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Isolation and Identification of *Escherichia coli* and *Staphylococcus aureus* from animal and detection of their antibiotics susceptibility pattern

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***Abstract*— Food-borne infections and diseases remain one of the major concerns of public health and food safety caused by a wide range of pathogens contaminating food and food products. *Staphylococcal* food poisoning is one of the most economically important foodborne diseases in public health programs worldwide while the most common and important species of Escherichia genus which consists of five species, these species that cause human infection. A total of 44 swab samples were collected from different animals (Cow, Buffalo, and Goat). These samples were collected from Al Qurnah farm, animal farm of Basrah veterinary collage. These samples were collected mainly from 4 locations in the animal body (nose, mouth, rectum, and vagina). The results indicates that the most E. coli isolation comes from the cows followed by goat and the most contaminated areas was mouth (100 %) followed by nasal cavity (90.9 %) , rectum (72%) and finally vaginal cavity (54.5 %) while the results of S. aureus infected revealed that high percentage , were rectal samples (90.9%); nasal samples; (81.8%)and (72.7%) for both vaginal and oral samples. All animals were infected approximately equally with S. aureus. Antibiotic susceptibility test for E. coli concluded that the bacteria were sensitive to (Clindamycin, Cefoxirim and Erythromycin) and were resistance to Vancomycin and Tetracycline While Antibiotic susceptibility result for S. aureus showed susceptibility to Clindamycin, and Tetracycline and complete resistance to Cefotaxime and Vancomycin.**

***Keywords*—E. coli, S. aureus, antibiotic, susceptibility, foodborne, nasal, rectal, cow, buffaloes, cot**

I. INTRODUCTION

Food-borne infections and diseases remain one of the major concerns of public health and food safety caused by a wide range of pathogens contaminating food and food products. The genus of Escherichia consists of five species: *Escherichia coli, Escherichia fergusonii, Escherichia hermanii, Escherichia vulneris and Escherichia blattae*. E.coli is the most common and important species of these species that cause human infection. E.coli is also subdivided into O, H, and K-based biotypes and serotypes. *E.coli* is a natural resident of all mammals ' large intestine. In carnivores and omnivores, it usually occurs more frequently than in herbivores. *E.coli* is excreted for weeks or months in fecal and fecal particles (Fratamico and Smith, 2006). In body sites such as mammary (mastitis) and uterine (metritis) glands, *E.coli* strains normally considered non-pathogenic may cause opportunistic infections. *E.coli* strain that cause enteritis have been classified as Enterotoxigenic E.coli (ETEC) which the fimbrial adhesions K88 , K99 or others. The production of these colonisation factors correlates with enterotoxin production. These strains cause the majority of cases of neonatal colibacillosis. Enteropathogenic (EPEC) strains don't appear to produce enterotoxins or shiga-like toxins but they can cause enteritis and diarrhea by other mechanisms. These strains have been recovered from lambs with diarrhea. Enter invasive (EIEC) stains adhere to cells of the distal small intestine; invade the enterocytes and deeper layers of the intestinal mucosa. They reach the lymphatic system where there is multiplication .The death of some *E.coli* cells occurs and endotoxin is released. The virulence factors such as capsules, adhesions, siderophores and alpha-haemolysins are important survival factors for these invasive strains which are responsible for colisepticaemia. Attaching and effecting *E.coli* (AEEC) strains colonies the small intestine, attach it target cells and kill them. The Shiga-like toxins (Vero toxins) destroy the microvilli by unknown meabs. These strains have been isolated from calves and rabbits with enteric disease (Kaper et al. 2004).

Staphylococcal food poisoning is one of the most economically important foodborne diseases in public health programs worldwide [Shimizu et al., 2000]. also found in food animals, and dairy sheep , goat and cattle especially if affected by subclinical mastitis, are likely contaminants of milk [Stewart, et al 2005]. Enterotoxins are also considered to be the major reason of food poisoning. They are associated with a form of gastroenteritis (Silva et al., 2001; Vandecasteele et al.., 2003). It is usually transferred to food and food products because of their poor handling, the growth of the bacterium is favored by protein-rich foods with high salt content,

which are often charged with *staphylococcal* food poisoning including meat and meat products, eggs and poultry products, fish and fish products if the contaminated food is stored at a temperature that promotes the growth of these organisms, enterotoxins are produced in the food and after food digestion it will spread rapidly in humans. *S. aureus* is able to grow in a various range of temperatures (optimum of 30º C), PH (4.2 to 9.3) with an optimum of to (7.5) and sodium chloride concentrations up to 15.(Shimizu et al., 2000). This study aims to investigate the presence of E. coli and *S. aureus* in animal samples and determine its susceptibility against several antibiotics.

