ISSN: 2356-7767

The role of lesser grain borer in transmission of pathogenic bacteria to poultry and animal feed

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Received in 5/1/2016
Accepted in 17/2/2016

Abstract

Insects have been considered as the most diverse species for animals living on earth. The majority of insects were directly related to humans, animal and to the environment. The goal of the present study was to detect the role of lesser grain borer as stored product insect in harboring and potentiating virulent bacteria. The study included both whole and homogenized form of insect for microbial detection where *S. equi, Streptococcus pyogenes, S. aureus, S. epidermidis, Salmonella, E. coli* and Enterococcus were identified. The positive PCR results obtained for eqbe, smeZ, clfA, gseA, invA, phoA and 16SrRNA genes were capable to confirm the isolation of these microbes, respectively. The virulence profile of the isolated bacteria was strengthened by the positive results encountered for the different virulence genes tested by PCR. The isolated bacteria were resistant to almost tested antibiotics.

Key words: *Lesser grain borer, Enterococcus, E. coli, Salmonella, Staphylococcus.*

Introduction

Stored-product insects are cosmopolitan in distribution **(Hagstrum and Subramanyam, 2009)** and are adapted to infest raw and processed cereal products, posing a constant threat to stored-food commodities worldwide. Numerous stored-product insect species are associated with stored-grain elevators, feed mills, and retail stores **(Larson *et al.*, 2008a)**. These pests cause significant quantitative and qualitative losses to the multibillion dollar grain, food, feed, and retail industries each year through their feeding and product adulteration. In addition, several stored-product insects had been reported to harbor potentially pathogenic bacteria. For example, the darkling beetle, *Alphitobius diaperinus* (Panzer), a cosmopolitan general stored-product pest from poultry brooder houses was reported to contain *Salmonella spp., Escherichia coli, Campylobacter spp.* **(Templeton *et al.*, 2006)**, *Micrococcus spp., Streptococcus spp., and Bacillus subtilis* **(De Las Casas *et al.*, 1972)**. *Escherichia intermedia, Proteus rettgeri, Proteus vulgaris, B. subtilis, Serratia marcescens, Streptococcus spp., Micrococcus spp.,* and members of the *Klebsiella–Aerobacter* group were isolated **(Harein and De Las Casas, 1968)**. However, very few studies **(Channaiah *et al.*, 2010)** have addressed the importance of stored-product insects carrying bacteria.

The main objective of this study was to determine the prevalence, number of microorganism per insect, and potentially virulent bacteria associated with stored product insects collected from a feed-manufacturing facility, grain storage silo, and retail store.

Materials and Methods

Samples:

Lesser grain borer derived from sex different feed mills located at Giza Governorate were aseptically collected, transported in sterile plastic bags to the laboratory for microbial and molecular examination.

Microbial examination

The Lesser grain borer was cultivated according to **Kamelia *et al.* (2014)** as whole and homogenized form with sterile enrichment broth [lactose broth for *E. coli*, Rappaport Vassiliadis soy peptone (RVS) broth for Salmonella, and peptone water for *Staphylococcus spp.*, peptone water with 5% serum for *Streptococcus spp.* and Tryptic soya broth for mycotic examination) and a loopful was subcultivated on selective medium [Eosin methylene blue agar (EMB) for *E. coli*, xylose lysine deoxycholate (XLD) agar for Salmonella, Mannitol salt agar for Staphylococcus, Blood agar for Streptococcus, Sabouraud dextrose agar (SDA) for mycotic cultivation]. Single colony was selected from each isolate for further biochemical analysis according to **Bergey's Manual of Systematic Bacteriology (2005).**

Antimicrobial susceptibility tests

The antimicrobial sensitivity was determined by standard agar disc diffusion technique in accordance with the Clinical and Laboratory Standards Institute **(CLSI, 2008).** The panel of antibiotic disks (Becton, Dickinson and Company) were used in the panel screens belonged to certain drug classes ranked by **FAO, OIE and WHO (2003)**. The antimicrobial agents tested and their corresponding concentrations were shown as follows in Table (4).

Molecular detection

DNA purification: DNA extraction from bacterial cultures was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH).

