J. Appl. Zool. Res. (2017) **28**(2): 138-146
ISSN 0970-9304
NAAS Rating: 4.22

OCCURRENCE OF ANTIBIOTIC-RESISTANT *ENTEROCOCCI* IN SOME INSECTS FROM STORED FOOD PRODUCTS IN BOTSWANA

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Received: 5 July, 2017 **Accepted: 6 September, 2017**

ABSTRACT: The occurrence of antibiotic-resistant enterococci in some insects from stored food products from fourteen geographical areas in Botswana was investigated in the present study. Eleven insect species were identified from a total of 737 stored food products. *Oryzaephilus surinamensis* was the most predominant. It comprised 65% and 51.1% of the speciesin food products from households and the selected warehouse respectively; followed by *Tribolium castaneum*, which comprised 17.1% of the species in stored foods from households and 32.3% of the species in stored food products from the warehouse. There was no significant difference ($p<0.05$) in the distribution of the insect species from the geographical areas sampled.Sixty-eight per cent (68%) of the total insect numbers harboured *Enterococcus* species. *Enterococcus* species were isolated from *Prostephanus truncatus* and *Cryptolestes ferrugeneus*, obtained from laboratory stored maize cultures. The isolation rate of enterococci was significantly higher ($p<0.05$) in laboratory stored product insects as compared with insects collected from stored foods sampled from the selected geographical areas. Enterococci showed total resistance to erythromycin, fusidic acid, oxacillin, novobiocin, and penicillin G but were all sensitive to chloramphenicol. This shows the capacity of some stored product insects to carry antibiotic-resistant and potentially pathogenic *Enterococcus* species. The latter is of public health importance and therefore there is need to monitor the occurrence of *Enterococcus* species in insects infesting stored products. These insects can act as vectors of pathogenic microorganisms & cause health hazards to consumers if proper pest management strategies are not applied to stored products.

Key words: *Oryzaephilus surinamensis, Prostephanus truncatus, Cryptolestes ferrugineus, Tribolium castaneum, Enterococcus* species, Enterococci, antibiotic resistance

INTRODUCTION

Cereals and pulses are important food staples in Botswana and represent significant portions of the daily dietary intake (ALLOTEY, 1991; ALLOTEY and RAMONGALO, 2011). Insect species such as *Sitotroga cerealella* (Olivier), *Ephestia cautella* (Walk.), *Callosobruchus maculatus* (Fabr.), *Rhyzopertha dominica* (Fabr.), *Sitophilus zeamais* Motsch, *Tribolium castaneum* (Herbst.), and *Tribolium confusum* (J. du Val.) have been reported in food commodities in Botswana (ALLOTEY *et al*., 2010; MOHALE *et al*., 2010; ALLOTEY *et al*., 2011; ALLOTEY, 1991; ALLOTEY and MOLOKO, 2015). They cause significant food losses during storage (ALLOTEY *et al*., 2010; ALLOTEY and MOLOKO, 2015) and their control is of paramount importance to food security (ONIANG'O and ALLOTEY, 1999). Some stored products insects are known to harbour pathogenic and potentially pathogenic bacteria (CHANNAIAH *et al*., 2010). *Sitophilus oryzae* (L.) has been reported to carry *Salmonella* sp. (ALLOTEY, 2011; BLAZAR *et al*., 2011). *Staphylococcus* and *Bacillus* species have been isolated from the gut of *Callosobruchus maculatus* (SEVIM *et al*., 2016).CHANNAIAH *et al*. (2010) have addressed the importance of stored product insects carrying enterococci.

Enterococci have previously been isolated from some perishable food products such as milk, chicken, beef and beverages in Botswana (COLLISION and JOHANNES, 1999; CHINGWARU *et al.*, 2003; AAKU *et al.*, 2004; MATSHEKA *et al.*, 2013). Prior to the present study there has been no report on enterococci in stored product insects in Botswana. *Enterococcus* species are Gram positive cocci and occur in diverse environments such as soil, animal food products, gastrointestinal tracts of animals and animal excreta (FRANZ *et al.*, 1999). These bacterial species are established pathogens especially incausing hospital-acquired infections (LINDEN *et al.*, 1999) and are also opportunistic pathogens in case of immune-suppression such as HIV/AIDS infection (AWANDA *et al.*, 1999). They also cause infectious diseases such as bacteraemia, urinary tract infections, endocarditis, wound and tissue infections (KAYSER, 2003). Enterococci are known for their resistance to a wide array of antibiotics (MURRAY, 1990) and they are also specifically recognized as important reservoirs of antibiotic resistance genes that can spread horizontally to other bacterial pathogens (DEVRIESE *et al.*, 1992). The objectives of the present study were to identify insects associated with some stored products and to determine their potential of carriage of antibiotic-resistant enterococci.

