Wayamba Journal of Animal Science – ISSN: 2012-578X; P270 - P274, 2012
First Submitted March 07, 2012; Number 1332386621

INCIDENCE OF ANTIBIOTIC-RESISTANT ENTEROCOCCI IN THREE EDIBLE LAND SNAILS CONSUMED IN NIGERIA

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Food animals have been implicated to be the reservoir or carriers of so many anthropogenic pathogens. In the recent time snails have been brought close to man and the rate of snail faming has drastically increase. The possibility of this animal being a source of zoonotic pathogens to farmers and consumers inform this study. Standard microbiological methods were used to isolate and identify enterococci isolated from three edible land snails comprises of *Archachatina marginata* , *Achatina fulica* and *Limicolaria* sp. Disc diffusion method was used to determine the antibiotic susceptibility of the isolates to different antibiotics. A total of 191 enterococci isolates were recovered from land snail. *Enterococcus faecalis* was the most abundant species followed by *Enterococcus feacium*. *A. marginata* had the highest enterococcal load followed by *A. fulica* while the least was detected in *Limicolaria* sp**.** except for erythromicin, augmentin and amoxycillin the highest percentage resistance to the tested antibiotic was observed in isolates recovered from *Limicolaria* sp. No gentamicin-resistant enterococci was detected in the three land snail samples. Antibiotics susceptibilities were in the following decreasing order: GEN<CHL<TET<ERY<COT<STR<AM X<AUG<CXC. This is an evidence that snail could be an important public health risk to snail farmers, sellers and consumers alike.

Key Words: Enterococci, snail, pathogen, *Archachatina marginata, AchatinA fulica* and *Limicolaria* sp.

Enterococci are facultative anaerobic Gram-positive cocci. They are widely distributed in nature. Enterococci have been recovered from man and other mammals like cattle, pigs, dogs, cats, sheep, horses, goats and rodents. Enterococci have also been isolated from lower animals like birds, reptiles and insects (Deibel,1964). Although the primary source of enterococci is the intestine of humans and warm-blooded animals, they are frequently isolated from environmental sources such as air, soil, surface waters, sewage and vegetation (Devriese *et al*., 1996).

Enterococci survive in environmental conditions that destroy other microorganisms of sanitary significance (Ksoll *et al*., 2007). Unlike other faecal bacteria, enterococci survive for a long time outside their natural intestinal hosts hence its current choice as better indicator of faecal pollution. Enterococci have surfaced as organisms of importance due to the emergence of multiple-drug-resistant strains. Their ability to acquire resistance to antibiotics and transfer the same to other related organisms present a significant challenge for therapeutic measures (Huycke et al. 1998; Fisher and Phillips 2009).

In recent times in Nigeria, snail has been brought closer to man than before and its farming is gaining importance. This is due to its increasing consumption as a result of its high nutritive qualities (Adeyeye, 1996; Adeyeye, 1998, Ademolu *et al*., 2004; Babalola and Akinsoyinu, 2009). Although land snails feed mainly on fresh plants, they also feed on leaf litters (Odaibo, 1997; Iglesias and Castillejo, 1999; Chevalier et al., 2003; Hatziioannou et al., 1994). Consequently, they take in along some of the microfloral of the soil and plants.

There is no information on the association of enterococci with land snails commonly found in Nigeria hence this study. We investigated the occurrence and distribution of enterococci in the three types of land snails and the susceptibility pattern of the isolates to common antibiotics used in clinical practices.

MATERIALS AND METHODS

Collection of the snail samples

Snail samples comprises of *Archachatina marginata, Achatina fulica* and *Limicolaria* sp. were purchased from local markets in Ado-Ekiti, Akure and Osogbo, all in the western part of Nigeria. For each of the three snail samples, a total of five were purchased from each location. The samples were purchased between the months of September and October, 2010. Samples were then identified in the Department of Zoology, Ekiti State University Ado-Ekiti.

