Cairo University
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Genotypic characterization and toxigenic potential of *Bacillus* species isolated from different sources

A Thesis Presented
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Under The Supervision Of

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Abstract

In this study a total number of 225 retail meat and offal samples (156 chicken meat and offal samples and 99 beef meat samples) were collected from tow governorates (Giza and Qalubia) for isolation of *Bacillus* spp samples were obtained either from butchers shops (chicken and local beef meat) and supermarkets (frozen imported meat) for one year. The meat samples consisted of 156 chicken samples (65 breast meat, 48 thigh meat, 29 livers and 14 hearts), 59 local beef meat (44 meat cut and 15 minced meats) and 40 imported frozen beef meat. *Bacillus* spp isolates were 48.6% from total samples (124 isolates), divided into 66 (42.3%) from chicken samples (26 from breast, 24 from thigh, 10 from liver and 6 from heart) and 58 (58.6%) from beef meat samples (23 from local meat cut ,7 from local minced meat and 28 from imported frozen meat cuts). Results of virulence assay showed that the majority of *Bacillus* isolates showed slime formation (63.7%) and biofilm formation (84.7%).The prevalence of the hemolytic activity of *Bacillus* spp isolates showed the total β-hemolysis activity of *Bacillus* spp reached 77.4% while the α- hemolysis activity and γ– hemolysis reached 16.1% and 6.5%respectively. *Bacillus* spp isolates showed the total Lecithinase activity of *Bacillus* spp reached 53.2%. The prevalence of production of α-amylase *Bacillus* spp isolates showed the total hydrolysis 87.1% and all isolates of *Bacillus cereus* having ability to starch hydrolysis except one isolates with 96.3%. The cytotoxic activity of 94 *Bacillus* spp isolates supernatant fluid indicated 100% cytopathic effect on the Vero cell line with different degree 46.8%, 41.5% and 11.7% for (+++), (++) and (+) respectively. The highest percentage of resistance against examined antimicrobial agents was recorded in penicillin 100%, resistance against Cephalothin, Oxacillin, Sulfamethazole /Trimethoprim, and Nalidixic acid were 95.2% 94.4%, 77.4%, and 62.9% respectively. for genotypic detection of different distribution of virulence factors gene of isolated *Bacillus* spp revealed that the amplification for *CytK, nheA, nheB, nheC, plc, entFM, hblA, hblC* and *hblD* gene were 58.9%, 33.9%, 13%, 25%,40.3%, 35.5%, 45.2%, 29.8% and 20% respectively. Each isolates regardless of their origin harbored at least one of the enterotoxin. genes indicating their pathogenic nature, which must be considered as serious health hazard.

Dedication To

My mother

…… My father

……..My sister

…….My brother

…… Dr. Mahmoud Sharawy

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AHLs	Acyl-homoserine lactones
AMPs	Antimicrobial peptides
API	Analytical profile index
ATP	Adenosine triphosphate
BceT	Enterotoxin-T gene
C	Capsule
CAN	Columbia agar with nalidixic acid and colistin
CDC	Cholesterol-dependent cytolysin
CerAB	Cereolysin AB
ces	Emetic toxin gene
CFU	Colony forming unit
CLO	Cereolysin O
CLSM	Confocal laser scanning microscopy
CRA	Congo red agar method
CTM	Christensen's tube method
cytK	Cytotoxin K gene
CytK 1	Cytotoxin K
CytK 2	Cytotoxin K
ent FM	Enterotoxin FM
Epr	Extracellular protease
EPS	Exopolymeric substances
Fnr	regulatory gene for fumarate and nitrate reduction
hblA	Hemolysin BL A gene
hblC	Hemolysin BL C gene
hblD	Hemolysin BL D gene
hlyII	Haemolysin II
hlyII	Haemolysin II gene
HlyIII	Haemolysin III
HlyIII	Haemolysin III gene
hlyIIR	Haemolysin II regulators
m-DAP	*meso*-diaminopimelic acid
nheA	Non hemolytic enterotoxin A
nheB	Non hemolytic enterotoxin B gene