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Isolation of Bacterial contamination agents in imported animal feed in Iraq

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Abstract
This study assessedin order to feed safety evaluationby determining bacterial contamination of imported animal feed in Iraq, because it causes animal diseases that often causing death and great economic losses.
Six types of feeds (A total of 300 samples) were collected and analyzed, the results showed that no relatively bacterial contamination was however detected by the methods used in (163) samples at (54.3%),whereas occurrence of contamination regard to Enterobacteriaceae family (Gram-negative bacteria) was in (137) samples at (45.7%) for 12 bacterial species as it shown in table (1 and 2), therefore we concluded that must be assessed the microbial quality of imported animal feed by manufacturers and health authorities to ensure feed safety.

Keywords: Bacterial contamination, animal Feed, *Escherichia coli*, *Salmonella* spp., *Proteus* spp. *Shigella spp*. *Klebsiella sp.*

Introduction
The feed safety is a great concern in developed countries because it is an essential requirement for animal health. Unsafe feed may causes great economic losses because of destroying an infected flock of animals. (Nahid Ahmed, 2002) [1] Feeds to meet the complex nutrient requirements of animalsare formulated from both animals and plants sources and are mostly agro wastes.
The normal flora of the feed which obtained from more than one environmental sources such as dust, soil and insects, cross contamination and water quality are important factor form icroorganisms presence in the feed, therefore probiotics could be deliberate incorporation of these (Lactic acid bacteria) into the feed (Osaro Matthew *et al*, 2017) [2].
Feed processing type and storage conditions are important factors thathave an effect on the population levels and species of pathogens in the feed. Since long time has been known that infections agents can be transmitted to animals by feed contamination. (Gangarosa, E. J., 1973) [3]
Feed materials(raw materials and finished products) may be contaminated with pathogens during growing, harvesting, processing, mixing of ingredients, by handling, transport and storage.(Onyeze R. *et al* , 2013) [4]. Animal diseases and death occur as a result of consumption of contaminated feed.

Materials and Methods
Preparation of media used
The media were prepared according to the manufacturer's instructions on the media labels and autoclaved at 121°C for 15 min.

Isolation
A total of 300 samples were collected. For *Salmonella* isolation, 25g from feed sample was pre-enriched in 225ml peptone water, mix and incubated, 1ml was transferred to 9ml of **S**elenite Cysteine Broth and incubated at 37°C for 24hours. After the incubation period, differential media; Xylose lysine Deoxycholate agar, Hektoen enteric agar were streaked and incubated at 37°C for 24hours. *Salmonella* colonies were subcultured on Nutrient Agar and incubated at 37°C for 24hours. Purification was achieved by sub-culturing and *Salmonella* suspect colonies were picked up for biochemical tests and agglutination test. (Holt JG, 1994) [5] For isolation of *Escherichia coli* used Eosin Methylene Blue agar (EMB), Nutrient agar and macconkey agar.

Identification of the Isolates
Identification of isolates by API kit (remel one rapid system).

Statistical analysis
It carried by Microsoft Excel (2010) to find STDEV. P

Results and Discussion
This study was conducted in order to determine and investigate the Bacterial contamination to ensure health and welfare of animal via feed safety (one of requirements of Animal Health Organization (OIE)) to ensure human health by consumption of safe animal products(one of requirements of World Health Organization (WHO)), which meaning feed safety is necessary to achieving food safety because of bacterial contamination of these pro ducts is an entry pathway of pathogens into the human food supply.(Maciorowski, K. G. *et al*, 2009) [6]
Six types of feeds (A total of 300 samples) were collected and analyzed ,the results showed that no relatively bacterial contamination was however detected by the methods used in (163) samples at (54.3%),this could be attributed to increased antibiotics concentration in these feed samples, or collected in proper storage of feed Supplies (Madaki Chat *et al*, 2019)[7] Where asoccurren ceofcontamination regard to Enterobacteriaceae family (Gram-negative bacteria) was in (137)samples at (45.7%) as it shown in table (1 and 2). The

results in Table (1) shows the distribution of isolates according to sample types was as following: poultry feed (12%), fish feed (9 %), dog feed (3%), bird feed (4%), corn (8.3%) and soya bean meal (9.7%).

There was no significant association (P= 0.1, p >0.05) between feed sample types.

This indicated that samples of poultry feed, fish feed, soybean meal and corn are "critical feed material" among other feed ingredients, because these samples were rich source of nutrient for microbial growth especially when the environmental conditions are favorable.

Table (2) shows number of bacterial species was isolated from each feed type. The organisms were: *Salmonella* spp. (17) isolates at (12.4%), *Escherichiacoli* (31) isolates at (22.6%), *Klebsiella* spp. (18) isolates at (13.1%), *Shigella* spp. (12) isolates at (8.8%), *Proteus* spp.(9) isolates at (6.6%),*Pseudomonas* spp. (8) isolates at (5.8%), *Citrobacter* spp. (5) isolates at (3.6%) , *Pantoea* spp. (15) isolates at (10.9%), *Yersinia* spp. (7) isolates at (5.1%), *Acinetobacter* spp. (10) isolates at (7.2%),*providencia* spp. (6) isolates at (4.4%), *Enterobacter* spp. (1) isolates at (0.7 %), There was no significant association (P= 0.06, p >0.05) .

