Original Research

Detection of *Enterococcus faecalis* as an Indicator Organism in Abattoirs´ Environment as well as Meat

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ABSTRACT

Occurrence of *Enterococci* as an indicator organism was investigated in 5 municipal slaughterhouses located in different provinces, Egypt. Altogether, 300 samples were taken, including slaughtered meat, water, and air samples beside swabs from floor, wall, and workers hand (50 samples/each). Statistical analytical results of *Enterococci* count showed that the highest mean value was recorded in the wall swabs (6.63×10^3 CFU/g) followed by hand swabs of workers then floor swabs then air samples then meat samples and lastly water samples. Additionally, it was recorded that the overall rate of *Enterococci* isolation was 12% (36 isolates); the highest rate of isolation was recorded in floor swabs (24%) followed by air samples (14%) then wall swabs and air samples (12% for each) and lastly meat samples (4%). Moreover, 20 *Enterococcus faecalis* isolates were biochemically identified and PCR was employed successfully to confirm the identification of *E. faecalis* isolates by detection of 16S rRNA specific for *E. faecalis.* Finally, antibiogram pattern of 20 *E. faecalis* isolates was investigated realizing that *E. faecalis* were resistant to Cefotaxime (60%), Amikacin and Linezolid (55 %), Rifampin (50%), Amoxiclav (45%) and Gentamycin and Vancomycin (40%) while it was observed that 80% of isolates were sensitive to Ciprofloxacin. Furthermore, molecular detection of Vancomycin resistance gene A (*van*A) was performed by PCR, and it was amplified at 885 bp in 8 isolates only with percentage of 40%. Based on the recorded result, increased *enterococci* count in meat as well as abattoirs environment would have a clear influence on increasing the microbial load of meat so to ensure a high level of safety and lowering the carcasses contamination, HACCP program must be applied.

Keywords: *Enterococcus faecalis*, Enumeration, Identification, Antibiogram, VanA gene

INTRODUCTION

Abattoirs are described as any suitable sites in which animals are slaughtered, eviscerated and dressed for man consumption and are officially permitted by the veterinary authority, **(CAC, 1993).** The main function of an abattoir is the provision of wholesome, healthy halal meat under the veterinary authorities supervision with the application of hygienic measures in avoidance of contaminated environmental exposure facing infectious agents **(Zailani et al., 2016).**

The abattoir components should include slaughter hall, lairage, detained meat room, gut room, offal room, condemned meat room, and hide room. Other slaughter houses include refrigeration room, cutting room, cold and hot water under pressure supply, disinfection room, veterinary supervision room, veterinary office, and facilities for condemned meat offal, incinerator partition, chemical treatment and disposal. Unmaintained and Substandard abattoir infrastructures will seriously hamper standard operations to produce wholesome meat for human consumption, thereby, posing problems of meat hygiene threatening human health **(Lawan et al., 2013).**

Contaminated meat is one of the seriously sources of foodborne diseases caused by the accidental ingestion of bacteria and their toxins that are inactivated even after meat cooking **(Bersisa, 2019).** Pathogenic microorganisms are

normally found on live animals skin contaminated with feces and also present in the digestive tract of the apparently healthy cattle. After slaughter, soil and fecal matter adherent to hides can be transmitted to sterile meat during processing of meat particularly if applied in absence of a carcass hanging system on dirty floor **(Abdelwahed and Abdelgadir, 2019).**

Upon unclean evisceration, contents of intestine may be contaminating the meat surface so that the meat undergoes spoiling quickly if it is not correctly handled and offered to consumers making illness **(Diyantoro and Wardhana, 2019).** There are several extrinsic sources of agents contamination in abattoirs including unsanitary worker handling, the use of contaminated knives and other tools used in cutting process, and polluted air during air droughts inside the hall **(Birhanu et al., 2017).** Also, using unsanitary water for washing of both carcasses and equipment during slaughtering can predispose or add microbial contamination **(Parvin et al., 2017).**