MATERIALS AND METHODS

Collection of insects: Insect infested foods were collected from households in 12 different places, mostly from the southern part of Botswana, the Botswana Agricultural Marketing Board (BAMB) warehouse in Gaborone and from the Insectary in the Department of Biological Sciences, University of Botswana. Localities sampled were mostly from southern Botswana (Gaborone, Mochudi, Otse, Lobatse, Molepolole, Tlokweng, Kopong, Good hope and Pitsane) and northern Botswana (Mahalapye and Dibete). Insect-infested food products were sifted using standard sieves (Humboldt, Chicago, Illinois, USA) to obtain live insects. The live insects were weighed and thereafter insect species were identified. The present study was conducted from November 2014 to May 2015.

Isolation and identification of enterococci: Each identified insect was surface sterilized using 10% sodium hypochlorite in a Petri dish and further sterilized by immersion into 70% ethanol. The surface sterilized insect was homogenized in 1ml phosphate buffered saline. The homogenate (5µl) was then drop plated onto mEnterococcus agar (HiMedia, Bangkok, India) and the agar plates incubated at 37^{0}C for 24h.To identify enterococci, red and pink colonies on mEnterococcus agar (characteristic of *Enterococcus* species) were Gram stained and the catalase test performed. Gram positive and catalase negative cocci were tested for β-hemolysis on blood agar and β-hemolytic colonies were presumed to be *Enterococcus* sp. To confirm these isolates as enterococci, the bile esculin test, growth in 6.5% NaCl and L-pyrrolindonyl-β-naphthlyamide (PYR) tests were performed. Isolates that tested positive for all the three tests were confirmed as *Enterococcus* sp. The isolates were not identified to the species level.

Antimicrobial susceptibility testing: To perform antibiotic susceptibility, the test isolates were standardized to 0.5 McFarlands in 0.1% physiological saline. Using a swab, the bacterial suspension was spread throughout Mueller Hinton agar (Oxoid, Basingstoke, UK) plates before performing antimicrobial susceptibility testing for all the positive isolates using the disc diffusion test. All the antibiotics were obtained from Mast Group (Merseyside, UK). The eight antibiotics used were: Chloramphenicol (25µg), Erythromycin (5µg), Fusidic acid (10µg), Oxacillin (5µg), Novobiocin (5µg), Penicillin G (1U), Streptomycin (10µg) and Tetracycline (25µg). Plates were then incubated at 37°C for 24h. After incubation diameters of zones of clearing around colonies were measured and recorded and the results were interpreted using the criteria from the Clinical & Laboratory Standards Institute (2006).

The data was analysed using Statistical Package for Social Science (IBM SPSS 21.0). Pearson correlation was employed to demonstrate relationship between sampling locations, food commodity type, identified insect species and percentage of isolates that were positive for enterococci. One-way ANOVA was utilized to separate the means of the percentage of the positive enterococci isolates according to the zones sampled and insect species.

RESULTS AND DISCUSSION

Oryzaephilus surinamensis was the predominant insect species found in food samples from Extension 4, Goodhope, Pitsane, Mochudi, Otse, Broadhust and Mahalapye (65.8% of locations had *O. surinamensis*). Out of the 8 infested food samples from the warehouse, 6 had *O. surinamensis* which made it the most common insect species (Table-1). All households collected foods had one insect species except the farinaceous soup product from Mochudi which had two species, *Tribolium castaneum* and *O. surinamensis.*Insects from household food samples constituted 35.2% of the total insect population recorded, while those from the warehouse constituted 18.6%.

Ten (10) infested food commodities from the insectary were utilized for this study. *Prostephanus truncatus* and *Cryptolestes ferrugineus*from maize were the dominant species constituting 28.6% and 24.2%, respectively (Table-2). However, the food commodity did not have any statistical significance on the identified insect species ($p<0.05$; $p=0.000$). Only 1 (0.3%) *Anthrenus* sp. was found from biltong. Maize had multiple infestations of 3 species *viz.*, *P. truncatus, Rhyzopertha dominica* and *C. ferrugineus* whereas most food commodities had one species.