The presence of these pathogens may suggest fecal contamination as well as environmental contamination due topoor hygiene, especially the presence of rodents that could be responsible for spreading of *Salmonella* species, which it well known as pathogens of birds and farmed animal. The finding in this study shown (17) samples which contaminated with *Salmonella* spp. (17) isolates at (12.4%) were rejected because it does not fulfill

the Iraqi specification and European Union limit of *Salmonella* contamination also because a high nutritional value of soybean meal we observed thissoy bean meal samples were the most contaminated with *Salmonella* spp. among other material, butit were absentin fish feed addition to dog and bird feed samples.

*Escherichiacoli*was higher frequently contamination rat comparing with other pathogens because its prevalence rate was (22.6%), as it known it is an indicter to fecal contamination especially feces of infected rodents in feed (Ashida H *et al*, 2011) [8] which causing a human infection via consumption of animal food products.

Our study reported prevalence rate of *Klebsiella* spp. was (13.1%), study by (Maung S. Myint *et al*, 2007) [9]. reported corn plants in the field could serve as a reservoir for *Klebsiella pneumonia*and it may infect plants, animals and probably humans. Whereas prevalence rate of *Shigella* spp. (8.8%), which is a public health significance because it can cause human–poultry cross-infection as well as(10)(Run Shi *et al*, 2014) [10] was suggest that.

Proteus spp. prevalence at (6.6%), as it known *Proteus* spp. iscausing acute and chronic infections in all or most types of birds and animals, This organism can survive and multiply at refrigerator temperatures and in a wide range of pH, hence even a small amount of contamination may besignificant (11) (Aisha Danbappa *et al*,2018) [11].

Pseudomonas spp. recorded prevalence rate at (5.8%), that is least than study by (12)(Satish and Priti, 2015) who reported *Pseudomonas* spp. prevalence rate at (12.5%)and *Pseudomonasaeruginosa* causing an infection in both, man and animals

The finding were *Citrobacter* spp. prevalence at(3.6%) in fish feed only, this may be suggest to contamination of water used in processing with *Citrobacter* spp. which it was considered the intestinal inhabitants of human and animals, and commonly existed in sewage, water (Honglian Chen *et al*, 2018) [13] Prevalence rate of *Pantoea* spp. (10.9%),which it recognized as a plant pathogen and commonly found in water, soil, seeds and foodstuffs, as well as reported as opportunistic pathogens of animals and humans and may cause severe and occasionally fatal infections.(Jalal Mardaneh *et al*, 2013)[14]

Prevalence rate of *Yersinia* spp. (5.1%) in poultry feed only this may be suggest to contamination of water used in the manufacturing processes with this microbe,

(Aulisio *et al*., 1983) [15] Prevalence rate of *Acinetobacter* spp. at (7.2%), its presence associated with environmental conditions and it emerging opportunistic bacteria for aquaculture in different parts of the world.(16) (Dadar M. *et al*, 2016) [16] Prevalence rate of *providencia* spp. at (4.4%) in fish feed only, as it known it prevalence in animal feces (Huiling DI. *et al*, 2018) [17] Prevalence rate of*Enterobacter* spp. at (0.7 %) in soya bean meal only, which is a lower percentage compared with the other bacteria, *Enterobacter cloacae*is hospital-acquired and it contributes to the following ailments: bacteremia, endocarditis, septic arthritis, osteomyelitis, skinof tissue infections, and lower respiratory tractinfections (Leticia Amoakoah - Twum *et al*, 2019) [18].

In the finding of this study, the occurrence of contamination was in (137) samples at (45.7%), higher compared to the results of Brenner *et al*, (2010) [19] who reported an occurrence rate of bacterial contamination (17%) from different feed samples in Nigeria, this indicate some feed business operators are still ignoring the rules of good practices of producing, manufacturing, hygiene at the primary production stage and transporting which lead to occurred an contamination of feed materials

Conclusion

We conclude from these results, some samples analyzed in this study showed the presence of pathogenic microorganisms, which is a health hazard for animals and humans through contaminated food of animal origin, therefore it is necessary to evaluate feed safety by bacteriological examination of imported animal feed to ensure safety and quality of feed to prevent animals diseases and great economic losses.

Table 1: Distribution of Isolates According to Sample Types.

	Feed type	No. of feed samples collected	No. of Positive samples (%)	No. of negative samples (%)	Total isolates
1	Poultry feed	50	36(12%)	14(4.7%)	36
2	Fish feed	50	27(9%)	23(7.7%)	27
3	Dog feed	50	9(3%)	41(13.7%)	9

4	Bird feed	50	12(4%)	38(12.7%)	12
5	Corn	50	25(8.3%)	25(8.3%)	25
6	Soybean meal	50	29(9.7%)	21(7%)	29
	Total	300	137(45.7%)	163(54.3%)	137

STDEV. P= 0.1

Table 2: Number and percentage for each bacterial species.

Feed type	*Salmonella* spp.	*E. coli*	*Klebsiella*	*Shigella*	*Proteus*	Pseudomonas	Citrobacter	Pantoea	Yersinia	Acinetobacter	providencia	Enterobacter	Total isolates
Poultry feed	5	9	2	3	-	2	-	-	7	8	-	-	36
Fish feed	-	7	-	5	2	-	5	-	-	2	6	-	27
Dog feed	-	-	2	-	-	-	-	7	-	-	-	-	9
Bird feed	-	-	-	4	-	-	-	8	-	-	-	-	12
Corn	4	5	9	-	7	-	-	-	-	-	-	-	25
Soybean meal	8	10	5	-	-	6	-	-	-	-	-	-	29
Total isolates	17	31	18	12	9	8	5	15	7	10	6	1	137
%	12.4	22.6	13.1	8.8	6.6	5.8	3.6	10.9	5.1	7.2	4.4	0.7	

STDEV. P= 0.06

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