II. MATERIALS AND METHOD

A. Samples collection and Bacterial isolation

All the samples were collected through period extended from November 2015 to January 2016. A total of 44 swab samples were collected from different animals (Cow, Buffalo, and Goat). These samples were collected from Al Qurnah farm, animal farm of Basrah veterinary collage. These samples were collected mainly from 4 locations in the animal body (nose, mouth, rectum, and vagina). Samples were collected and transferred immediately to the lab at 4°C and examined bacteriologically by culturing on both selective and differential media to determine the strain of the isolates. Each swab was enriched in nutrient broth and incubated at 37 °C for 18-24h. Swabs also were enriched in Tryptone soy broth (TSB) supplemented with 4mg/ L vancomycin and incubated at 37ºC for 18-24 h. For isolation of E. coli. there samples was taken and cultured in Eosin methylene blue (EMB) agar in which they give special metallic sheen, and MacConkey agar in which they give bright pink colonies. All suspected colonies were streak on the surface of pre-dried nutrient agar plates, in a manner which allowed well isolated colonies to develop. The inoculated plates were incubated at 37˚C for 24 h. Thus the pure culture obtained was used for primary identification.

The samples show positive results (turbidity in the broth) were streak on the surface of plated of mannitol salt agar and incubated at 37 ºC for 24 h for S. aureus selection. All suspected colonies from primary cultures for S. aureus were purified by subculture onto mannitol salt agar (MSA) medium and incubated at 37 ºC for 24- 48 h . (Talan et al., 1989)

B. Identification of E. coli and S. aureus

Smear of fresh culture swabbed on slid and stained with Gram stain, then examined under light microscope. All colonies appear on previous media were subjected for Api system identification.

C. Api Identification of E.coli:

The API (Analytab Products, Inc., Plainview, N. Y.) system consists of a 20 microtubes plastic strip containing dehydrated biochemicals. The strips were inoculated (with Muller Hinton agar's 24-h culture). Positive reactions were converted to a seven - digit profile number, identifying with the API Profile Index (fourth edition) and comparing with the identification of the diagnostic laboratories.

D. Api Staph system

The Api Staph (BioMerieux - france) is the identification system for Staphylococcus and Micrococcus. This test applied according to the company instructions. After checking the purity of tested bacteria, the isolate with 18-24 hr. growth was transferred to Api Staph Medium, mixed well to prepare a homogenous bacterial suspension with a turbidity equivalent to(0.5McFarland's standard).An incubation box prepared by distribution about 5 ml of tap water to wells of tray to create a humid atmosphere. By using micropipette, the micro tubes filled with inoculated medium, the tip of the pipette was placed against the side of the capsule (upper part) to avoid bubbles formation at the base of the tube. Filling only the lower part of tube, with the exception of the two tests of arginine hydrolysis and urease production (ADH, URE) which the lower part filled with bacterial inoculums and the capsules filled with mineral oil. Then the incubation box was closed by the lid and incubated at 37° C for 18- 24 h.

E. Antibiotic susceptibility test

This test was performed using Kirby et al., (1966) method. Pure colony transferred to clean tube containing 4 ml heart infusion broth and 4-5 h incubated at 37oC. After the swab in the broth culture had been moistened from the center to the border on the surface of the Muller-Hinton agar, the plate was left to dry for 2-5 min. The antibiotic disks were fixed on the plate (average 4-5 disks in each plate) using sterile forceps after 24 hour incubation at 37oC. The inhibition zone was measured against the special standard table.

III. RESULTS

A. Isolation of E. coli

A total of 44 swab samples were collected from different animals (cow, buffalo, and goat).These samples were collected mainly from 4 locations in the animal body (nose, mouth, rectum, and vagina). After culturing on TSB-Vancomycin. Using Gram stain, E.coli shows Gram-negative bacillus under light microscope. The isolates were screened on EMB agar in which they give special metallic sheen and on MacConkey agar in which they give bright pink colonies. Using of API 20 E system revealed that all isolates were identified as E. coli (figure 1).

Fig. 1: E.coli on Api 20 system

The results indicates that the most E. coli isolation comes from the cows (table 1) followed by goat (figure2) and the most contaminated areas was mouth (100 %)

followed by nasal cavity (90.9 %) , rectum (72%) and finally vaginal cavity (54.5 %) , (table 2)(Figure 3).

