Effect of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* isolated from polluted drainage water on rats

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Received 3 November 2015
Accepted 15 May 2016

Journal of The Arab Society for Medical Research 2016, 11:1–8

Background/aim
Water pollution not only damages the environment but also kills wildlife. The present study aimed to investigate the effect of *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, which were isolated from drainage water at El-Rahawey area, Giza, Egypt, on experimental rats.
Materials and methods
Water samples were collected from the subsurface layer for bacteriological examination, stored in an ice box, and delivered to the laboratory and analyzed to count the total viable bacterial counts/ml using the most probable number technique. The isolated bacteria were given to rats for 21 days at a dose equal to 10^3/ml and the liver and kidney were dissected for histopathological and histochcemical studies.
Results
Results showed that the average counts at 22 and 37°C were 10^5 and 10^6 CFU/ml, respectively, whereas the average counts by using the most probable number technique/100 ml were 10^5, 10^4, 10^3, 10^3, and 10^2 for total coliforms, fecal coliforms, *E. coli*, *Salmonellae* group, and total staphylococci, respectively. Isolation of pathogens from water sources were identified by using membrane filter technique on specific media. The histopathological examination of the liver of treated rats with *E. coli*, *S. typhi*, and *S. aureus* revealed swollen hepatocytes with decreased sinusoidal spaces and widely distributed necrotic foci. In the kidney, renal tubules showed extensive epithelial swelling with decreased lumen space and generalized necrotic changes with interstitial hemorrhage in renal cortex. The histochemical study indicated the depletion of staining of protein and polysaccharides.
Conclusion
E. coli, *S. typhi*, and *S. aureus* that are isolated from polluted drainage water cause histological and histochemical changes in the liver and kidney of rats.

Keywords:
drainage water, Escherichia coli, histochemical, histopathological, rats, Salmonella typhi, Staphylococcus aureus

J Arab Soc Med Res 11:1–8

1687-4293

Introduction

In Egypt, the river Nile is the main source of drinking water, and is also used for various other purposes. It now faces rising sources of pollution despite all the programs for pollution control. Discharging industrial and domestic wastewater, return drainage of irrigated water, and flash flood into the river Nile represent the major sources of pollution [1]. It was found that some sites along the river Nile, such as the El-Rahawy drain, have no accepted parameters for different uses the river is put to [2]. In Egypt, some villagers rear birds in the drain mentioned above, and consequently may infect the water with different types of microorganisms (like bacteria), which may result in the outbreak of diseases in the surrounding human population [3].

El-Rahawy drain (Giza area, Egypt) is one of the main drains with an outlet to Rosetta branch of the river Nile, and receives considerable wastewater from the greater Cairo area. There are two main sources of pollution that potentially affect and deteriorate the water quality of Rosetta branch: first, the agriculture and the domestic wastes from villages distributed along the drain discharge their wastes without any treatment process; and, second, the wastewater treatment plants, particularly Abu Rawash and Zenein, significantly affect the water quality of the branch [4].

Water pollution not only damages the environment and kills wildlife but can also sicken and kill people [5]. Water must be safe and free of risk factors. Risk factors

related to water pollution are divided into two basic categories: chemical and biological pollutants. Both categories derive from human activity, which inevitably tends to modify water composition with respect to its original state in nature [6,7].

In the Fayoum governorate, Medani *et al.*[8] found that gross and histopathological lesions were seen in liver, lung, kidney, and heart.

In their study, Wang *et al.*[9] noted that subtilase cytotoxin caused extensive microvascular thrombosis and other histologic damage in the brain, kidneys, and liver, as well as dramatic splenic atrophy. Peripheral blood leukocyte levels were increased at 24 h; there was also a significant neutrophil infiltration in the liver, kidneys, and spleen and toxin-induced apoptosis at these sites.

Escherichia coli are bacteria that live in the guts of animals and people, and can be shed in feces, causing serious disease. On the other hand, contamination can also occur through contaminated food and through direct contact with infected animals or people. Infection with E. coli can cause diarrhea and in some cases a severe complication called hemolytic uremic syndrome, which commonly occurs in young children and the elderly [10–12].