P. truncatus and *C. ferrugineus* were the two insect species that were positive for enterococci, both from the insectary stored maize (Table-3). Of the 98 *P. truncatus* and 83 *C. ferrugineus*, 34% and 20% were positive for *Enterococcus* sp., respectively. Overall, the occurrence of enterococci in the two species was significantly higher than that of other insect species, with 95% confidence ($p<0.05$; $p=0.000$). The two insect species positive for enterococci constituted 6.8% of the total live insects which were identified. All the enterococci isolates from the two insect species were resistant to novobiocin, methicillin, erythromycin, fusidic acid, and penicillin G but susceptible to chloramphenicol. Ninety (90) per cent and 30% of the isolates were resistant to tetracycline and streptomycin, respectively whereas 10% and 70% of the isolates were susceptible to tetracycline and streptomycin, respectively (Table-4). None of the enterococci isolates was intermediate to any antibiotic.

From the above results, the most predominant insect species associated with stored products in this study was *O. surinamensis* which comprised of 65.8% and 51.1% of species in the households and the Botswana Agricultural Marketing Board (BAMB) warehouse, respectively. *Tribolium castaneum* was the second predominant insect species in both the warehouse (32.3%) and households (17.1%).

Table-1: Insect species identified from various stored-product foods collected from households and a warehouse in Botswana

Sampling location	Stored-Product sample (N_h=23, N_w=8)*	Insect species identified	No. of insects (%)
Households (h)			
Goodhope	Sorghum	*Oryzaephilus surinamensis*	12 (4.7)#
Lobatse	Samp	*Tribolium confusum*	2 (0.8)
	Flour	*Tribolium castaneum*	8 (3.1)
Gaborone (Extension 4)	Sorghum	*O. surinamensis*	32 (12.5)
	Samp	*T. castaneum*	2 (0.8)
Pitsane	Sorghum	*O. surinamensis*	24 (9.3)
Dibete	Samp	*T. castaneum*	13 (5.1)
	Cowpeas	*Callosobruchus maculatus*	6 (2.3)
Mochudi	Samp and beans combination	*T. castaneum*	9 (3.5)
	Farinaceous soup	*T. castaneum*	5 (1.9)
		O. surinamensis	28 (10.9)
	Sorghum	*O. surinamensis*	16 (6.2)
Otse	Wheat	*T. castaneum*	7 (2.7)
	Rice	*O. surinamensis*	14 (5.4)
	Sorghum	*O. surinamensis*	8 (3.1)
Gaborone (Broadhurst)	Whole maize	*Sitophilus zeamais*	2 (0.8)
	Crushed maize	*O. surinamensis*	12 (4.7)
	Green peas	*C. maculatus*	9 (3.5)
Tlokweng	Maize meal	*T. confusum*	6 (2.3)
Molepolole	Black eyed peas	*C. maculatus*	4 (1.6)
Kopong	Haricot beans	*C. maculatus*	15 (5.8)
Mahalapye	Sorghum	*O. surinamensis*	16 (6.2)
	Maize meal	*O. surinamensis*	7 (2.7)
Total (T_h)			**257 (34.9)**
Warehouse (w)	Maize rice	*O. surinamensis*	3 (2.2)
		T. castaneum	1 (0.7)
	Samp	*O. surinamensis*	12 (8.8)
		T. confusum	8 (5.8)
	Rice	*O. surinamensis*	16 (11.7)
		T. castaneum	3 (2.2)
	Cowpeas	*C. maculatus*	13 (9.5)
	Homemade brew	*T. castaneum*	31 (22.6)
		O. surinamensis	21 (15.3)
	Flour	*T. castaneum*	4 (2.9)
	Maize meal	*T. castaneum*	6 (4.4)
		O. surinamensis	12 (8.8)
	Sorghum	*O. surinamensis*	7 (5.1)
Total (T_w)			**137 (18.6)**

*N_h= No. of insect infested food from households; N_w= No. of insect infested food from the warehouse; T_h= Total number of insects from households; T_w= Total number of insects from the warehouse; #Numbers in parentheses indicate percentages.