Table 1: Samples showing positive results for E. coli on Tryptone soy broth

Sample's type	Animals		
	cow	buffaloes	Goat
Vaginal	4	2	0
Rectal	4	2	2
Oral	4	3	4
Nasal	4	2	4
Total	16	9	10

Table 2: Summary of animal samples with its bacterial isolation of E. coli

Sample	Positive result
Vaginal	6/11 (54.5 %)
Rectal	8/11 (72%)
Oral	11/11 (100%)
Nasal	10/11 (90.9%)
Total	35/44 (79.5%)

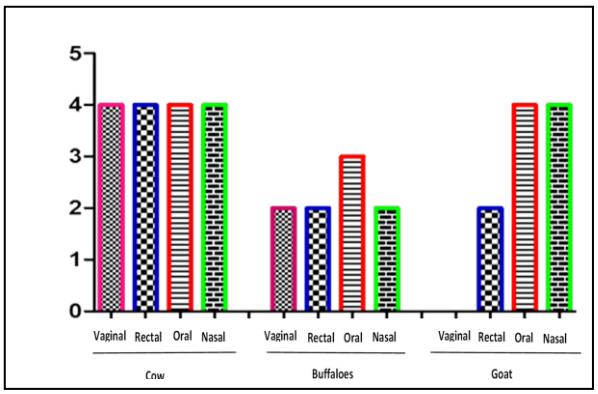

Fig. 2: The positive results of *E. coli* on Tryptone soy broth _Vancomycin

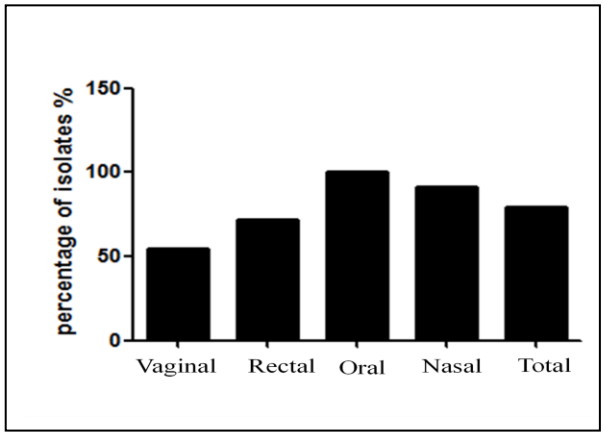

Fig. 3: The percentage of *E. coli* isolates of animal samples

B. Isolation of S aureus

By Gram stain, the smear of suspected colonies of S aureus showed grape like clusters or different irregulars shape Gram positive cocci. Using of API Staph System revealed that all suspected isolates were identified as S.aureus (figure4)

Fig. 4: *S.aureus* on Api Staph.

The results revealed that cow and buffaloes approximately equally infected with S. aureus (table 3, figure 5) and high percentage isolation gave positive result (able to grow) on mannitol salt agar were rectal samples (90.9%); nasal samples; (81.8%)and (72.7%) for both vaginal and oral samples and all animals were infected approximately equally with S. aureus (table 4)(Figure 6).

Table 3: Cow samples showing positive results for *S. aureus* on Mannitol salt Agar

Sample's type	Animals		
	cow	buffaloes	Goat
Vaginal	4	2	2
Rectal	3	4	3
Oral	2	3	3
Nasal	3	3	3
Total	12	12	11

Table 4: Summary of animal samples with its bacterial isolation of *S. aureus*

Sample	Positive result
Vaginal	8/11 (72.7 %)
Rectal	10/11 (90.9%)
Oral	8/11 (72.7 %)
Nasal	9/11 (81.8%)
Total	**35**/44 (79%)