Lack of awareness of some workers by the hygienic significance of personal hygiene results in contamination of meat products by contaminated workers' hands **(Pradhan et al., 2018).** so, staff dealing with food industry must practice a high standard of personal hygiene such as washing hands thoroughly before preparing foods, wear clean footwear, clean clothing, never drink or eat while working **(Slobodan et al., 2017).**

Enterococci are normal inhabitants of the GIT of animals and man found mainly in water, food and soil. They are enteric streptococci but differ from the streptococcus species. For several years, they were considered as normal micro-flora and not harmful to human **(Wierzchowska et al., 2012).** However, enterococcus has emerged as major nosocomial pathogens, representing an alarming problem for public **(Sood et al., 2018).**

The main objective of the following study was to determine the degree of contamination and microbial quality of meat produced by some local abattoirs in Egypt based on *Enterococci* count beside isolation and identification of *Enterococcus faecalis* and determination of antibiogram profile of isolates as well as molecular detection of antibiotic resistance genes.

MATERIALS AND METHODS

Study area This study was carried out in 5 slaughterhouses present in different provinces, Egypt. Abattoirs are manually operated slaughterhouses. They well-constructed and consisted of a quarantine partition, slaughtering partition, emergency slaughtering hall, condemnation room, and eviscerated rooms. Slaughtering capacity is around 200 cattle/ day. The slaughtering was started after dawn at 6:00 am lasting between 10:00 to 12:00 pm depending on the number of slaughters admitted for slaughtering. The hall cleaned routinely at the end of slaughter day. **Sampling** A total of 300 samples were collected as following: meat, water, and air samples beside swabs from floor, wall, and workers hand (50 samples/each). Samples were collected during twice visits per week then labeled and transferred in icebox to the laboratory of Animal Health Research Institute, Cairo, Dokki, Egypt **Meat samples:** About 25 gm from all sample were aseptically transferred into sterile blender flask containing 225 ml of sterile brain heart infusion broth and homogenized at 1400 rpm for 2-5 minutes to obtain a homogenate 1/10 dilution and then allowed to hold at room temperature for 6 minutes. The flask contents were thoroughly mixed well by shaking thoroughly and 1 ml of the homogenate was pipetted to another tube containing 9 ml of sterile brain heart infusion broth, from which 10-fold serial dilutions were prepared up to 10^{-6} **(APHA, 2002)**. **Water samples** Water samples were sampled from water taps and the identified tanks in sterile plastic screw capped bottles (500 ml capacity). The sampling bottles were thoroughly shaked and one ml was pipetted to sterile tube containing 9 ml of sterile brain heart infusion broth, from which 10-fold serial dilutions were prepared **(APHA, 1998)**. **Air samples** Air samples were Aspirated using impinge filled impinger with 225 ml sterile brain heart infusion broth. The sampling bottles contents were thoroughly mixed, and one ml was sterilely pipetted to tube containing 9 ml of sterile brain heart infusion broth, from which 10-fold serial dilutions were prepared up to 10^{-6} **(Hamet et al., 1991). Wall and Floor swabs** Swabs collection from walls and floors were conducted using cotton swabs by swabbing on the walls and floor surfaces in nearly 1 cm^2 surface area, and then swabs insertion in sterile brain heart infusion broth and transport to the laboratory under the chilling condition. The swab tubes contents were thoroughly mixed, and one ml was sterilely pipetted to sterile tube containing 9 ml of sterile brain heart infusion broth, from which 10- fold serial dilutions were prepared up to 10^{-6}. **Hand swabs of slaughterhouse workers** Swabs were gathered from workers hand of the investigated 5 abattoirs (10/each). They were obtained in sterile swabs placed in a sterile brain heart infusion broth test tube and each tube was covered with sterile cotton plug then they were transferred immediately in an ice box to the laboratory. The swab tubes contents were thoroughly mixed, and one ml was sterilely pipetted to sterile tube containing 9 ml of sterile brain heart infusion broth, from which 10- fold serial dilutions were prepared up to 10^{-6}**. Enumeration of *Enterococci* (Facklam et al., 2002)** From each examined sample, 0.1 ml of the prepared dilutions was dispensed into duplicate plates of Bile Esculin Azide agar and evenly distributed till complete absorption using surface plating technique. The inoculated plates were incubated at 35° C ± 1° C for 48 hours then red colonies were observed, counted and recorded as *Enterococci* count. **Isolation, identification, molecular characterization and antibiogram pattern of *E. faecalis.* Bacterial isolation and identification** *Enterococcus* spp. Isolation and identification were performed following the protocols of Facklam et al., (2002). Samples were pre-enriched on brain heart infusion broth (Oxoid) and incubated for one day at 37 °C. A loopful from the pre-enriched samples was streaked on the Bile Esculin Azide agar (Oxoid) with addition of Cefotax antibiotic and incubated

