Detection of Some Virulence and Resistance Genes of *S. aureus* and *B. cereus* Isolated from Some Meat Products

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Abstract: A total one hundred forty (140) random samples of minced meat, sausage, chicken breast and chicken liver (35 for each) obtained from retail outlets were screened bacteriologically for the occurrence of *S. aureus* and *B.cereus*. A total of 14/140 (10%) isolates of *S. aureus* and 16/140 (11.42%) isolates of *B. cereus* were recovered. The isolated *S. aureus* were highly resistant for erythromycin (90%) followed by amoxicillin-clavulanic, cefotaxime and doxycycline (60% for each), gentamicin and vancomycin (50% for each) and ciprofloxacin (30%). Meanwhile, *B. cereus* were highly resistant for amoxicillin-clavulanic and cefotaxime (100% for each) followed by ciprofloxacin, erythromycin and vancomycin (80% for each) and gentamicin (20%). Polymerase chain reaction (PCR) was applied on *S.aureus* to detect staphylococcalenterotoxins (*sea, seb, sec, sed, see*) none of tested isolates harbored these genes and resistance genes *bla*Z, *mec*A and *van*A which were detected by a percentage (100%, 100%, 0%) respectively. Meanwhile, *B.cereus* were screened for detection of toxin genes *hbl*, *nhe* and *ces* which were detected by a percentage (20%, 100%, 0%) respectively. While resistance genes *tet* A, *bla* and *erm*A were detected by a percentage (100%, 100%, 0%) respectively. In conclusion, The results suggest that meat and poultry products represent threat to public health through transmission of enterotoxigenic and antibiotic resistant *S.aureus* and *B.cereus.*

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1. Introduction

Food-borne diseases represent a serious threat to public health all over the world **(Jay, 2005).** It have become a major public health problem worldwide due to the significantly increased incidence of food borne diseases over the last 20 years **(Oliver et al., 2005**). The most common pathogens which are responsible for most food borne disease outbreaks are *L.monocytogenes, E.coli* O157: H7, *S. aureus, B. cereus, Vibrio spp., C.jejuni, C.perfringens,* and Shiga toxin-producing *E. coli* (STEC) **(Zhao et al., 2014).**

S. aureus are Gram-positive cocci ranging from 0.5 to 1.5 µm in diameter, which may or may not contain a polysaccharide capsule, non-motile, non-spore forming (**O'Riordan and Lee, 2004).**

Staphylococcal food poisoning (SFP) is an intoxication that results from the consumption of foods containing sufficient amounts of one (or more) preformed enterotoxin (**Le Loir et al., 2003**). *S.aureus* can grow without change in odour or taste of the food and producing heat-stable enterotoxins which lead to food poisoning **(Plaatjies et al., 2004).** Symptoms of SFP have a rapid onset (2–8 h), and include nausea, violent vomiting, abdominal cramping, with or without diarrhea (**Murray,2005**).

B. cereus is a Gram-positive, aerobic-to-facultative, spore-forming rod widely distributed environmentally (**Ash et al., 1991).**

B. cereus is considered one of the most important causes of food poisoning in the world **(Per and terje, 2006)** due to its ability to release two core toxins, a heat-labile diarrheal enterotoxin and heat- stable emetic enterotoxin (**Stenfors et al., 2008).** The diarrheal syndrome manifested via the release of one or three diarrheal enterotoxins: the tripartite toxins hemolysin BL (HBL) and non-hemolytic enterotoxin (Nhe), the two forms of cytotoxin K (cytK-1 and cytK2) and possibly enterotoxin T and enterotoxin FM while emetic type is due to the production of heat-stable emetic toxin (cereulide**) (Granum, 2001; Moravek et al., 2006).**

The objective of this study was to apply bacteriological and molecular studies on *S. aureus* and *B.cereus* isolated from minced meat, sausage, chicken breast and chicken liver.

2. Materials and Methods

2.1. Samples

A total of 140 random samples of meat products (minced meat, sausage, chicken breast and chicken

liver) (35 for each) were collected from different retail outlets. The collected samples were transferred directly in an ice box under complete aseptic conditions for bacteriological examination.

