on the petri dishes (Pyrex) of MacConkey medium and LB medium. Petri dishes were kept in the incubator for 24 hours at 37ºC (Hajna and Perry, 1939). After 24 hours, plates were studied for the colonies of microbes grown on the media. Microorganisms grown on MacConkey agar are capable of metabolizing lactose which produces acid by-products that lower the pH of the media which causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony (Koneman, 2005). Different biochemical tests (Werkman, 1930; O'Meara, 1931; Vaughn et al., 1939; Silva et al., 1980) were performed for the identification of *E. coli* in the waste effluents of food and beverage industry (Table 1).

**Serological tests**

Commercial latex kits are available for O157, O26, and H7 strains of *E. coli*. O157 antiserum has been shown to cross-react with other organisms including *E. hermanii* (frequently found in foods) (Hopkins and Hilton, 2000; Law, 2000). Tests incorporated positive and negative control organisms and control latex. Test was performed by a slide agglutination test using somatic (O) or flagella (H) antisera. Some pathogenic bacteria were nonmotile.

**Results**

*E. coli* was cultured on LB medium and MacConkey medium for morphological characterization. After 24 hrs, two types of colonies were isolated under microscopic examination. All the isolated colonies were pink on MacConkey medium, while creamy yellow on LB medium. *E. coli* was observed in highest concentration from wastewater samples of industry (Site A) whereas in wastewater samples of industry (Site B) six samples indicated the presence of *E. coli* which was confirmed by biochemical and serological test. Four samples were of gram positive bacteria which may be *Bacillus subtilus* or *Bacillus thuringiensis*. In industrial effluent (Site C) eight samples were of gram negative while two samples were of gram positive bacteria. It was observed that waste effluents of food industry (Site A) revealed greater percentage of gram negative bacteria.

In wastewater samples of industry (Site D) five were gram negative, while five were gram positive bacteria. Six samples in industrial effluent (Site E)
Table 1. Biochemical identification of *E. coli* in industrial wastewaters

<table>
<thead>
<tr>
<th>Samples Sources</th>
<th>Indole Test</th>
<th>Spot Indole Test</th>
<th>Kovacs Indole Test</th>
<th>Methyl Red Test</th>
<th>Voges Proskeur Test</th>
<th>Simmon's Test</th>
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<td>Ammonium acetate Test</td>
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<td>Industrial effluent(B)</td>
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<td>Industrial effluent(C)</td>
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<td>Industrial effluent(D)</td>
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<tr>
<td>Industrial effluent(E)</td>
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<td>Industrial effluent(F)</td>
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were gram negative and four were gram positive bacteria. In the wastewater samples of beverage industry (Site F) all samples were of gram negative bacteria (Figure 1 and 2).

The average value of gram negative bacteria in wastewater of food industry (Site A) was 5.10 ± 0.34. The average value of gram negative bacteria in wastewater of food industry (Site B) was 4.66 ± 0.66 while in wastewater of food industry (Site C) was 4.50 ± 0.50. The average value of gram negative bacteria in wastewater of beverage industry (Site D) was 5.0 ± 0.70, whereas in the wastewater of beverage industry (Site E) was 3.8 ± 0.60 and in the wastewater of beverage industry (Site F) was 4.0 ± 0.33. Student’s *t*-test revealed a non significant difference (*P* > 0.05) between gram positive and gram negative bacteria.

Figure 1. Percentage of gram negative bacteria in all industrial wastewater samples. Food Industry A: Rettigon road; Food Industry B: Township industrial area; Food Industry C: Township industrial estate; Beverage industry D: Multan Road; Beverage industry E: Multan road; Beverage industry F: Wahadat road
Figure 2. Number of *E. coli* colonies (mean ±SE) in wastewater samples of industries. Sites A, B, C: Food industries at Rettigon road, Township industrial area, Township industrial Estate Sites D, E, F: Beverage industries at Multan Road and Wahadat road.

Discussion

The bacterium *E. coli* is one of the best and most thoroughly studied free-living organisms. It is also a remarkably diverse species because some *E. coli* strains live as harmless commensals in animal intestines. *E. coli* is a widely used indicator of fecal contamination in water bodies. External contact and subsequent ingestion of bacteria from fecal contamination can cause detrimental health effects (Money *et al*., 2009).

Stomach cramps, nausea and vomiting are the symptoms caused by *E. coli*, however serious complications can also occur. Water samples were the only nonfecal samples that tested positive for *E. coli*. Water has been implicated in human outbreaks and the studies revealed that water may be an important source of 0157:H7 on farms (Karmali, 1989).

The present research work was conducted to isolate *E. coli* from food and beverage industrial effluents. Effluents are good primary reservoir for *E. coli*. Sixty different samples from food and beverage industries were processed for the isolation of *E. coli*. The food and beverage industries uses large volume of water as it is suitable, clean, and a quite inexpensive resource, both as a constituent of many products, and for other production requirements. Microbial growth in drinks due to contaminated water supplies or sugar syrups can cause discoloration, off flavors and shortened shelf-life, as well as increasing the risk of infection to consumers (Noronha *et al*., 2002).

However, selective media are universally used in water monitoring and were employed in the United States Environmental Protection Agency epidemiological investigations, suggesting that culturable fecal indicator counts are valid predictors of disease risk (Sinton *et al*., 1994). Sewage can serve as a vehicle for entering into human and nonhuman hosts either by direct contact or through contamination of drinking water supplies (Boczek *et al*., 2007).

The results revealed that the highest percentage of *E. coli* was observed in the waste effluents of industries (A and F). ANOVA showed non significant (P > 0.05) variation. Student’s *t*-test also revealed non significant difference between gram positive and gram negative bacteria.

According to Boczek and colleagues (2007) the occurrence of clonal group in wastewater demonstrates a potential mode for the dissemination of this clonal group in the environment, with possible secondary transmission to human or animal hosts. Chalmers and colleagues (2000) demonstrated that the effluent had a significant pollution potential, mainly due to the low pH and high concentration of *E. coli*. The results also
demonstrated that the wastewater presented significant acute toxicity, and could cause diseases if released into the receiving body without being adequately treated. This represents a dangerous public health risk, which needs future evaluation and control. Culture-independent analysis in various environmental samples has been used to catalog this species and also to assess the impact of human activity and interactions with microbes on natural microbial communities.

According to Barreto-Rodrigues and colleagues (2008), the objective of the work was to characterize the effluent originating from a Brazilian TNT production industry. Analyses were performed using physical, chemical, spectroscopic and ecotoxicological assays, which demonstrated that the effluent had a significant pollution potential, mainly due to the low pH and high concentration of TNT (156 ± 10 mg L⁻¹). The results also demonstrated that the effluent causes significant acute toxicity, and could cause countless damages if released into the rivers without being properly treated. The observed pollution potential justifies studies to evaluate treatment technologies or recover the residue generated in the TNT industry. From a total of 149 E. coli strains, 87 E. coli strains were from raw wastewater and 62 strains from treated wastewater by stabilization ponds. Within these strains two and four positive serological reaction to E. coli 0157 were found for raw and treated wastewater, respectively.

In the same direction, Muller and his colleagues (2001) carried out a study on E.coli 0157:H7 strains in water sources in South Africa and they did not find any evidence of EHEC 0157 while virulence factors present in the 96% of analyzed samples (196), however 8 isolates from 8 samples demonstrated the presence of Stx1 and Stx2.

References