at 37 °C for 24 – 48 hours under microaerophilic condition. Suspected colonies of *Enterococcus* spp. were subjected to identification tests using the following characteristics: fermentation of sucrose, growth and hydrolysis of bile-esculin agar, and absence of catalase.

Biochemical tests	Reading	Result
Bile esculin hydrolysis	Convert from yellow to black	Positive
Sucrose	Convert from red to yellow	Positive
Catalase	Colorless – no foaming 18 – 24 hrs	Negative

Determination of antibiogram pattern of *E. faecalis* the test was carried out by applying the bacterial inoculum on the surface of Muller Hinton agar medium. Then commercially prepared, fixed concentration antibiotics discs were placed on the inoculated agar surface. The plates were incubated at 37˚C aerobically for 24 hours. Specific rulers used for measuring the inhibition zones around each antibiotic disk. The inhibition zone diameter is related to the rate of the drug's diffusion through the agar medium and the isolate's susceptibility. The zone diameters of each drug were interpreted using the parameters published by the Institute of Clinical and Laboratory Standards **(CLSI, 2017). Molecular detection of *Enterococci*, *E. faecalis* and detection of Van resistance gene** Molecular reactions were performed at the Central Laboratory, Faculty of Veterinary Medicine, Cairo University. Twenty isolates of *E. faecalis* were further investigated by PCR for presence of *16S rRNA* and *van*A.

Oligonucleotide primers (Midland Certified Reagent Company_ Oilgos, USA):

Target	Gene	Primer Sequence (5'-3')	Product size	Reference
E. faecalis	*16S rRNA*	GTT TAT GCC GCA TGG CAT AAGAG	310 bp	**Zoletti *et al.*, (2006)**
		CCG TCA GGG GAC GTT CAG		
Vancomycin resistant gene	*van*A	CATGACGTATCGGTA AAATC	885 bp	**Patel *et al.*, (1997)**
		ACCGGGCAGRGTAT TGAC		

Cycling conditions of the primers during cPCR:

Target	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	No. of cycles
Enterococcus	*16S rRNA*	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	35
E. fecalis	*16S rRNA*	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	35
Vancomycin resistance gene	*van* A	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec.	72˚C 50 sec.	72˚C 10 min.	35

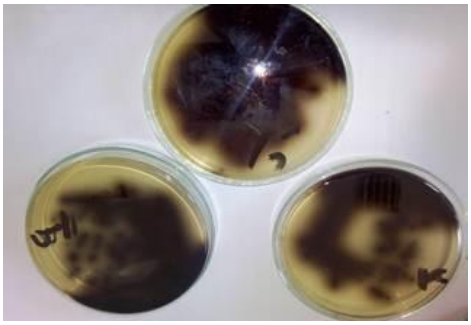
RESULTS

Table (1): Statistical analytical results of *Enterococci* count (CFU/g) of samples:

Sources of samples (n=50 of each)	Minimum	Maximum	Mean ± S.E*
Beef meat	**1×10**	**6.2×10^3**	**$3.02\times10^3 \pm 3.33\times10^2$**
Water	**1×10^3**	**3.0×10^3**	**$1.66\times10^3 \pm 6.6\times10^2$**
Air	**2×10**	**2.2×10^4**	**$3.19\times10^3 \pm 5.87\times10^2$**
Floor swabs	**1×10^2**	**7.5×10^3**	**$4.25\times10^3 \pm 1.97\times10^3$**
Wall swabs	**4×10**	**5×10^4**	**$6.63\times10^3 \pm 2.36\times10^3$**
Hand swabs of workers	**2×10**	**4×10^4**	**$5.33\times10^3 \pm 2.37\times10^3$**

Table (2): Rate of isolation of *Enterococci* and *E. faecalis* from different examined samples

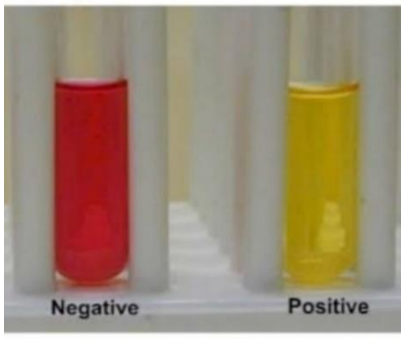
Enterococci	Meat (n=50)		Water (n=50)		Air (n=50)		Floor swabs (n=50)		Wall swabs (n=50)		Hand swabs (n=50)		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Enterococci	2	4.0	6	12.0	7	14.0	12	24.0	6	12.0	3	6.0	36	12.0
E. faecalis	1	2.0	2	4.0	4	8.0	6	12.0	4	8.0	3	6.0	20	6.7



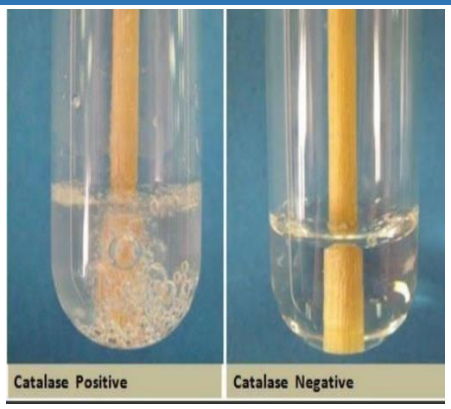
Esculin hydrolysis by Enterococci on Bile Esculin agar is indicated by media blackening around the colonies

Positive **Negative**

Bile Esculin agar test result for identification of *Enterococcus* spp. showing; Black colour (Positive) and Yellow colour (Negative)



Sucrose test result for identification of *Enterococcus* spp. showing Red colour (Negative) and Yellow colour (Positive)



Catalase test result for identification of *Enterococcus* spp. showing Foaming (Positive) and Colorless – no foaming (Negative)

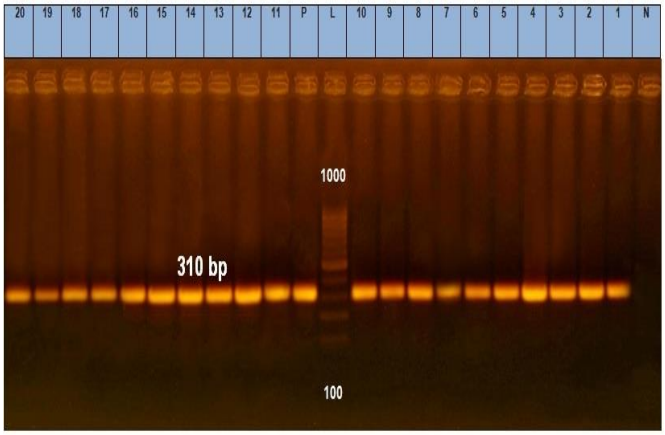


Fig. (1): PCR products specific for E. faecalis 16S rRNA (310 bp)

All E. faecalis isolates (n = 20) were positive for presence of 16S rRNA.