Salmonellosis, a s*almonella* infection, is caused by drinking, eating, or coming in contact with objects contaminated with the bacteria. There are many animals that carry Salmonella spp. These include livestock and poultry, reptiles, amphibians, rodents, and even fish in aquariums. Salmonella spp. can cause serious disease, especially in young children, the elderly, and people with weakened immune systems [13–15].

In addition, Staphylococcus aureus is a type of bacteria that is resistant to certain antibiotics. It causes skin infections, and infected people and animals can transmit it to each other. Direct skin-to-skin contact is the common way for its spread. People and pets can carry S. aureus on their skin or in their noses and still not show signs of illness. Transmission involving animals is rare compared with person-to-person transmission [16,17].

Therefore, the present study was designed to evaluate the histopathological and histochemical changes in rats who were given *E. coli*, *Salmonella typhi,* and *S. aureus* isolated from polluted drainage water.

Materials and methods

Sampling procedure

Animals were maintained according to National Research Centre Ethics Committee rules and recommendations for experiments on animals. Water samples were collected from the Rosetta branch of the river Nile (mixing point between the El-Rahawy drain and the Rosetta branch) during April 2014 according to American Public Health Association [18]. Samples were collected from the subsurface layer (at depth 30 cm) in stopper polyethylene plastic bottles. Samples collected for bacteriological examination were stored in an ice box and delivered to the Laboratory of the National Research Center for analyses within 2 h.

Bacteriological analyses

Using the membrane filter and most probable number (MPN) techniques the tested bacteria (*E. coli* O157: H7, *S. typhi*, and *S. aureus*) were isolated, purified, and identified according to the American Public Health Association [18] from water samples, as well as using HiMedia (Mumbai, Maharashtra, India). MPN values for the *Salmonellae* group, *S. aureus*, *E. coli*, and coliphage were determined according to American Public Health Association [18]. All detected microorganisms were confirmed by using the membrane filter technique and specific chromogenic media (HiMedia). The bacteria was suspended in a sterile and aqueous buffer solution, provided that the number were 2.1×10^3, 3.2×10^2, 3.5×10^3 and 1.1×10^3 CFU/100 ml for, salmonellae group, *Staphylococcus aureus*, *E. coli*, and coliphage, respectively [18].

The experimental animals

Female albino rats (Laboratory Animal Colonies; National Research Center, Cairo, Egypt) weighing 100–150 g were used in this study. The animals were housed in groups of seven in stainless steel community cages at 25 ± 2°C with a 12 h light–dark cycle, and allowed to acclimatize for a period of 7 days before experimental use. Throughout the experiment, the rats were allowed free access feed (rats dietary pellets prepared by Cairo Company of Oil & Soap, Cairo, Egypt) and water.

The experimental design

Twenty-eight rats were used in the present study and were classified into four groups (seven rats each) as follows.

Group I, which served as the control group; group II, in which the rats were given an oral dose of staph bacteria (10^3/ml) for 21 days; group III, in which the rats were

given an oral dose of *Salmonella* bacteria (10^3/ml) for 21 days; and group IV, in which the rats were given an oral dose of *E. coli* bacteria (10^3/ml) for 21 days.

At the end of the experiment, liver and kidneys samples were dissected, and then fixed instantaneously in 10% formal saline for 24 h for histopathological and histochemical investigations.

(1) The histopathological study

The specimens were washed under tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax (melting point 55–60°C). Sections of 6 μm thicknesses were prepared and stained with hematoxylin and eosin [19].

(2) The histochemical studies
   (a) Total proteins: the mercury bromophenol blue method [20] was used for the histochemical demonstration of total proteins.
   (b) The polysaccharide inclusions: visualization of the polysaccharide materials was carried out using the periodic acid Schiff method (PAS) [21].

Results

Bacteriological results

Bacteriological results of water samples collected from the mixing point between El-Rahawey drain and the Rosetta branch during 2014 are presented in Table 1. The data represented showed that the densities of total viable counts at 22 and 37°C in raw Nile water samples were about 10^5 and 10^6 CFU/ml, respectively. The total and fecal coliforms were recorded to be 10^5 and 10^4 MPN-index/100 ml, respectively.