Table-2: Infestation of stored products by insects in the insectary at University of Botswana

Food commodity	Insect species	Number (%) of insects
Biltong	*Lasioderma serricorne*	10 (2.9)[#]
	Anthrenus sp.	1 (0.3)
Phane	*Stegobium paniceum*	23 (6.7)
Maize	*Prostephanus truncatus*	98 (28.6)
	Rhyzopertha dominica	12 (3.5)
	Cryptolestes ferrugineus	83 (24.2)
Cowpeas	*Callosobruchus maculatus*	17 (5.0)
Maize meal	*Tribolium castaneum*	21 (6.1)
	Tribolium confusum	6 (1.7)
Rabbit feed	*Sitophilus zeamais*	2 (0.6)
Flour	*Tribolium confusum*	18 (5.2)
	T.castaneum	4 (1.2)
Spices	*T.castaneum*	9 (2.6)
Sorghum	*Oryzaephilus surinamensis*	32 (9.3)
Bambara nuts	*C. maculatus*	7 (2.0)
Total		**343 (46.5)**

[#]Numbers in parentheses indicate percentages.

Table-3: Prevalence of enterococci in stored product insects isolated in three localities

Insect species isolated from:	Total no. of insects	No. (%) of isolates positive for enterococci
Households and Warehouse		
1. *O. surinamensis*	240	0 (0)[#]
2. *C. maculatus*	38	0 (0)
3. *T. castaneum*	96	0 (0)
4. *T. confusum*	16	0 (0)
Insectary		
1. *L. serricorne*	10	0 (0)
2. *S.paniceum*	23	0 (0)
3. *P. truncatus*	98	33 (33.6)
4. *C. ferrugineus*	83	17 (20.5)
5. *O. surinamensis*	32	0 (0)
6. *C. maculatus*	17	0 (0)
7. *T. castaneum*	34	0 (0)
8. *T. confusum*	18	0 (0)
9. *S. zeamais*	2	0 (0)
10. *R. dominica*	12	0 (0)
11. *Anthrenus* sp.	1	0 (0)
Total	**730**	**50 (6.8)**

[#]Numbers in parentheses indicate percentages

These results are in agreement with a previous study by LARSON *et al.* (2008), who reported *T. castaneum* to be the second predominant insect species in feed mills in the Midwestern United States. From the insectary *P. truncates* (29.1%) was the predominant species in maize followed by *Cryptolestes ferrugineus* (24.6%). These two species have been reported to be associated with maize infestation (BELL and WATTERS, 1982; WHITE *et al.*, 1995).

Table-4: Antibiotic susceptibility of *Enterococcus* sp. isolated from *P. truncatus* and *C. ferrugineus*

Antibiotic	% Resistant	% Intermediate	% Susceptible
Novobiocin	100	0	0
Streptomycin	30	0	70
Methicillin	100	0	0
Erythromycin	100	0	0
Tetracycline	90	0	10
Penicillin G	100	0	0
Fusidic acid	100	0	0
Chloramphenicol	0	0	100

Notably, *P. truncatus* and *C. ferrugineus* were the only stored product insects from which antibiotic resistant *Enterococcus* sp. were isolated. The association of antibiotic resistant enterococci with maize is significant considering the fact that maize forms one of the main dietary staple foods in Botswana. It can be noted that enterococci were not isolated from stored product insects in households and the warehouse. Samples from the warehouse and households represent fresh harvest from the farm for immediate consumption whilst samples from the insectary are for teaching purposes and have been in some instances stored and handled for up to 10 years. It is therefore likely that the keeping time of samples in the two settings could have had a bearing on the differences in the occurrence of antibiotic resistant enterococci. This study profiles the occurrence of antibiotic resistant *Enterococcus* sp. in stored product insects in Botswana. Antibiotic resistance in enterococci to a wide range of antimicrobials is a significant cause of concern globally, and this resistance can either be acquired or intrinsic (KAYSER, 2003). Enterococci have been found to be resistant to β-lactams, cephalosporins, sulphonamids, chloramphenicol and vancomycin (KAYSER, 2003; RADU *et al.*, 2001).

Enterococcus species in the present study displayed high resistance to antibiotics employed, and only showed complete sensitivity to chloramphenicol. Total resistance was encountered for erythromycin, fusidic acid, novobiocin, penicillin G and methicillin. Resistance to β-lactam antibiotiotics such as penicillin G and methicillin was not unexpected because enterococci have an intrinsic mechanism of resistance to these antimicrobials through the overproduction of the essential target, the low affinity penicillin binding protein 5 (PBB5), or mutations of different residues at its active site (ZORZI *et al.*, 1996). It is highly probable that a significant portion of these resistant isolates represent vancomycin resistant enterococci (VRE) which have been implicated in 8% of nosocomial infections in the USA (CENTERS FOR DISEASE CONTROL AND PREVENTION, 1993). Of public health importance is the finding that VREs are capable of transferring antibiotic resistance genes horizontally to more serious human pathogens such as *Staphylococcus aureus* (FRANZ *et al.*, 1999).