PCR amplification: Different primers used in PCR were supplied from Metabion (Germany) and Biobasic (Canada) and are listed in Tables (1) and (2). A 25- µl master mix reaction containing 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara, Japan), 1 µl of 20 pmol conc. of each primer, 4.5 µl of water, and 6 µl of the DNA template. The reactions were performed in Applied biosystem 2720 thermal cyclers. Each PCR product was loaded in a separate well in 1.5% agarose gel. The PCR photos were photographed and analyzed using a gel documentation system (Alpha Innotech, Biometra, Germany) through its computer software (BioDocAnalyze 2.64.8.1).

Results and Discussion

Microbial detection

The digestive tract of adult insects provides an ideal microclimate for bacterial growth and development **(Dillon and Dillon, 2004)** and several authors have also reported acquisition, retention, and transmission of bacterial pathogens in adult stored-product insects **(Larson *et al.*, 2008a)**. The study summarized that the whole form of lesser grain borer harbored no microbial agents, while the enriched sample of homogenized form of lesser grain borer revealed metallic sheen colony of *E. coli* after cultivation onto EMB, Salmonella red colonies with black centers onto XLD, Staphylococci yellow colony onto Mannitol salt agar and tiny colony with variable hemolysis onto blood agar. A single colony from each isolate was biochemically identified that revealed the detection of *E. coli*, Salmonella, *S. aureus, S. epidermidis*, *Enterococcus* and *Streptococcus species* as (Table 3). These isolates were confirmed by PCR. No mycotic growth could be obtained in both whole and homogenized forms of lesser grain borer.

Table (1). Sequences and cycling conditions of the different used PCR primers for amplification of *E. coli*, Salmonella, *S. pyogenes* and *S. equi* genes.

Gene	Bacterial agent	Primers sequences (5'-3')	Amplified segment (bp)	Amplification (35 cycles)			Reference
				Secondary denaturation	Annealing	Extension	
eaeA	***E. coli***	GACCCGGCA-CAAGCATAAGC	384	94˚C 45 sec.	54˚C 45 sec	72˚C 45 sec	**Wen-jie JIN *et al.*, 2008**
		CCACCTGCAG-CAACAAGAGG					
iss		ATGTTATTTTCTG CCGCTCTG	266	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	**Yaguchi *et al*., 2007**
		CTATTGTGAG-CAATATACCC					
iutA		GGCTGGACATGGG AACTGG	300	94˚C 30 sec.	63˚C 30 sec.	72˚C 30 sec.	
		CGTCGGGAACGG GTAGAATCG					
phoA		CGATTCTGGAAAT GGCAAAAG	720	94˚C 45 sec.	58˚C 40 sec.	72˚C 45 sec.	**Hu *et al*.,2011**
		CGTGAT-CAGCGGTGACTAT GAC					
tsh		GGTGGTGCACTGG AGTGG	620	94˚C 45 sec.	54˚C 45 sec.	72˚C 45 sec.	**Delicato *et al*., 2003**
		AGTCCAGCG TGA-TAG TGG					
invA	***Salmonella***	GTGAAAT-TATCGCCACGTTC GGGCAA	284	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	**Oliveira *et al*., 2003**
		TCATCG-CACCGTCAAAGGA ACC					
avrA		CCT GTA TTG TTG AGC GTC TGG	422	94˚C 30 sec.	58˚C 30 sec.	72˚C 30 sec.	**Huehn *et al*., 2010**
		AGA AGA GCT TCG TTG AAT GTC C					
sopB		TCA GAA GRC GTC TAA CCA CTC	517	94˚C 45 sec.	58˚C 45 sec.	72˚C 45 sec.	
		TAC CGT CCT CAT GCA CAC TC					
mgtC		TGACTAT-CAATGCTCCAGTG AAT	677	94˚C 45 sec.	58˚C 45 sec.	72˚C 45 sec.	
		ATTTACTGGCCGC TATGCTGTTG					
stn		TTGTGTCGCTAT-CACTGGCAACC	617	94˚C 1 min.	59˚C 1 min.	72˚C 1 min.	**Murugkar *et al*.,2003**
		ATTCG-TAACCCGCTCTCG TCC					
Pef		TGTTTCCGGGCTT GTGCT	700	94˚C 55 sec.	55˚C 55 sec.	72˚C 55 sec.	
		CAGGGCATTTGCT GATTCTTCC					
SmeZ	***S. pyogenes***	TTTCTCGTCCTGT GTTTGGA	246	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	**Borek *et al*., 2012**
		TTCCAAT-CAAATGGGACGG AGAACA					
eqbe	***S. equi subsp. equi***	ATGTAGCTATGGC AAATGTGGC	110	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	**North *et al*., 2014**

Table (2). Sequences and cycling conditions of the different used PCR primers for amplification of different genes of *S. aureus*, *S. epidermidis* and *E. faecalis*.