2.2. Bacterial isolation and identification of *S.aureus* and *B.cereus*

The detection and identification of *S.aureus* were performed according to **(APHA, 2001; Quinn et al., 2002)** using nutrient broth as enriched and Baird Parker agar, Mannitol salt agar **(Oxoid)** as a selective media. Meanwhile *B.cereus* were identified according to **(APHA, 2001; Sandra & Tallen, 2012)** using brain heart infusion broth (BHIB) as enriched and polymyxin – pyruvate - egg yolk – mannitol - bromothymol blue agar (PEMBA) as a selective media **(Oxoid).**

2.3. Antimicrobial susceptibility testing:

The obtained bacterial isolates were tested in vitro for their susceptibility to the following antimicrobial discs: amoxicillin-clavulanic (AMC) 30µg, cefotaxime (CTX) 30µg, ciprofloxacin (Cip) 5 µg, Erythromycin (E) 15µg, gentamicin (CN) 10 µg, doxycycline (DO) 5µg and vancomycin (VA) 30µg according to (**Koneman *et al.,* 1997)** and the degree of sensitivity was interpreted according (**NCCLS, 2002; NCCLS, 2016).**

2.4. Detection of virulence and resistance genes of S.aureus and B.cereus

2.4.1. Extraction of DNA:

DNA was extracted from the isolated *S.aureus* and *B.cereus* using QIAamp DNA mini kit. It was applied on 5 random isolates. PCR Master Mix and cycling conditions of the primers during PCR was prepared according to Emerald Amp GT PCR mastermix (Takara) kit. Oligonucleotide primers used in PCR have specific sequence and amplify a specific product **(table,1)**. DNA samples for uniplex PCR were amplified in a total of 25µl as follows: 12.5µl of Emerald Amp GT PCR mastermix, 1µl of each primer of 20 pmol concentrations, 4.5 µl of grade water and 6 µl of template DNA. Meanwhile, for enterotoxins multiplex PCR, DNA samples amplified in 50µl as follows 25µl of Emerald Amp GT PCR mastermix, 1µl of each primer of 20 pmol concentrations, 7µl of grade water and 8 µl of template DNA. The reaction was performed in a Biometra thermal cycler. Temperature and time conditions of the primers during PCR were applied. Aliquots of amplified PCR products were electrophoresed in 1.5 % agarose gel (ABgene) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of PCR products were loaded in each gel slot. A 100 bp DNA ladder (QIAGEN Inc, Valencia, CA, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Table (1): Designing of primers used for detection *S.aureus and B.cereus* enterotoxins and resistance genes

Target M.O	Target Gene	Primer sequence (5'-3')	Length of amplified product	Reference
S.aureus	*Sea*	F- GGTTATCAATGTGCGGGTGG R- CGGCACTTTTTTCTCTTCGG	102	Mehrotra et al., (2000)
	Seb	F- GTATGGTGGTGTAACTGAGC R- CCAAATAGTGACGAGTTAGG	164	
	Sec	FAGATGAAGTAGTTGATGTGTATGG R- CACACTTTTAGAATCAACCG	451bp	
	Sed	F-CCAATAATAGGAGAAAATAAAAG R- ATTGGTATTTTTTTTCGTTC	278bp	
	See	F- AGGTTTTTTCACAGGTCATCC R- CTTTTTTTTCTTCGGTCAATC	209bp	
	mecA	F- GTA GAA ATG ACT GAA CGT CCG ATA A R- CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al*., (2006)
	blaZ	F- ACTTCAACACCTGCTGCTTTC R-TGACCACTTTTATCAGCAACC	173bp	Duran et al., (2012)
	vanA	F- CATGACGTATCGGTAAAATC R- ACCGGGCAGRGTATTGAC	885bp	Patel *et al*., (1997)
B.cereus	*Ces*	F- GGTGACACATTATCATATAAGGTG R-GTAAGCGAACCTGTCTGTAACAACA	1271	Ehling-Schulz et al., (2006)
	Nhe	F- AAG CIG CTC TTC GIA TTC R- ITI GTT GAA ATA AGC TGT GG	766bp	
	Hbl	F- GTA AAT TAI GAT GAI CAA TTTC R- AGA ATA GGC ATT CAT AGA TT	516bp	
	tet A	F- GGCGGTCTTCTTCATCATGC R- CGGCAGGCAGAGCAAGTAGA	502bp	Rather et al., (2012a)
	Bla	F- CATTGCAAGTTGAAGCGAAA R- TGTCCCGTAACTTCCAGCTC	680bp	Chen et al., (2004)
	ermA	F- TCTAAAAAGCATGTAAAAGAA R- TTCGATAGTTTATTAATATTAGT	652bp	Adimpong et al., (2012)

3. Results

3.1. Incidence of *S.aureus and B.cereus.*

According to phenotypic and biochemical identification, 14/140 (10%) *S.aureus* isolates were isolated from minced meat, sausage, chicken breast and chicken liver samples with the isolation rates of 11.42%, 5.71%, 14.28% and 8.57% respectively. Meanwhile, 16/140 (11.42%) *B. cereus* isolates were isolated from minced meat, sausage, chicken breast and chicken liver samples with the isolation rates of 20%, 17.14%, 5.71% and 2.85% respectively.