The reasons for the high antibiotic resistance of enterococci in this investigation are not clear, but resistance in these species has been linked to the use of antibiotics in feed as growth promoters (WITTE, 2000). It is important to note that in Botswana, there are no standard regulations on the use of antibiotics in feed and as a result antibiotic resistance due to this problem cannot be precluded. To confirm this association however, further studies are warranted. Enterococci are known indicators of food sanitary quality while their presence in insects is thought to be of animal fecal matter origin (JAY *et al.*, 2005).The absence of enterococci in stored product insects in households as well as the warehouse may indicate good sanitary practices in these environments. The source of enterococci in the insectary could be a result of cross-contamination by personnel through extensive handling over a long time, especially where aseptic techniques were not enforced. Because the insect species detected in this study are highly mobile and capable of flight (e.g., *T. castaneum* and *P. truncatus*), one cannot rule out the possibility of cross-contamination of other stored food products with pathogenic antibiotic-resistant *Enterococcus* sp.

Thus the present study shows that stored product insects are capable of being important carriers of potentially pathogenic enterococci. Although stored food produce from the households and warehouse were not associated with enterococci in this study, strategic pest control measures and effective food management practices, including good sanitary practices and enacting regulations on the use of antibiotics in animal feeds can act as the panacea in reducing the dissemination of enterococci through insect pests/vectors in stored food products.

ACKNOWLEDGEMENTS: The University of Botswana is acknowledged for financial support.

REFERENCES

AAKU, E.N., COLLISON, E.K., GASHE, B.A.and MPUCHANE, S. 2004. Microbiological quality of milk from two processing plants in Gaborone, Botswana. *Food Control* **15:**181-186.

ALLOTEY, J. 1991. Storage insect pests of cereal in small scale farming community and their control. *Int. J. Trop. Insect Sci.***12:**679-693.

ALLOTEY, J., NKWENTI, V., MAKATE, N. and MPHO, M. 2010. Interaction between pulse beetle, *Callosobruchus maculatus* (Fabr.) and fungi species associated with the deterioration and spoilage of pulses in Botswana. *J. Appl. Zool. Res.***21:**133-148.

ALLOTEY, J. 2011. Food safety and the environment: consumer issues. *Afr. J. Food Agric. Nutr. Dev.* **11:** 5358-5374.

ALLOTEY, J.and RAMONGALO, B. 2011. Development of *Ephestia cautella* (Walk.) on some food commodities in Botswana.*J. Appl. Zool. Res.***22:**119-130.

ALLOTEY, J., SEAGO, M. MAKATE, N. and MPHO, M. 2011. Development of *Callosobruchus maculatus* (F.) on some pulses in Botswana. *J. Appl. Zool. Res.***22:**131-137.

ALLOTEY, J. and MOLOKO, A.2015. Development of *Sitotroga cerealella* (Oliver) on certain cereal grains in Botswana. *J. Appl. Zool. Res.***26:**179-186.

AWANDA, A., VAN DER AUWERA, P., MEUNIER, F., DANEAU, D. and KLASTERSKY, J. 1999. Streptococcal and enterococcal bacteremia in patients with cancer. *Clin. Infect. Dis.***15:**33-48.

BLAZAR, J.M., LIENAU, E.K. and ALLARD, M.W. 2011. Insects as vectors of foodborne pathogenic bacteria. *Terr. Arthropod Rev.* **4:**5-16.

BELL, R.J.and WATTERS, F.L. 1982. Environmental factors influencing the development and rate of increase of *Prostephanus truncatus* (Horn)(Coleoptera: Bostrichidae) on stored maize. *J. Stored Prod. Res.* **18:**131-142.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 1993. Nosocomial enterococci resistant to vancomycin. *MMWR*, **42:**597-99.

CHANNAIAH, L.H., SUBRAMANYAM, B., MCKINNEY, L.J.and ZUREK, L. 2010. Stored-product insects carry antibiotic-resistant and potentially virulent enterococci. *FEMS Microbiol. Ecol.* **74**:464-471.