Table 1 Counts of classical bacterial indicators [total bacterial counts at 22 and 37°C (CFU/ml)] and coliforms bacteria (MPN-index/100 ml) as well as *Salmonella typhi* (CFU/ml)

Bacteria	Total viable bacterial counts (CFU/ml) 22°C	37°C	MPN-index/100 ml
Total counts	10^5	10^6	=
Total coliforms	=	=	10^5
Fecal coliforms	=	=	10^4
Escherichia coli	=	=	10^3
Salmonella typhi	=	=	10^3
Staphylococcus aureus	=	=	10^2

CFU, colony forming unit; MPN, most probable number; =, not detected.

In addition, the data demonstrated that, all samples were detected coliforms from all collected water samples. The counts were being 2.1×10^3, 3.2×10^2 and 3.5×10^3 CFU/100 ml for for, salmonellae group, *Staphylococcus aureus*, and *E. coli*, respectively.

Histopathological results

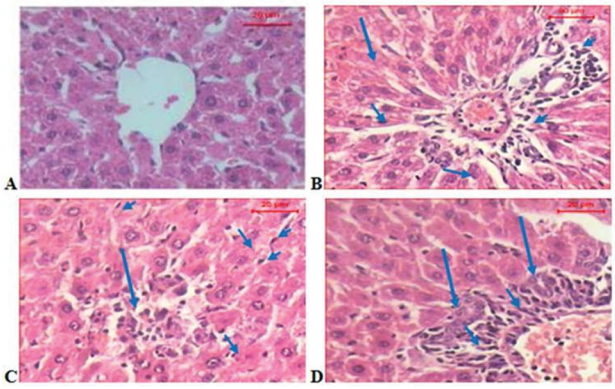
The results of the histopathological studies of the liver and kidney of treated and untreated rats are shown in Figs. 1 and 2, respectively. The liver and kidney of the untreated rats showed no visible histological changes (Figs. 1a and 2a, respectively).

In the liver of the staph-infected rats, there was a glass and eosinophilic appearance cytoplasm, dilated sinusoids, and lymphocytic infiltration in the portal and periportal areas (Fig. 1b). In the kidney, the glomeruli and renal tubules were degenerated (Fig. 2b).

In rats infected with *Salmonella* bacteria, apoptosis in the hepatocytes was found, and few of the hepatocytes nuclei showed karyolysis. The activated Kupffer cells were noticed (Fig. 1c). In the kidney, degeneration or swelling of the renal tubules and multiple foci of hemorrhage in the interstitium were found (Fig. 2c).

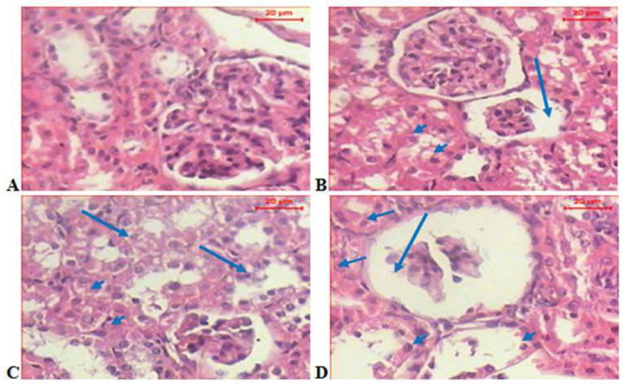
On the other hand, *E. coli* showed necrosis of the hepatocytes around the central vein, which appears associated with inflammatory infiltration (Fig. 1d). Infection with *E. coli* revealed degeneration of the