3.2. Antimicrobial susceptibility of the tested isolates:

Results of antibiotic sensitivity test showed that 90% of tested *S.aureus* isolates exhibited resistance against erythromycin, 60% against amoxicillin-clavulanic, cefotaxime and doxycycline, 50% against gentamicin and vancomycin and 30% against ciprofloxacin. Meanwhile, 100% of tested *B.cereus* isolates showed resistance against amoxicillin-clavulanic and cefotaxime, 80% against ciprofloxacin, erythromycin and vancomycin, 70% against doxycycline and 20% against gentamicin.

3.5. PCR results

3.5.1. Detection of enterotoxin and resistance genes in S. aureus

S. aureus isolates were examined for detection of staphylococcal enterotoxins (*sea, seb, sec, sed, see*) by multiplex PCR. None of the examined isolates harbored these genes. Also these isolates were tested for the detection of *bla*Z, *mec*A and *van*A genes by uniplex PCR. The results revealed that all the tested isolates 100% harbored *bla*Z and *mec*A while *van*A failed to be detected.

3.4. Detection of enterotoxin and resistance genes in B.cereus:

B.cereus isolates were examined for the detection of virulence genes by uniplex PCR. *Nhe* gene was detected in all the examined samples 100% while, *hbl* gene was detected in 20% of the examined samples. On the other hand *ces* gene failed to be detected. Also *B. cereus* was examined for the detection of resistance genes by uniplex PCR. *Tet* A and *bla* genes were detected in all the examined samples 100% while none of the examined samples harbored *erm* A.

4. Discussion

The incidence of *S.aureus* in the present study was *(*10%). Similar results were obtained **by EI-Jakee et al., (2013)** (12.8%), Conversely, this result is lower than that obtained by **Abdalrahman et al., (2015)** (53.8%). *S.aureus* was isolated from minced meat, sausage, chicken breast and chicken liver samples at an incidence of 11.42%, 5.7%, 14.28% and 8.57% respectively. These results were not different from other studies reported by **Hanson et al., (2011)** (17.8%) for chicken breast and **Dewedar et al., (2016)** (7%) for chicken liver, while higher results were obtained by **Abd El Tawab et al., (2018) (24%)** for minced meat**, Shylaja et al., (2018)** (53.33%) for sausage, **Abdul Ameer (2017) (58%) Darwish et al., (2018) (15%)** for chicken liver. The lowest incidence rate was recovered from sausage this may be due to the addition of some additives that have antibacterial activity **(Musa and Okande 2002).**

B. cereus was isolated from minced meat, sausage, chicken breast and chicken liver samples at an incidence of 20%, 17.14%, 5.71% and 2.85% respectively. This outcome is nearly similar to **Shawish and Tarabees** (2017) (22.5%) for minced meat, **Hassanien (2004) (16**%) for sausage, conversely, higher results were obtained by **Mohamed and Ghanyem (2015)** (65%) for minced meat, **Ibrahim et al., (2014)** (40%) for sausage, **Zakki, (2017)** (26.6%) for chicken breast and **Tahmasebi et al., (2014)** (11%) **for** chicken liver. The high frequency of isolation from minced meat and sausage may be attributed to processing of minced meat also additives and spices that added to sausage, which can increase the number of Bacillus spores. Therefore it is important to use additives from a trustful source during processing of raw meat and test these additives regularly for the presence of bacillus spore **(Shawish and Tarabees, 2017).**