N= negative control l=ladder p= positive control 1-20=samples

Table (3): Antibiogram pattern of *E. faecalis* (n =20 isolates) obtained from different samples

Antibiotics	Sensitive		Resistant	
	No.	%	No.	%
Amikacin (AK)	9	45.0	11	55.0
Amoxiclav (AMC)	11	55.0	9	45.0
Cefotaxime (Cfm)	8	40.0	12	60.0
Ciprofloxacin (CIP)	16	80.0	4	20.0
Gentamycin (Cn)	12	60.0	8	40.0
Linezolid (LZD)	9	45.0	11	55.0
Rifampin (RA)	10	50.0	10	50.0
Vancomycin (VA)	12	60.0	8	40.0

Molecular detection of vancomycin resistance gene A (*van*A) was performed by PCR reactions for 20 confirmed isolates to *E. faecalis*. The *van*A gene was amplified at 885 bp in 8 isolates only as shown in Fig., (2).

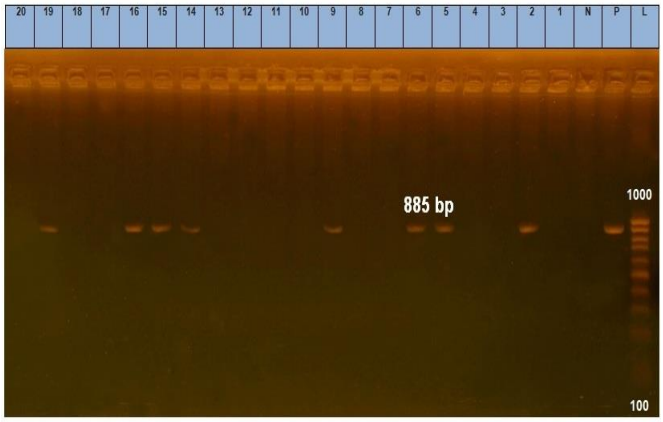


Fig. (2): PCR products specific for *van*A *gene* (885 bp) N= negative control p= positive control l=ladder 1-20=samples

DISCUSSION

The slaughterhouse may be the main source of meat microbial contamination in case of bad sanitary state. Analyzing the microbial load of the slaughterhouse reflects the slaughterhouses hygienic quality and determines the meat quality and the public health risk of food poisoning.

The slaughterhouses should have aplenty of clean safe water (free from microbial load or chemicals) **(Gracey *et al*., 1999).** Unhygienic disposal of abattoir offal may pollute underground water **(Adebowale et al., 2010).** Un-adequate removal of carcasses' blood from the ground results in offensive odor and threaten human health living around by respiratory complications and spreading the microorganisms, which gain access to the meat surface and slaughterhouses water supplies **(Magaj and Chup, 2012).** Meat contamination occur usually due to improper handling in slaughterhouses and inadequate hygienic conditions **(Koo et al., 2013).** Since one major task of animal hygiene is to protect both meat and the meat handlers from cross-contamination, thus, this study was carried out to investigate an important food safety issue related to the control of hygienic measures in local abattoirs.

Due to their significance in environmental and clinical samples, *Enterococci* counts have become an important issue in current research activities. In addition, **Torres et al. (2018)** reported that *Enterococcus* spp. have emerged as the cause of 12% of nosocomial infections, with only two species, *E. faecium and E. faecalis*, causing about 90% of clinical manifestations.

The obtained data in **Table (1)** showed that statistical analytical results of *Enterococci* count of the examined samples. It was found that the highest mean value was recorded in the wall swabs (6.63×10^3 CFU/g) followed by hand swabs of workers (5.33×10^3 CFU/g) then floor swabs (4.25×10^3 CFU/g) then air samples (3.19×10^3 CFU/g) then meat samples (3.02×10^3 CFU/g) and lastly water samples (1.66×10^3 CFU/g).

