

## Isolate and diagnose the Gram-Negative bacteria from feces and bed of laboratory animal

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### Abstract

An attempt was made to isolate Gram-Negative bacteria from a total of 100 feces and beds voided by four species of laboratory rodents mice ,rats, guinea-pigs and rabbits (25 samples for each) was carried out by using selective as well as nonselective culture techniques , strains were tentatively grouped and presumptively identified on the basis of morphology and Gram stain , physiological and biochemical tests. The result showed (10 %) isolated of *Salmonella spp.* (66 %) *Enterobacter cloaca*, (24 %) *Proteus sp.* , *E. coli* (23 %), *Klebsiella* (11 %), *Pseudomonas* (3 %), *Acinetobacter* (18 %) and Nil of *Vibrio spp.* and *aeromonas* in 100 fecal sample of laboratory animal . Rigid , high husbandry standards and the screening of a new arrivals, existing animals and animal care personals will reduce the possibility of outbreaks, birds wilds rodents and contaminated feed must be excluded elimination of the infection from conventional colonies is extremely difficult, killing, disinfection and restocking are more practical approaches.

### Introduction

This present study of the occurrence of Gram-Negative bacteria in the intestinal tract of laboratory animal has been assessed for two reasons , first , the laboratory animals are used for pathogenicity studies with these organisms. Second the epidemiology of diseases involving Gram-Negative bacteria has been adequately determined and the role of laboratory rodents as a source of infection has been assessed (1). An examination of fecal swabs and beds microflora of mice, rats, rabbits and guinea-pigs was made with regard to the efficiency of several roll tube media in enumeration and recovery of anaerobes, the effects of medium constituents on recovery and isolation and identification of the

predominant kinds of bacteria (2). Predisposing factor causes some of the enterobacteriaceae bacteria cause outbreak in laboratory animal (3) which found *Citrobacter rodentium* that causes disease in laboratory animal , other researcher isolated *Citrobacter rodentium* from thickened colon and resulted in high morbidity and mortality in laboratory animal (4,5). Coliform bacteria, *Proteus sp.*, *Pseudomonas sp.* and *Bacteroides sp.* isolated by using selective media (6), *Salmonella spp.* isolated from Guinea-pig (7,8) and mice and rat (9,10,11,12,13) while (14) conclude that *Salmonella spp.* can be isolated from mice and it's no species specific and can infect variety animal.

### Material and methods

1. **Laboratory animals:** the experimental animals used in these experiments were rats, mice, rabbits and Guinea-pigs fecal samples and beds were taken.

2. **Collection of samples:** freshly voided fecal pellets were collected from laboratory mice, rat, guinea-pig and rabbit from April 2011 to February 2012 manually with a glove moistened with glycerol collected fecal material was placed in a plastic bag ,

closed tightly to exclude air , kneaded for a few minutes to mix the digest.

3. **Isolation:** a fecal pellet was suspended in brain heart infusion broth , incubated at 37°C in 24 hour after that one loopfull transfer in Nutrient and Blood agar and incubated at 37°C in 24 hour then transfer the colony on selective media such as MacConkey, XLD , Manitol salt agar and alkaline peptone water and subculture on TCB agar (15).



4. Characterization of isolates: strains were tentatively grouped and presumptively identified on the basis of morphology Gram stain, physiological and biochemical tests for

catalase production, gelatin liquefaction, oxidase, sugar fermentations, H<sub>2</sub>S production were performed as by (16).