Figure 1



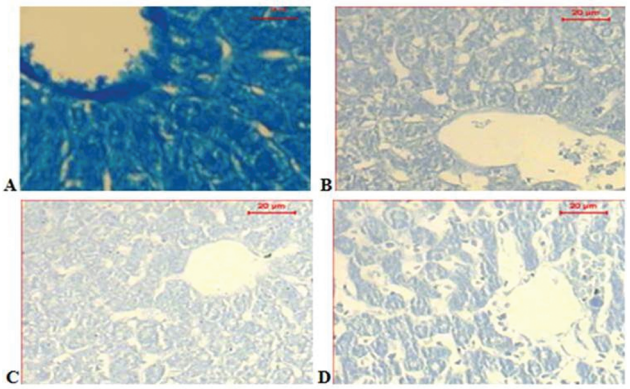
Micrographs of liver of (a): control rat liver shows the normal architecture of the hepatic lobule, (b): rat given *Staphylococcus* bacteria showing the cytoplasm of the hepatocytes has a ground glass and eosinophilic appearance (arrow), dilated sinusoids (short arrows), and lymphocytic infiltration in the portal and periportal areas (arrowheads) is seen, (c) rat given *Salmonella* bacteria shows apoptosis in the hepatocytes (arrow). Few of the hepatocytes nuclei show karyolysis (short arrows). Notice the activated Kupffer cells (arrowheads), (d) rat given *Escherichia coli* shows necrosis of the hepatocytes (arrows) around the central vein that appears associated with inflammatory infiltration (short arrows) (hematoxylin and eosin, scale bar: 20 μm).

Figure 2



Micrographs of the cortical tissue of the kidney of (a) control rat shows normal renal corpuscle (arrow) and renal tubules (arrowheads), (b) rat given *Staphylococcus* bacteria shows degeneration of the glomerulus (arrow) and renal tubules (arrowheads), (c) rat given *Salmonella* bacteria shows degeneration (arrow) or swollen of the renal tubules (arrowheads), (d) rat given *Escherichia coli* shows degeneration of the glomerulus (arrow) and renal tubules (arrowheads). Notice swelled renal tubules (short arrow) (hematoxylin and eosin, scale bar: 20 μm).

Figure 3



Sections of liver of (a) control rat shows the proteinic contents in irregular particles of various sizes that are equally distributed in the cytoplasm of the liver cells, (b) rat on a daily, oral dose of *Staphylococcus* bacteria shows the relatively diffused staining of the proteinic inclusions in both cytoplasm and nuclei, (c) rat on a daily, oral dose of *Salmonella* bacteria shows a marked diminution of the proteinic inclusions in many liver cells. Notice that the staining is mostly diffused, and (d) rat administered with of a single dose of *Escherichia coli* bacteria shows relatively few proteinic inclusions in the hepatocytes (bromophenol blue reaction, scale bar 20 μm).

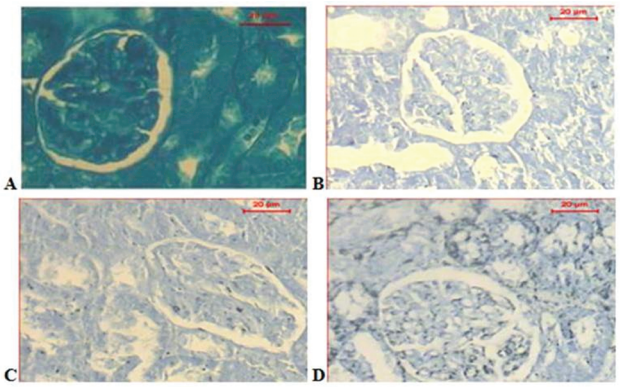
glomeruli and renal tubules. Swollen renal tubules were also detected (Fig. 2d).

Histochemical results

Total protein

Examination of the sections of the liver and kidney of the control rats displayed the normal distribution of proteinic inclusions as grayish blue irregular particles in both cytoplasms and nuclei (Figs. 3 and 4Figs. 3a and 4a, respectively).

Figure 4



Sections of the kidney of a (a) control rat shows the normal distribution of protein inclusions in the renal corpuscles and tubules, (b) a rat on a daily, oral dose of staph bacteria shows that the proteinic inclusions display faint blue staining in the glomerulus and exhibit diffused staining in most of the tubules; (c) rat on a daily, oral dose of *Salmonella* bacteria shows the proteinic inclusions with diminution and acquiring pale staining in the renal tubules and glomeruli, (d) rat given a single dose of *Escherichia coli* bacteria shows the distribution of the proteinic inclusions that display marked decrease in the cells of the renal tubules; many cells appeared almost unstained (bromophenol blue reaction, scale bar 20 μm).

Daily treatment with an oral dose of staph bacteria for 21 consecutive days revealed relative diffusion of the staining of the proteinic content in the hepatocytes (Fig. 3b). In the kidney, the proteinic inclusions displayed faint blue staining in the glomeruli and exhibited diffused staining in most of the cells of both proximal and distal convoluted tubules (Fig. 4b).