S=sensitive :≥20 mm , M=moderate : 10-20 mm , and R=resistance: 9mm

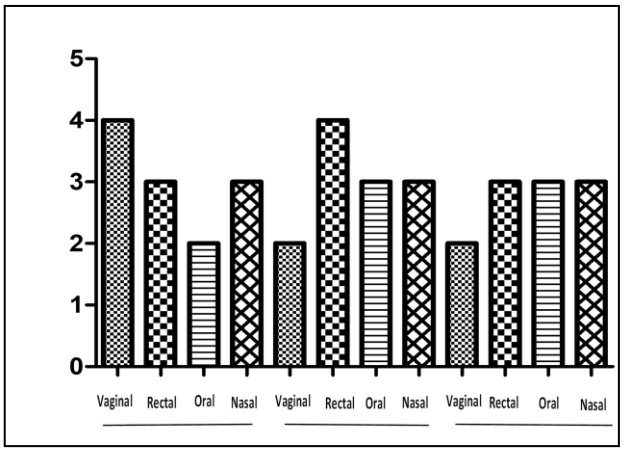

Fig. 5: *S. aureus* isolates gave positive result (able to grow) on mannitol

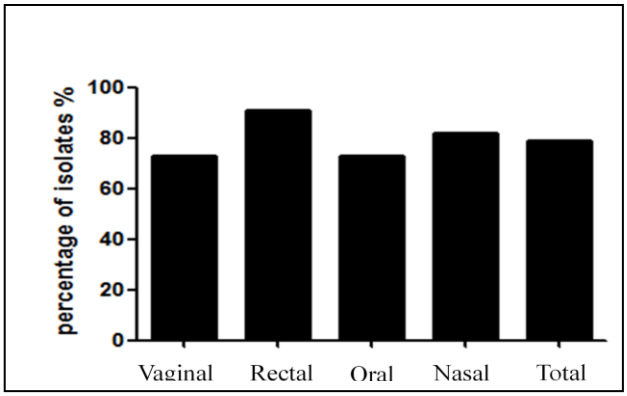

Fig. 6: The percentage of *S. aureus* isolates of animal samples

C. Antibiotic susceptibility test for E. coli

Antibacterial activity determination was carried out using the filter paper disc diffusion method (Figure7).The results of antibiotic susceptibility are shown in table 5. From the results it can be concluded that the bacteria were sensitive to (Clindamycin, Cefotaxime and Erythromycin) while gave moderate susceptibility to (Gentamycin and Streptomycin) and were resistance to Vancomycin and Tetracycline.

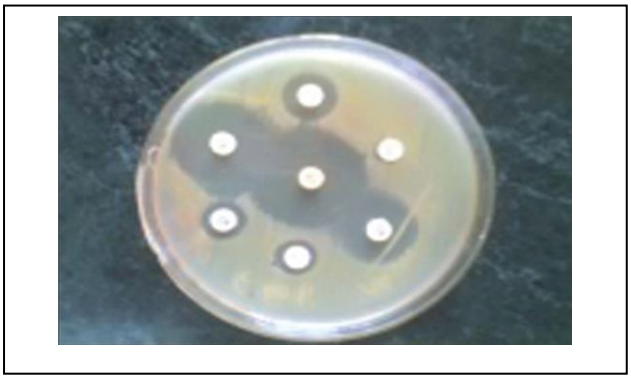

Fig. 7: Antibiotic susceptibility test of E coli isolates to several antibiotic disc

Table 5: Antibiotic susceptibility test results for E coli

Antibiotic (µg)	**Susceptibility (mm)**
Vancomycin (Van)	R
Gentamycin (Gen)	M
Clindamycin (Clin)	S
Streptomycin (St)	M
Tetracycline (Tet)	R
Cefoxirim (Cef)	S
Erythromycin (Ery)	S

D. Antibiotic susceptibility result for S. aureus

The isolates showed susceptibility to Clindamycin, and Tetracycline. The less susceptible results were showed to Gentamycin, Erythromycin, and Streptomycin, and complete resistance to Cefotaxime and Vancomycin as show in Table (6) and Figure (8).

Table 6: The results of antibiotic susceptibility test for S .aureus

Antibiotic (µg)	**Susceptibility (mm)**
Vancomycin(Van)	R
Gentamycin(Gen)	M
Clindamycin(Clin)	S
Streptomycin(St)	M
Tetracycline(Tet)	S
Cefotaxime(Cef)	R
Erythromycin(Ery)	M