Enterococci survived in exterior habitats including sediments, soil, aquatic vegetation, and ambient waters (rivers). They may also be considered as heterothermic (temperatures are variable) habitants, in contrast to the gastrointestinal tract of warm-blooded animals, where the temperature is approximately constant. In addition, they primarily colonized in the gastrointestinal tract and represents 1% of the gut microflora **(Tedim et al., 2015)**.

Occurrence of *Enterococci* as an indicator organism was presented in **Table (2)**. It was recorded that the overall isolation rate of *Enterococci* from the examined samples was 12% (36 isolates out of 300 samples) and the highest rate of isolation were recorded in floor swabs (24%) followed by air samples (14%) then wall swabs and air samples (12% for each) and lastly meat samples (4%). In addition, 20 *Enterococcus faecalis* isolates were identified biochemically out of 36 isolates of *Enterococci* in the examined samples. Moreover, PCR was employed successfully confirming the identification of *E. faecalis* isolates by detection of 16S rRNA specific for *E. faecalis* **(Fig., 1)**.

E. faecalis had a symbiosis to the intestinal microflora as a commensal; however, serine proteases and *gel*E produced by *E. faecalis* can cause inflammatory reaction in intestinal epithelium and disrupt the normal function. This led to translocation of normal flora, including *E. faecalis* to systemic circulation causing bacteremia **(Tan et al., 2013)**.

Antimicrobial resistance can involve both pathogens and commensals, which may act as a reservoir of antimicrobial resistance genes. Therefore, avian infections with antibiotic resistant Enterococcus could reservoirs and disseminators for MDR Enterococcus to humans (**Miller et al., 2014)**. Antibiogram pattern of *E. faecalis* (n =20 isolates) obtained from different examined samples was presented in **Table (3)**. It was realized that *E. faecalis* were resistant to Cefotaxime (60%), Amikacin and Linezolid (55 %), Rifampin (50%), Amoxiclav (45%) and Gentamycin and Vancomycin (40%) while it was observed that 80% of isolates were sensitive to Ciprofloxacin.

These results opposed **Stępień-Pyśniak *et al.,* (2016)** who recorded that *Enterococcus* spp. were resistant vancomycin (0.11%). These results indicate that the overuse of antibiotics and chemotherapy in animal production may lead to the population of multidrug resistant *Enterococci* **(Ruzauskas et al., 2009)**.

In addition to the historical applications (examination of foods, water, intestinal and other clinical specimen), the detection of vancomycin-resistant *enterococci* (VRE) has become an important task, where VRE have observed to be frequently involved in nosocomial infections. Finally, molecular detection of Vancomycin resistance gene A (*van*A) in the 20 isolates to *E. faecalis* was performed by PCR and *van*A gene was amplified at 885 bp in 8 isolates only with percentage of 40% **(Fig., 2)**. These results were in accordance with **Hassan et al., (2008)** who revealed that *van*A was observed in 22.4% of *E. faecalis* and overall *van*A detected in 36.4% *Enterococcus* isolates. *Enterococci* inhabiting livestock appeared to play an important role in the spreading of AMR determinants **(Daniel et al., 2015).**

CONCLUSION

In the current work, the microbiological examination revealed the presence of increased microbial counts in meat as well as abattoirs environment which have a clear influence in increasing the microbial load on meat surfaces. Upon poor hygienic sanitary conditions, slaughtering of animals on the

floor followed by skinning and evisceration in the same hall are considered as the principal risk factors for heavy bacterial load of carcasses which seriously threaten food safety and public health to foodborne diseases. Therefore, it requires a serious precaution from all health authorities to apply and maintain the standard hygienic slaughterhouse practices in attempt to prevent the carcasses bacterial contamination preventing food borne diseases. To ensure a high level of safety and lowering the carcasses contamination, abattoirs should be constructed with high level of sanitation to minimizing the initial bacterial count with application of mechanical techniques in slaughtering to minimize human intervention and antibiotic treatment of animals should be applied only with veterinarian prescription and after sensitivity test with diagnosis of bacteria to minimize the risk of MDR bacteria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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