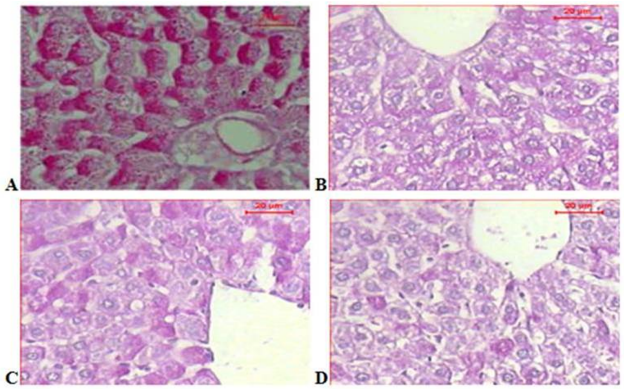
In rats administrated with *Salmonella* bacteria for 21 successive days, the proteinic inclusions showed marked diminution in many liver cells and the staining was mostly diffused (Fig. 3c). In the kidney, the protein inclusions of some proximal and distal convoluted tubules acquired a pale staining. However, the degenerated glomeruli exhibited dense stainability (Fig. 4c).

After 21 days of oral administration of *E. coli* bacteria, the proteinic particles in the liver cells were relatively few in number in both cytoplasm and nuclei. Most of the liver cells showed almost normal staining (Fig. 3d). In case of the kidney, marked decrease in the proteinic inclusions of the cells of the renal tubules and many of these cells appeared almost unstained (Fig. 4d).

Polysaccharides

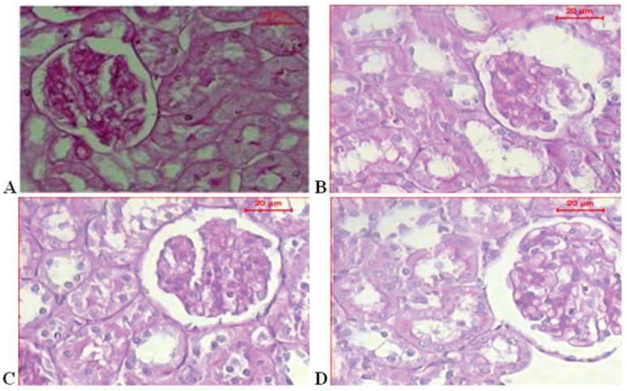
Examination of the liver and kidney sections of control rats showed the abundance of polysaccharide materials

Figure 5



Sections of liver of (a) control rat showing the abundance of glycogen in the cell of the hapatic lobule, (b) liver of rat daily receiving an oral dose of *Staphylococcus* bacteria shows the polysaccharides inclusions that displayed diffuse staining. A few number of the hepatocytes display dense staining, (c) liver of a rat on a daily, oral dose of *Salmonella* bacteria shows faint homogeneous staining of the polysaccharide inclusions, and (d) liver of a rat receiving a single oral dose of *Escherichia coli* bacteria shows marked depletion of the polysaccharide inclusions (PAS/H, scale bar 20 μm). PAS/H, periodic acid Schiff/hematoxylin.

Figure6



Sections of kidney of (a) control rat showing the normal distribution of the polysaccharide inclusions in the renal corpuscles, the basement membrane of the proximal and distal convoluted tubules, and the brush border of the proximal convoluted tubules, (b) rat on an oral dose of *Staphylococcus* bacteria shows marked diminution in PAS reaction in the basement membranes of the distal and proximal convoluted tubules and the brush borders of the proximal convoluted tubules and in the glomerulus, (c) rat on an oral dose of *Salmonella* bacteria shows heterogeneous staining of the cortical tissues. Notice the glomerulus displayed strong staining. The degenerated tubules are devoid of stainable materials and the healthy show strong reaction in their brush borders and basement membranes, and (d) rat on an oral dose of *Escherichia coli* bacteria shows a moderate and weak reaction of PAS-positive materials in the glomerulus and renal tubules, respectively (PAS/H, scale bar 20 μm). PAS/H, periodic acid Schiff/hematoxylin.