Antimicrobials are widely used in the veterinary field nowadays. The unrestricted use of antimicrobials or their use in sub-therapeutic dosing can lead to the development of antimicrobials resistant strains **(Beninati et al., 2015).** In the present study all isolated strains were resistant to at least one or more of the used antibiotics. A total of 10 *S.aureus* isolates were further tested for their antimicrobial susceptibility 90% of tested isolates were resistant to erythromycin, 60% to amoxicillin-clavulanic, cefotaxime and doxycycline,50% to gentamicin and vancomycinand30% to ciprofloxacin. Nearly similar results were obtained by **Abd El Tawab et al., (2018**) for cefotaxime (58.3%) **Saleh et al., (2016)** forvancomycin (55.5%); **Fan et al., (2015)** for gentamicin (63.4%); **Abd El Tawab et al., (2018**) for ciprofloxacin (20.8%); **Abd El Tawab et al., (2015**) for doxycycline (57.5%), **Sallam et al., (2015)** for erythromycin (73.6%), **Abd El Tawab et al., (2014)** for amoxicillin-clavulanic (61%). Conversely, these results disagreed with **Hanson et al., (2011**) for erythromycin (14.8%) with **Sallam et al., (2015)** forvancomycin (5.9%) **Abd El Tawab et al., (2018)** for gentamicin (8.3%) **Khalifa et al., (2014)** foramoxicillin-clavulanic (16%) **Abd El Tawab et al., (2015**) for cefotaxime (10%) **Akbar and Anal (2013)** for ciprofloxacin 7.8%**Miranda et al., (2008)** for doxycycline (23.8%). The low susceptibility of *S.*

aureus to beta-lactam antibiotics observed in this study may be due to the production of beta-lactamase enzymes **(Canton and Valverde, 2008).**

Meanwhile (10) *B.cereus* isolates were tested for antimicrobial susceptibility. The most common drug resistance was to amoxicillin-clavulanic and cefotaxime (100% for each), ciprofloxacin, erythromycin and vancomycin (80% for each), doxycycline (70%) and gentamicin (20%). Nearly similar results were obtained by **Guven *et al*., (2006)** forgentamicin (27%) **Naas et al., (2018)** foramoxicillin-clavulanic and cefotaxime (100%) **Avşar et al., (2017)** for vancomycin (71.9%) and gentamicin (23.1%). While these results disagreed with **(Shawish and Tarabees 2017)** for vancomycin (0%) **Naas et al., (2018)** for doxycycline (0%), **Bashir et al., (2017)** forerythromycin (27.2%), gentamicin (13.6%) **Jawad et al., (2016)** for gentamicin (57%) and ciprofloxacin (20.9%). Variations in the percentages of antibiotic susceptibility may be attributed to the differences in the concentrations of antibiotic agents, locally approved drugs and misuse or overuse of antibiotics **(Agwa et al,.2012).**

PCR has emerged as a high sensitive and specific method for identifying pathogens **(Lim et al., 2004).** In the following study none of the examined samples harbored staphylococcal enterotoxins (*sea, seb, sec, sed, see)* they might have other types of SEs which are family of 21 serological types of heat stable enterotoxins. this came in accordance with **Saleh et al., (2016)** who failed to detect *sea* and *sed* genes and disagreed with **Kitai (2005)** who detected *seb* (50 isolates), *sea* (14), *sec* (8), *sed* (2), *sea*+*seb* (2), and *sea*+*sec* (2).

In the current study the results revealed that 100% of tested isolates of *S. aureus* harbored *bla*Z gene this result agreed with **El Seedy et al., (2017) (100%).** Also 100% of tested isolates harbored *mec*A gene this came in accordance with **Momtaz et al., (2013) (82.92%)** and disagreed with **podkowik et al., (2012)** who failed to detect *mec*A gene. Meanwhile vanA gene failed to be detected and this agreed with **Ma et al., (2018)** while **Okolie et al., (2015)** detected *vanA* gene in 14.2% of examined samples. MRSA has been reported as an emerging problem in veterinary medicine **(Leonard and Markey, 2008)**, the isolation of MRSA from food products in markets confirms that MRSA not only associated with problems for hospitals but also they entered the food chain **(Otalu et al 2011, and Karmi 2013).**

In this study 100% of tested *B.cereus* isolates harbored *nhe* this agreed with **Anderson et al., (2001)** (100%), While 20% of tested isolates harbored *hbl* gene this came in accordance with **Torkarand Seme (2009) (**31.7%). Mean while none of tested isolates harbored *ces* gene this agreed with **Ankolekar *et al.,*** (2009) and disagreed with **Aubaidand Dakel (2017)** who detected *ces* gene in (41.6%) of examined isolates. Also 100% of tested B.cereus harbored *tet*A gene and this result agreed with **Rather et al., (2012)** (92.3%). Meanwhile, *Bla* gene was detected in 100% of examined isolates this agreed with **Avsar et al., (2017)** who detected bla $_{CMY-2}$ in 45% of examined isolates.

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