Bacterial agent	Gene	Primers sequences (5'-3')	Amplified segment (bp)	Amplification (35 cycles)			Reference
				Secondary denaturation	Annealing	Extension	
S. aureus	***clfA***	GCAAAATCCAGCACAACAGGAAACGA CTTGATCTCCAGCCATAATTGGTGG	638	94˚C 1 min.	55˚C 1 min.	72˚C 1 min.	**Mason *et al.*, 2001**
	hlg	GCCAATCCGTTATTAGAAAATGC CCATAGACGTAGCAACGGAT	937	94˚C 1.5 min.	55˚C 1.5 min.	72˚C 1.5 min.	**Kumar *et al.*, 2009**
	mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	**McClure *et al.*, 2006**
	Coagulase	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	Four different types of bands may be detected 350 430 570 630	94˚C 1 min.	55˚C 1 min.	72˚C 1 min.	**Iyer and Kumosani, 2011**
	spa	TCA ACA AAG AAC AAC AAA ATG C GCT TTC GGT GCT TGA GAT TC	226	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	**Wada *et al.*, 2010**
	Sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	**Mehrotra *et al.*, 2000**
	Seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	
	Sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	
	Sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTTCGTTC	278	94˚C 30 sec.	48˚C 30 sec.	72˚C 30 sec.	
	See	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	
S. epidermidis	***gseA***	ATGAAAAAGAGATTTTTATCT GTTTGGTGACACTCTTAAG	503	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	**Ikeda *et al.*, 2004**
E. faecalis	**16SrRNA**	GTTTATGCCGCATGGCATAAGAG CCGTCAGGGGACGTTCAG	310	94˚C 30 sec.	60˚C 30 sec.	72˚C 30 sec.	**Zoletti *et al.*, 2006**

Table (3). Prevalence of microbial population isolated from feed mills.

Microbial population	No. of Microbial population / feed mills (n=6)
E.coli	1/6 (16.6%)
Salmonella	1/6 (16.6%)
S.aureus	3/6 (50%)
S.epidermidis	1/6 (16.6%)
Enterococcus species	1/6 (16.6%)
Streptococcus species	4/6 (66.6%)

Table (4). Antibiotic susceptibility for different isolates.

Antibiotic	*E. coli*	Salmonella	*S. aureus*	*S. epidermidis*	*Enterococcus species*	*Streptococcus species*
Amoxicillin (AX) 25 mg	R	R	R	R	R	R
Nalidixic acid (NA) 30 µG	R	R	R	R	R	R
Flumoquine (UB) 30 µG	R	R	R	R	R	R
Tetracycline (T) 30 µG	R	R	R	R	R	R
Gentamycin (CN) 10 µG	R	R	R	R	R	R
Colistin sulphate (CT) 10 µG	S	S	R	R	R	R
Streptomycin (S) 10 µG	R	R	R	R	R	R
Penicillin 10 µG	R	R	R	R	R	R
Enrofloxacin (ENR) 5 µG	R	R	R	S	R	R
Norofloxacin (NX) 10 µG	R	R	R	R	R	R
Danofloxacin (DO) 30 mg	R	R	R	R	R	R
Ciprofloxacin (CF) 5 µG	S	S	R	R	S	R
Ampicillin (Amp) 10 µG	R	R	R	R	R	R

The result of antibiotic susceptibility testing declared that all the tested strains were resistant to almost all used antibiotics.

Molecular identification of isolates:

The 1st PCR step aimed to confirm the results of bacteriological examination, followed by the testing for different virulence genes of Salmonella, *E. coli* and *S. aureus*. The results of different genes are shown in Tables (5, 6 and 7) and Photos (1 and 2).