(glycogen) in the hepatocytes and glomeruli and the lining cells of the renal tubules. The nuclei gave negative result on PASs reaction, indicating the absence of polysaccharides (Figs. 5 and 6Figs. 5a and 6a).

Administration of oral dose of staph bacteria revealed diffuse stainability of the positive PAS materials of the hepatocytes. A few hepatocytes displayed dense stainability than did the others (Fig. 5b). Kidney sections showed marked diminution in PAS-positive materials in the basement membranes of the renal tubules and the brush borders of the proximal convoluted tubules. The glomeruli showed a picture, which was more or less similar to that obtained from the control rats (Fig. 6b).

In rats infected with *Salmonella* bacteria, faint homogeneous stainability of the polysaccharide inclusions in the hepatocytes was present (Fig. 5c). In the kidney, PAS-positive materials displayed weak stainability in the parietal and visceral layers of Bowman's capsules and the glomeruli. The polysaccharide inclusions in the tubules showed heterogeneous stainability where the degenerated tubule cells were weakly stained, whereas the necrotic ones were devoid of stainable materials. A moderate to strong reaction in the brush borders of the healthy cells was also observed (Fig. 6c).

In the liver of rats given an oral dose of *E. coli* bacteria, the polysaccharide inclusions showed marked depletion of the polysaccharide inclusions (Fig. 5d). Examination of the kidney showed moderate and weak PAS-positive material in the glomeruli and the renal tubules, respectively. Heterogeneous staining was encountered in the cells of the lining of the renal tubules; the degenerated cells were weakly stained, whereas the necrotic cells were devoid of stainable inclusions (Fig. 6d).

Discussion

The indicator bacteria level in drainage water is a common problem in rural areas that often leads to impairment of human consumption. Our bacteriological analyses revealed that drainage water was contaminated with coliforms and some pathogens like *E. coli*, *Salmonella* spp., and *S. aureus*. In this study, water samples were found to have high total viable bacterial counts (10^5 and 10^6 at 22 and 37°C, respectively). These counts were not in agreement with those obtained by Sabae and Rabeh [22]. Osman *et al.*[23] found that the counts were around 10^7 CFU/ml for both 37 and 22°C in the samples from the Damietta branch of the river Nile and from the Giza district, respectively. On the other hand, Ezzaat *et al.*[24] found that the log total viable bacterial counts at 22°C fluctuated between 10^3 and 10^6 CFU/ml and

from 10^4 to 10^6 CFU/ml at 37°C at 10 sites of the Rosetta branch.

Similarly, the findings of this study were in agreement with that of a study by El-Leithy [2], who demonstrated that the average values of total viable bacterial counts at 37 and 22°C were 1.0×10^5 and 1.4×10^5 CFU/ml, respectively, in the water samples collected from 10 sites along the Rosetta branch of the river Nile.

Findings of this study, investigating total and fecal coliforms, were nearly replicated in a study by Kumarasamy *et al.*[25], in samples collected from the Cauvery river, South India, in which they found that total coliforms were about 10^5/100 ml. On the other hand, the results in this investigation were higher than those obtained by Shash *et al.*[26], who reported that the total and fecal coliforms were detected in Nile water in Greater Cairo in 100% of the tested samples, reaching 10^4 and 10^3 CFU/100 ml, respectively. In addition, the results were lower than those obtained by Osman *et al.*[23], who found that in the samples from the river Nile in the Giza area, Egypt, the total and fecal coliforms were 10^4 and 10^2 MPN-index/100 ml, respectively. The results were in agreement with that of a study by El-Leithy [2], who reported the total and fecal coliform counts to be 5.8×10^4 and 3.3×10^4 MPN-index/100 ml, respectively, in the Rosetta branch of the river Nile.

Data demonstrated that, in Egypt, the total viable bacteria and coliforms bacteria at the mixing point between the El-Rahawey drain and the Rosetta branch were higher than that in other sites along the river Nile. This phenomenon is due to human activity. Moreover, Niemi and Niemi [27] reported that domestic and industrial wastewater and agriculture wastage are the chief sources of fecal bacteria into rivers. In addition, Geldreich *et al.*[28] reported that the outbreaks of water-borne diseases can be attributed to the consumption of *E. coli* O157: H7-contaminated water. In addition, Pant [29] reported that WHO estimates that 80% of all diseases in the world can be attributed to inadequate potable water supplies and poor sanitation. In Egypt, El-Jakee *et al.*[30] found that *E. coli* isolated from different water sources causes human and animal infections and some diseases common to both. Moreover, Khalifa and Sabae [31] reported that the Rosetta branch and the indicator bacteria of pollution exceeded the acceptable levels at some sites.