S= sensitive: ≥20 mm, M= moderate : 10-20 mm , and R=resistance <9 mm

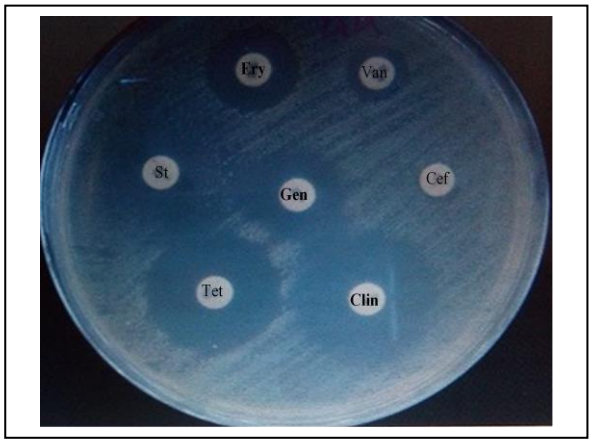

Fig. 8: Antibiotic susceptibility of S. aureus to different antibiotic

IV. DISCUSSION

E. coli is hemolytic and beta-forming bacteria. It usually ferments lactose on MacConkey Agar or produces pink colonies with precipitated bile salts surrounding areas (Mahon et al., 2007). It also presents on EMB agar with a green shine and produces tryptophan indole; it does not produce hydrogen sulfide and urease and cannot use citrate as the sole source of carbon (Mahon et al., 2007). The results indicates that the most contaminated areas was mouth (100 %) followed by nasal cavity (90.9 %), rectum (72%) and finally vaginal cavity (54.5%).Our result agree with J.M. Fairbrother & É. Nadeau (2006) and . Caprioli A et al(2005) when found the intestinal tract of ruminant species, including cattle, sheep, goats, pigs, water buffalo, are shed in their faeces a higher percentage rate of E coli and can contaminated their food or water and environment. (Jarvis and Martone (1992) recorded *E. coli* as the most commonly reported nosocomial pathogen in surveillance at some hospitals in the US. Pathogenic strains of *E. Coli* are responsible for three types of human infections: urinary tract infections, neonatal meningitis and intestinal diseases (Todar, 2008). Multiple antibiotic-resistant strains can be transmitted from animal to human by contaminated food. Carter et al., (1990) reported multiple resistant bacterial strains transmitted to humans by raw meat and milk. Cattle feces are a potential source of antibiotic resistant bacteria. If released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to human health (Chiuet al., 2004).Antibiotic resistance has become a major clinical and public health problem during the lifetime of most people (Levy,2002). Our study referred that *E coli* appeared resistant for some antibiotic and susceptibility for other. There are many reasons for this problem, one of which is an over use of antibiotics (Webster, 2002) in addition to the chromosomal changes or the exchange of the genetic material via plasmid and transposons which help in the transmission and spread of drug resistance. (Durso LM .et al 2011, and Levy, 2002)

Various samples from bovine (cattle and buffaloes) and ovine (goat) (healthy and infected) were collected in this study for isolation of *S. aureus* and the study of the properties of one of the most important pathogen in different major host samples and bordering microorganisms responsible for economic loss and public health problems. This microorganism was isolated in the present study rectum swab 90.9, nasal swab 81.8%, oral swab 72.7%, and vaginal swab 72.7%. The present results are in line with the results of many studies on animals. In AL- Kafaje, (2008), Mustafa, (2007) and AL- Marsomy (2008), report that the isolated S. aureus from clinical and subclinical mastitis in cows was the percentages of 53.33%, 43.5% and 46.24% respectively. Abd- AL- Rahman (1989) isolated S. aureus from camel in a percentage of 42.8%. That showed difference from the results of Ismaile (1986) who isolated S. aureus from different animals (29.88%) and from cows (22.29%).S. aureus isolates in this study were similar in some biochemical tests results with percentage of 100%.On the other hand the *S. aureus* isolates normal inhabitants of skin and mucus membranes, the source of contamination may be due to environment, milking utensils and the personals Kuehnert et al., (2006), Markham et al., (1966).This study revealed different percentage of susceptibility to different antibiotics; we find the isolates appeared susceptibility to Clindamycin and Tetracycline. Less susceptibility the results were showed to Gentamycin, Erythromycin, and Streptomycin, as well as resistance to Vancomycin and Cefotaxime. The present study was agreed with Shanmugam et al., (2008), who referred that sensitivity to Ciprofloxacin, Clindamycin and Oxacillin, and agreed with AL-Kafaji, (2008) to susceptibility to ciprofloxacin and Chloramphenicol, and agreed to (AL- Marsomy, 2008) who referred to the sensitivity to Ciprofloxacin, Chloramphenicol, Gentamycin, Streptomycin, and differ with Khan et al., (2007), who referred high resistance to Penicillin 23%, and Tetracycline 27%.

V. CONCLUSION

From this study, we conclude that cows, followed by goats, are the source of *E. coli* infection and the most contaminated areas were the mouth followed by the nasal cavity, rectum, and finally vaginal cavity. While the results of infected by *S. aureus* concluded that a high percentage, were rectal, nasal, and oral. Antibiotic susceptibility test for *E. coli* concluded that the bacteria were sensitive to (Clindamycin, Cefoxirim, and Erythromycin) and were resistant to Vancomycin and Tetracycline While *S. aureus* showed susceptibility to Clindamycin, and Tetracycline and complete resistance to Cefotaxime and Vancomycin.

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