Table (5). Confirmatory PCR results of the bacterial isolates:

Strain	NO. of isolates	Gene	PCR Result
S. equi	2	*eqbe*	-
S. pyogenes	2	*SmeZ*	+
S. aureus	3	*clfA*	+
S. epedirmidis	1	*gseA*	+
Salmonella	1	*invA*	+
E. coli	1	*phoA*	+
E. faecalis	1	*16SrRNA*	+

The *eqbe* gene which is specific for *S. equi subsp. equi* was not detected in the lesser grain borer insect which indicates that the isolated strain is *S. equi subsp. zooepidermicus*. **Bisgaard *et al.* (2012)** reported that this bacterium has caused an outbreak and 80% mortalities in 11,000 free range chickens and when pulsed field gel electrophoresis was done, the isolate was identical to that isolated from horses. The infection with *S. zooepidemicus* in layer chickens has been proven to induce hemostatic alterations including hyperfibrinogenaemia, prolonged PT, and hypercoagulability **(Roy *et al.*, 2014).**

The positive PCR result of the glutamic acid-specific serine protease (*gseA* gene) was capable of the affirmation of the *S. epidermidis* isolate which is considered harmless because it is coagulase-negative and poor protein A producer as reported by **Jensen *et al.*, (1987)**. This was assured by **Kawano *et al.*, (1996)** who isolated *S. epidermidis* from the nares and skin of healthy chickens**. Scanlan and Hargis, (1989)** consider *S. epidermidis* to be opportunistic in chickens as they have isolated *S. epidermidis* from cases suffering from dermatitis and tenosynovitis.

clfA gene which encodes for the primary fibrinogen-binding protein as mentioned by **Mason *et al.,(* 2001)** was used as the verifying gene of the coagulase positive *S. aureus***.** The gene was positively amplified in the three *S. aureus* isolates**.** The three *S. aureus* isolates were considered too virulent as they harbored *hlg*, *spa* and *coagulase* virulence genes.

Table (6). PCR results of the different virulence genes of the *S. aureus* isolates.

Staph isolates	*hlg*	*mecA*	*spa*	*coagulase*	*Enterotoxins*				
					Sea	*Seb*	*Sec*	*Sed*	*See*
S. aureus	+	+	+	+	-	-	-	-	-
S. aureus	+	+	+	+	-	-	+	-	-
S. aureus	+	+	+	+	-	-	-	-	-

The *hlg* contains two synergistically acting proteins (hlgA and hlgB) that undergo their action on erythrocyte after initial binding of *hlgB* followed by *hlgA* and subsequent generation of a pore **(Dickinson and Bisno, 1993)**. Moreover, the protein A (encoded by spa gene) is one of the most important virulence factors as it prevents antibody mediated immune clearance of the organism through binding to the Fc receptor of IgG. Also, protein A interferes with the phagocytosis of opsonized bacteria via binding IgG **(Murray *et al*., 2002)**.

Coagulase is used as a marker for the virulence of *S. aureus*. The speculative role of coagulase in the pathogenesis of disease is the formation of a fibrin layer around a focal staphylococcal abscess, which leads to the localization of the infection and protecting the organism from phagocytosis **(Sawai *et al*., 1997)**.

All the tested *S. aureus* isolates were positive for *mecA* gene by PCR. This may increase the risk to the humans who might be infected by MRSA through feeding on these birds as reported by **Lee *et al*., (2003)** who isolated three MRSA strains from chickens, and to determine the ability of these strains to infect human, RAPD PCR was applied and the results showed that their genome was very closely related to some human strains considering these isolates may be a possible source of human infections caused by consuming contaminated food products.

Only one isolate out of the three *S. aureus* strains showed positive PCR results for one of the enterotoxins (particularly enterotoxin E). This result agreed with those of **Murray *et al*. (1998)** who reported that 30% to 50% of all *S. aureus* strains producing an enterotoxin. The detection of this entrotoxin is so threatening as they are resistant to hydrolysis by gastric and jejuna enzymes and are heat stable at 100°C for 30 minutes making the Staphylococcal food poisoning the leading cause of food-borne microbial intoxication worldwide **(Holmberg and Blake, 1984)**.