Moreover, in this investigation, the detected and enumerated fecal coliforms indicated the presence of pathogenic bacteria like *E. coli*, *Salmonella* spp., and *S. aureus*[5,32].

The obtained results about *S. aureus* are in agreement with those obtained by El-Taweel and Shaban [33], who estimated the count of *S. aureus* as 10^4 CFU/100 ml in raw Nile water samples from a branch point in Egypt. In addition, Mostafa *et al.*[34] detected and enumerated *S. aureus* at the Rosetta branch of the river Nile, and it flocculated between 10^2 to 10^5 CFU/100 ml. In a similar study, Yehia and Sabae [35] found that the density of total staphylococci in the Damietta branch at the end of the river Nile ranged from 10^4 to 10^6 CFU/100 ml. In addition, these results were in agreement with those obtained by Sayed [36], who counted *S. aureus* in water samples at the Roseatta branch of the river Nile during summer months, and found it to be 10^4.

In this respect, the results as regards *Salmonella* spp. are in agreement with those obtained by El-Leithy [37], who found that the count of *Salmonella* spp. ranged from 10^2 to 10^4 MPN-index/100 ml in the main river Nile along the sites in Cairo, from Helwan in the south to El-Glatma in the north. Moreover, in their study, El-Taweel *et al.*[38] reported the total count of *Salmonella* spp. to be10^3 MPN-index/100 ml in the Rosetta branch of the Rive Nile.

In Egypt, some villagers rear birds in the drains mentioned previously and consequently may be responsible for infecting the water with different types of microorganisms (like bacteria), as well as for the outbreak of diseases in the surrounding human population [39].

In the present study, the liver of the rats infected with the staph group, *Salmonella* spp. or *E. colli* showed a glass and eosinophilic cytoplasm, dilated sinusoids, lymphocytic infiltration in the portal and periportal areas, apoptosis in and necrosis of the hepatocytes. These results were confirmed by the results obtained by Pooneh *et al.*[40] in rats, and by Ajibade and Famurewa [41] in rabbits infected with *S. typhi* bacteria. Cater *et al.*[42] showed these changes with *E. coli*. In the liver, hepatocytes were swollen with decreased sinusoidal spaces, and widely distributed necrotic foci were seen [43].

In rats infected with *Salmonella* bacteria, degeneration or swelling of the renal tubules associated with foci of hemorrhage in the interstitium of the renal tubules were found in rabbits. There were few foci of tubular necrosis and presence of hyaline casts with interstitial

cellular infiltration by macrophages [41]. In addition, in a study by Ajibade and Famurewa, renal tubules showed extensive epithelial swelling with decreased lumen space and generalized necrotic changes with interstitial hemorrhage in the renal cortex [43]. On the other hand, no specific change in the kidney was observed in *E. coli*-infected broiler chicks [44].

The present study indicated that the administration of the used types of bacteria show a reduction in the polysaccharides and total protein contents in the liver and kidney of rats. These results were supported with histopathological results of the current study.

The mild diffuse inflammation that occurred in liver may be due to the endotoxin of bacteria, which leads to changes of the cells morphology [45]. The extensive epithelial swelling in renal tubules with decreased lumen space and necrotic changes with hemorrhage may be due to α and β – hemolysis that causes lysis of the renal cells [46]. The decrease in the sinusoidal spaces and focal necrosis may be due to *E. coli* infection as displayed in the hepatic cells in case of all bacterial infections [47]; moreover, there is hemorrhagic areas, which may be caused by intravascular hemolysis by toxin produced from *E. coli* causing vascular damage [42].

Conclusion

The oral administration of *E. coli*, *Salmonella* spp., and *S. aureus* isolated from polluted drainage water caused histological and histochemical alterations in the kidney and liver of rats.

Conflicts of interest

There are no conflicts of interest

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