CHINGWARU, W., MPUCHANE, S.F.and GASHE, B.A.2003. *Enterococcus faecalis* and *Enterococcus faecium* isolates from milk, beef, and chicken and their antibiotic resistance. *J. Food Prot.* **66:** 931-936.

CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2006. Performance standards for antimicrobial disc susceptibility tests: approved standard; *M2-A9.Wayne PA,Clinical and Laboratory Standards Institute.*

COLLISON, E., and JOHANNES, T. 1999. The prevalence of enterococci in processed milk sold in Gaborone. *Botsw. Notes and Rec.* **31:**155-158.

DEVRIESE, L. A., M. D. COLLINS, AND R. WIRTH. 1992. The genus *Enterococcus*, p. 1465-1481. *In* BALOWS, A.,TRAPER, H.G.,DWORKIN, M.,HARDER, W. andSCHLEIFER, K.-H. (eds.). The Prokaryotes. *A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, vols. I-IV. second ed. Springer, New York.*

FRANZ, C.M.A.P.,HOLZAPFEL, W.H.and STILES, M.E. 1999. Enterococci at the crossroads of food safety? *Int. J. Food Microbiol.* **47:**1-24.

JAY, J.M., LOESSNER, M.J. and GOLDEN, D.A. 2005. *Modern Food Microbiology.* Springer, New York. KAYSER, F.H. 2003. Safety of enterococci from the medical point of view. *Int. J. Food Microbiol.* **88:**255-262.

LARSON, Z., SUBRAMANYAM, B., ZUREK, L.and HERRMAN, T. 2008. Diversity and antibiotic resistance of enterococci associated with stored-product insects collected from feed mills. *J. Stored Prod.Res.* **44:**198-203.

LINDEN, P.and MILLER, C.1999. Vancomycin-resistant enterococci: the clinical effect of a common nosocomial pathogen. *Diagn. Microbiol. Infect. Dis.* **33:**113-120.

MATSHEKA, M.I., MAGWAMBA, C.C**.**, MPUCHANE, S.and GASHE, B.A.2013. Biogenic amine producing bacteria associated with three different commercially fermented beverages in Botswana. *Afr. J. Microbiol. Res.***7:**342-350.

MOHALE, S., ALLOTEY, J.andSIAME, B.A. 2010. Control of *Tribolium confusum* J. du Val by diatomaceous earth (Protect It™) on stored groundnut (*Arachis hypogaea*) and *Aspergillus flavus* Link spore dispersal. *Afr. J. Food Agric. Nutr. Dev.***10:**2678-2694.

MURRAY, B.E. 1990. The life and times of *Enterococcus*. *Clin. Microbiol. Rev.***3:**46-65.

ONIANG'O, R.and ALLOTEY, J. 1999. Food safety and the role of government, p. 264-297.*In* OGUNRINADE, A., ONIANG'O, R. AND MAY, J. (eds.). *Not by bread alone: food security and governance in Africa. Toda Institute, South Africa. ISBN 0 620 25016 5; Witwatersrand University Press.*

RADU, S., TOOSA, H., RAHIM, R.A., REEZAL, A., AHMAD, M., HAMID, A.N., RUSUL, G. and M. NISHIBUCHI. 2001. Occurrence of the *vanA* and *vanC2/C3* genes in *Enterococcus* species isolated from poultry sources in Malaysia. *Diagn. Microbiol. Infect. Dis.* **39:**145-153.

SEVIM, A., SEVIM, L., DEMIRCI, M. and SANDALLI, C.2016. The internal bacterial diversity of stored products pests. *Ann. Microbiol.***66:**749-764.

WHITE, N.D., DEMIANYK, C.J., KAWAMOTO, H.and SINHA, R.N.1995. Population growth of *Cryptolestes ferrugineus* and *C. pusillus* (Coleoptera: Cucujidae) alone, or in competition in stored wheat or maize at different temperatures. *Bull. Entomol. Res.* **85:**425-429.

WITTE, W. 2000. Selective pressure by antibiotic use in livestock. *Int. J. Antimicrob. Agents* **16:** S19-S24.

ZORZI, W., ZHOU, X.Y., DARDENNE, O., LAMOTTE, J., RAZE D., PIERRE, J., GUTMANN, L. and COYETTE, J. 1996. Structure of the low-affinity penicillin-binding protein 5 PBP5fm in wild-type and highly enicillin-resistant strains of *Enterococcus faecium*. *J. Bacteriol.* **178:**4948-4957.