The streptococcal mitogenic exotoxin Z (smeZ) gene was detected confirming the isolation of *S. pyogenes* (Photo 2) which represents the group A streptococci (GAS). The presence of this gene aids the microbe to produce a range of super antigenic toxins which is so important in the pathogenesis of invasive streptococcal infections. The possible infection of the birds through lesser grain borer can be so dangerous and may lead to zoonotic illness in the meat handlers in the chicken abattoir or in the meat processing plants. Actually, this occurred in 1978 in a chicken factory in Yorkshire, England where 82 workers (mostly in the packing department) were infected with *S. pyogenes* through skin lesions with attack rate of 44% **(Barnham *et al*., 1980).** *S. pyogenes* infection in human can cause several disease illnesses from which the most known are puerperal fever and scarlet fever.

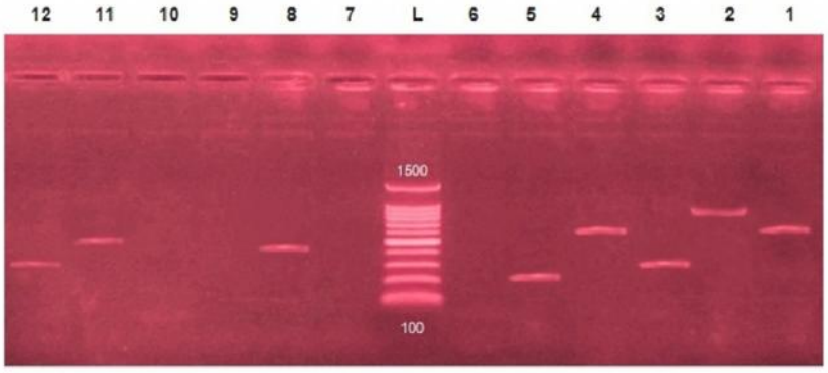

Photo (1). PCR results for the different *S. aureus* virulence genes, *S. epidermidis gseA* gene and *E. fecalis16SrRNA* gene. lanes 1 - 12 showing: *S. aureus* virulence genes: 1) *clfA* (638 bp), 2) *hlg* (937 bp), 3) *mecA* (310 bp), 4) *coagulase* (630 bp), L [Gelpilot100 bp plus ladder (Qiagen, 100-1500 bp)], 5) *spa* (226 bp), 6) *Sea*, 7) *Seb*, 8) *Sec* (451 bp), 9) *Sed*, 10) *See*, 11) *S. epidermidis gseA* (503 bp) and 12) *E. fecalis16SrRNA* (310 bp).

Several clinical disease problems could rise due to *E. faecalis* infection in poultry as endocarditis, systemic amyloidosis in chickens and hepatic granulomas in turkeys. In ducklings, it can cause arthritis and septicaemia. In the current study, a specific 310 bp of the *16SrRNA* gene of the *E. faecalis* was amplified by PCR to assure the transmission of *E. faecalis* by the lesser grain borer**. Lakshmikantha *et al.* (2006)** studied the association of Enterococci with stored products and stored-product insects and reported that the Lesser grain borer had the heighest incidence of Entorococci isolation. They Isolated 67 enterococci strains from lesser grain borer and upon further screening and identification by multiplex PCR, they were confirmed as *E. faecium* (23.2%), *E. gallinarum* (16.2%) and *E. casseliflavus* (60.4%). In a later study, **Lakshmikantha *et al.* (2010**) applied laboratory experiments to determine the survival of *E. faecalis* OG1RF:pCF10 in poultry feed and its acquisition and transmission by adults of *Tribolium castaneum* (Herbst) to sterile feed. *E. faecalis* survived in poultry feed during the7-day test period. *E. faecalis* persisted on the surface and within *T. castaneum* adults for 7 days when adults were released on *E. faecalis*–inoculated poultry feed.

The conserved 284 bp of the invasion gene (*invA*) recommended by **Olivera *et al.* (2003)** was detected to affirm the isolation of the Salmonella *invA* gene which exists in the majority of Salmonella strains and is related to intestinal mucosa invasion. **Crumrine *et al.* (1971)** made a design of an experimental study to determine the ability of seven stored products insects including lesser grain borer to transmit *Salmonella Montevideo* from experimentally contaminated wheat to clean wheat. The study disclosed the ability of all of the studied insects including lesser grain borer to the initial sample of clean wheat but not into a second or third sample. Although the progeny of lesser grain borer developed in the contaminated grain couldn't transmit Salmonella, other three insects could transmit Salmonella to clean grain.

