

College: Veterinary Medicine

Student's Name: Raneem Abdulkareem Lafta

Dept.: Microbiology & Veterinarian Parasitology

Supervisor's Name: Dr. Bassam y. khudaier

Certificate: Master

Specialization: Microbiology

Title:

Molecular characterization of Metallo β -lactamases bla *IMP*, bla *VIM* and bla *SPM* in *Escherichia coli* O157:H7 and *Klebsiella pneumoniae* from Animals and Patients in Basra City

Abstract:

The goal of this study was to define the occurrence of *Tem* and *Shv* beta-lactamases genes (extended-spectrum β -lactamases) in *Escherichia coli* O157:H7 and *Klebsiella pneumoniae* that isolated from buffalo feces and children.

During a period of five months (August 2015 to December 2015), a total of 250 samples were collected 125 from hospitalized children suffering from diarrhea, and 125 from buffalo feces, samples collected from different regions in Basra city (Abo alkaseeb, alqurna, karmat ali, alzobeer). All specimens were screened for the presence of *E. coli* O157H7 and *Klebsiella pneumonia* by cultured on MAC, EMB and SMAC.

A total of 104 (59.4%) of suspected *E. coli* isolates were obtained : 42 from buffalo feces and 62 from children stool, while in *Klebsiella* obtained 71 isolates 38 from children and 33 from buffalo, All suspected isolates were tested biochemically.

E. coli isolates were screened on SMAC to detect NSFEC. 4 out of 42 from buffalo feces 9.5% and 6 out of 62 from children stool 9.6% were NSFEC.

All isolates were tested for their antibiotic resistance against 10 antibiotics by the Kirby - Bauer disk diffusion test. All the isolates were found to be resistant to at least 7 antibiotics to which they were subjected. Therefore, all these four isolates were considered to be multidrug resistant.

The *E. coli* O157:H7 β -lactamase-producing isolates were further examined by PCR technique using two pairs of primers to amplify both *Tem* and *Shv* genes. The results revealed that all (66.6%) of the bacteria that isolate from children were positive for *Tem* gene, (50%) from the bacteria that isolate from buffalo were positive for *Tem* gene, while in *Klebsiella* isolate that isolated from children 83.3% were positive to *Tem* gene and 76% of the same bacteria that isolated from buffalo were positive to *Tem* gene, and there is no positive result yielded for *Shv* gene.

Twenty randomly selected PCR products of *tem* gene of the isolated bacteria were evaluated by Clustalw multiple sequence alignment. The homology of *tem* gene was done with 3 other accession numbers that selected with the percentage of homology 98%, 98%, 99% in *E. coli* and in percentage of homology 99%, 99%, 99% in *Klebsiella*.