### Results

The results of this work indicates that satisfactory isolation and cultivation of the predominant microflora in Rats, Rabbits, Mice and Guinea-pigs the result summarized the incidence of the most commonly encountered intestinal bacteria can be obtain many different kind of enterobacteriaceae in different percentage from 100 fecal sample, 25 sample for each kind of laboratory animal ( mice, rat, rabbit and Guinea-pig) when strict anaerobic culture. The following enteric bacteria were identified from fecal and beds samples in Rat, Rabbit, Mice and Guinea-pigs in

frequency that were positive for fecal coliforms *Enterobacter cloaca* (56%), (64%), (96%) and (44%), *E. coli* (20%), (28%), Nil in Mice and (44%), *Klebsiella spp.* (16%), (28%), Nil in Mice and Guinea-pigs, *proteus spp.* (20%), (8%), (20%) and (48%), *Salmonella spp.* (6%), (24%) and Nil in Mice and Guinea-pigs, *pseudomonas aeruginosa* (8%), Nil in Rabbit, (4%), and Nil in Guinea-pigs, and *Acintobacter* (12%), (28%), (32%) and Nil in Guinea-pigs, *Enterobacter cloaca* (56%), (64%), (96%) and (44%) and nil the growth of *vibrio* and *aeromonas* this result as shown in table (1).

Table(1) illustrated kind of enterobacteriaceae obtained from laboratory animal feces.

Bacteria	Rat (25 sample)	Rabbit (25 sample)	Mice (25 sample)	Guinea-pigs (25 sample)	Total percentage
<i>Enterobacter cloaca</i>	56%	64%	96%	44%	66%
<i>proteus</i>	20%	8%	20%	48%	24%
<i>E. coli</i>	20%	28%	Nil	44%	23%
<i>Acintobacter</i>	12%	28%	32%	Nil	18%
<i>Klebsiella</i>	16%	28%	Nil	Nil	11%
<i>Salmonella spp.</i>	6%	24%	Nil	Nil	10%
<i>Pseudomonas aeruginosa</i>	8%	Nil	4%	Nil	3%
<i>Alkaligenes fecalis</i>	4%	Nil	Nil	Nil	1%
<i>Vibrio</i>	Nil	Nil	Nil	Nil	Nil
<i>Aeromonas</i>	Nil	Nil	Nil	Nil	Nil

### Discussion

This is the first report of the isolation of Gram negative bacteria from feces of laboratory animals in Iraq, the results obtained indicate that different bacterial isolated depend on type of experimental animals there were marked variations in proportions of bacterial types isolated from the feces (2) and different of diet or nutritional deficiency, genetic predisposing factor of these animals (18) the majority of

samples contained *Enterobacter cloaca* and *E. coli* were the predominant fecal coliform species isolated and member of genus *salmonella*, *proteus*, *pseudomonas*, *Klebsiella*, *Citrobacter* (19) so the presence of Enterobacteriaceae in the feces of animal indicates the contamination of bed or animal house with these organisms (20) that understanding that the primary source of these bacteria in natural or environmental of



animal house has been regarded as the gastrointestinal tract of warm-blooded animals. Fecal samples contain a large variety of bacteria using conventional culture technique, only a small percentage of bacterial flora is identified (21) *Acintobacter* which is the first isolate in this study agreement with (22) and disagreement with (23) which was showed the infected of immune depression animals, but *Klebseillawas* isolated in this study that agree with (20) the salmonella isolate in rabbit 24% and rat 6% that disagree with (24) that called rabbit uncommon infected but rat and mice susceptible because the rabbit not laboratory and different in environment and feed. The pseudomonas isolate followed in rat 8% but mice 4% that disagree with (25) that called BALB miceresistant to *pseudomonas* infection the reason of it in this study immune system is

very develop in BALB mice to this infection (26) the end isolate *alkaligenesfecalis* in rat 4% also in this study search about vibrio and *aeromonas* and other enterobacteriaceae not found it may be discussion the reason of type of animal, different of environmental or deficiency of food (19). To decrease the outbreak which may be occur as a result of person to person spread of infection, through ingestion of infected food or contaminated water and from direct contact with laboratory animal like person deals with these animal or veterinarian (17) well contaminated with feces should be disinfected with liquid bleach, through flushed to remove bleach residue and retested due to potential for regrowth of bacteria in distribution lines these should also be disinfected and cleared period to retesting (19).

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