The Salmonella isolate was positive for four genes out of the five tested virulence genes (Table 7, Photo 2) indicating the riskiness of this isolate. In addition to that *stn* gene that encodes enterotoxin is a putative virulence factor and causative agent of diarrhea **(Chopra *et al.*, 1999)**, it is believed to have functions in the maintenance of membrane composition and integrity via regulation of OmpA membrane localization **(Nakano *et al.*, 2012)**. The positive result of the *pef* gene by PCR increases the risk of the isolated Salmonella as it encodes for the pef fimbriae. The Pef fimbriae mediate adhesion of Salmonella to the intestinal epithelium, which results in fluid accumulation (**Baumler *et al.*, 1996)**. The rule of pef fimbriae in the formation of biofilm was studied by **Ledeboer *et al.* (2006)** when the pef defective mutants were reported to lack the biofim formation on three tested surfaces.

Also, the *avrA* gene positive PCR result augments the virulence profile of the isolated Salmonella as it encodes for an effector protein of the TTSS (type III secretion system) complex that minimizes the host's inflammatory responses by the induction of cell apoptosis, especially of macrophages, and by the inhibition of pro inflammatory cytokines as IL-8 and TNF-α **(Ben-Barak *et al.* 2006)**.

The *SopB* protein is regarded as a novel bacterial enterotoxin **(Prager *et al.*, 2000)** encoded by the *SopB* gene. It is one of the important TTSS and associated with enteritis **(Wallis and Galyov, 2000)**. *SopB* is an inositol phosphate phosphatase that hydrolyzes several inositol phosphates resulting in high cellular level of Ins(1,4,5,6)P4 which induces electrolytes and fluid secretion and recruits the polymorphnuclear (PMN) cells in Salmonella infected intestinal mucosa **(Norris *et al.*, 1998)**.

As regards to the tested *E. coli* isolate, the 720 bp of the alkaline phosphatase (*phoA*) gene was amplified by PCR. *PhoA* gene is a housekeeping gene present in all *E. coli* strains (Table 7, Photo 2). The *eaeA* gene positive PCR result indicates the ability of the isolated *E. coli* to attach epithelial cells. The *eaeA* gene

encodes for intimin protein which is considered as a bacterial adhesion molecules that lead to the emersion of the A/E lesions **(Kilici *et al*., 2007)**.

E. coli isolate was confirmed to have the *iss* gene by PCR. *Iss* (increased serum survival gene) is considered as a promising virulence gene that is usually associated with the *Avian pathogenic Escherichia coli* (APEC) strains. **Lynne *et al*. (2007)** studied the role of *iss* gene when they developed an iss- mutant where a significant drop in *E. coli* resistance to serum was observed. A bor- mutant also showed a drop in serum resistance but the drop in serum resistance was more violent in iss- mutant which indicates that *iss* contributes more to serum resistance than bor in the *E. coli* strains. This effect was assured when the level of serum resistance was restored after the *iss* was reintroduced into the iss- mutant.

The virulence of the isolated *E. coli* was affirmed by the positive detection of the *tsh* (temperature-sensitive hemagglutinin) gene which is an auto transporter protein secreted by avian-pathogenic *E. coli* strains that colonize the respiratory tract and lead to airsacculitis, pericarditis, and colisepticemia **(Kostakioti and Stathopoulos, 2004).**

Table (7). PCR results of the different virulence genes of *Salmonella* and *E. coli* isolates.

Virulence genes								
Salmonella isolate					***E. coli* isolate**			
mgtC	***avrA***	***sopB***	***pefA***	***stn***	***eaeA***	***tsh***	***iutA***	***iss***
-	+	+	+	+	+	+	-	+

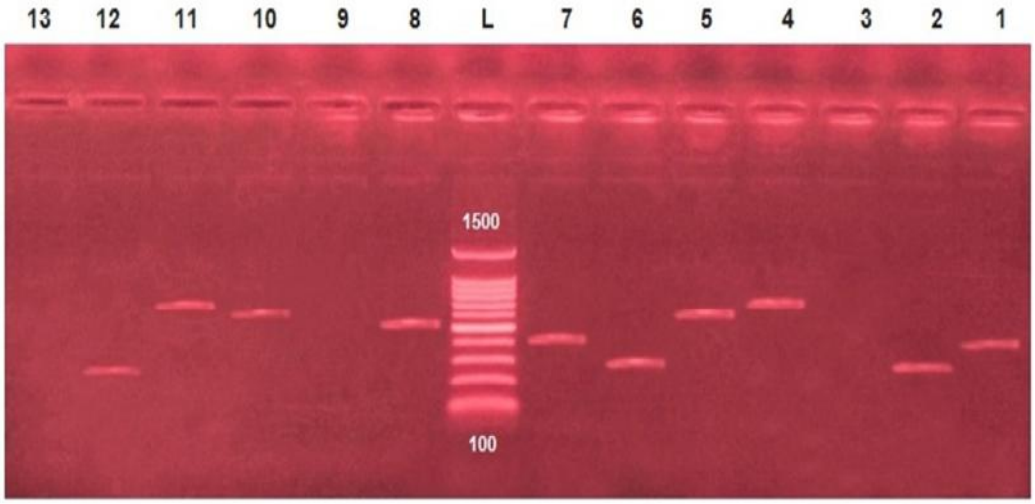

Photo (2). PCR results for the different *E. coli and* Salmonella virulence genes, *S. pyogenes* smeZ gene and *S. equi* eqbe gene. lanes 1 - 13 showing: *E. coli* virulence genes 1) *eaeA* (384 bp), 2) *iss* (266 bp), 3) *iutA*, 4) *phoA* (720 bp), 5) *tsh* (620 bp), L [Gelpilot100 bp plus ladder (Qiagen, 100-1500 bp)], *Salmonella* virulence genes 6) *invA* (284 bp), 7) *avrA* (422 bp), 8) *sopB* (517 bp), 9) *mgtC*, 10) *stn* (617 bp), 11) *pef* (700 bp), 12) *S.pyogenes smeZ* (246 bp) and 13) *S. equi* subsp. equi *eqbe*.

Research has shown that these insects can disperse at least 1000 m from a common release site **(Jia *et al*., 2008),** and so it is considering a major vector for transmission of many bacteria from feed mills to another.

Conclusion

The exposed findings provide evidence that stored-product insects harbor potentially virulent bacteria which may transfer into final feed and thus causing a major health problem for animal and poultry industries. The current data reinforces the need for preventing the infestation of grains with lesser grain borer as well as an integrated pest management to reduce the availability of insect vectors, particularly stored-product insects, in the feed mill and livestock environments.

Acknowledgment

This search was funded by the Reference Lab. For Veterinary Quality Control On Poultry Production (RLQP) and by the Microbiology department of the faculty of veterinary medicine, Cairo university.

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دور حفار الحبوب فى نقل االميكروبات الممرضة لاعلاف الدواجن والحيوانات

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باحث بالمعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى* ، مدرس بقسم الميكروبيولوجى بكلية الطب البيطرى – جامعة القاهرة** ، أستاذ مساعد بقسم الصحة كلية الطب البيطرى – جامعة القاهرة***

الملخص العربى

اعتبرت الحشرات من الأنواع الأكثر تنوعا للحيوانات التي تعيش على الأرض. معظم الحشرات لها علاقة مباشرة بالإنسان والحيوان والبيئة. وكان الهدف من هذه الدراسة الكشف عن دور حشرة حفار الحبوب كحشرة تعيش على الحبوب المخزنة في إيواء البكتيريا الفتاكة. وشملت الدراسة كلا من الحشرة الكاملة والمتجانسة للكشف عن الميكروبات حيث تم الكشف عن الميكروب السبحى من النوع الخيلى و الميكروب السبحى من النوع الصديدى و الميكروب العنقودى الذهبى و من نوع إبيديرميدس و ميكروب السالمونيلا و و إيشريشيا كولاى و إنتيروكوكاس. و كانت النتائج الإيجابية لاختبار تفاعل البلمرة المتسلسل لجينات eqbe ، smeZ ، clfA، gseA، invA، phoA، و ١٦SrRNA كافية لتأكيد عزل هذه الميكروبات على التوالى. وقد تم تعزيز ضراوة الميكروبات المعزولة من خلال النتائج الايجابية لجينات الضراوة باختبار تفاعل البلمرة المتسلسل. كما كانت البكتيريا المعزولة مقاومة لمعظم المضادات الحيوية المختبرة.