Preparation of Samples and Isolation of *Enterococcus* species

The shells of the snail were partially removed in order to take off the anterior part of the digestive tract. The intestine was removed and macerated in aseptically and transferred into 9 ml of normal saline [0.9% (w/v) sterile NaCl]. Each homogenate was serially diluted and plated on Bile aesculine azide agar (Oxoid) and incubated for 24h at 37 ^{o}C. The identification of *Enterococcus* spp. was carried out on 18h old culture using standard methods of Olutiola *et al.* (2000), Fawole and Oso (2001) and Facklam and Collins (1989). The results of the biochemical were interpreted according to Holt *et al*. (1994).

Antibiotic Susceptibility Testing

The isolates were grown at 37°C in Mueller-Hilton broth (Oxoid) for 16-18h and diluted to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625nm and stored at 4^{o}C. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (2008). The isolates were tested against eight commercial antibiotic disks (Abtek Biologicals Limited) with their concentrations (in μg): amoxicillin (25), gentamicin (10), cotrimoxazole (25), augmentin (30), tetracycline (30), erythromycin (5) chloramphenicol (30), cloxacillin (5) and streptomycin (10).

RESULTS

A total of 191 enterococci isolates were recovered from land snail and 187 were identified to the species level while the remaining isolates cannot be identified beyond the genera taxonomic level. As shown in Table 1, *E. faecalis* was the most common species isolated from the three samples, comprising 65 (74.71%) in *A. marginata,* 47 (79.66%) in *A. fulica* and 29 (64.44%) in *Limicolaria* sp. *E. faecium* accounted for 18.39% (n=16), 22.22% (n=10) and 13.56% (n=8) of the total enterococci isolated from *A. marginata, Limicolaria* sp. and *A. fulica* respectively. *A. marginata* had the highest enterococcal load followed by *A. fulica* while the least was detected in *Limicolaria* sp**.** Enterococcal isolates that can not be identified to the species level accounts for 2.09% of the total isolates.

The antimicrobial susceptibility pattern of the enterococci isolated from the land snail assumed a varying degree. Except for ERY, AUG and AMX the highest percentage resistance to the tested antibiotics was observed in isolates recovered from *Limicolaria* sp. The highest resistance to ERY and AUG was observed in *A. fulica* isolates while

isolates from *A. marginata* shows the highest resistance to AMX.

Table 1: Distribution of enterococci load in the land snail screened

Enterococci	Land snail type			Total (n=191)
	A. marginata (n=87)	*A. fulica* (n=59)	*Limicolaria* sp (n=45)	
E. faecalis	65(74.71 %)	47(79.66%)	29(64.44%)	141(73.82%)
E. feacium	16(18.39 %)	8(13.56 %)	10(22.22)	34 (17.80%)
E. gallinarum	4(4.60%)	2(3.39 %)	4(8.88 %)	10(5.24 %)
E. durans	1(1.15%)	0	1(2.22 %)	2(1.05)
Enterococcus spp	1(1.15%)	2(3.39 %)	1(2.22 %)	4(2.09%)

No gentamicin-resistant enterococci was detected in the three land snails while a total of 78(40.8%) were resistant to CLX. Highest resistance was observed against CXC (97.9%) closely followed by AUG and AMX with 91.6% and 91.1% respectively as shown in Table 2. The antibiotic resistance profile of enterococci from land snail to the antibiotics was in the following decreasing order: GEN<CHL<TET<ERY<COT<STR<AMX< AUG<CXC.

Table 2: Antibiotic resistant pattern of enterococci isolated from different land snail samples in Nigeria

Antibiotics	Land snail type			
	A. marginata (n=87)	*A. fulica* (n=59)	*Limicolaria* sp (n=45)	Total (n=191)
GEN	0 (0)	0(0)	0(0)	0(0)
TET	42(43.3%)	36(61.0%)	31(68.9%)	109(57.1%)
COT	60(69.0%)	27(45.8%)	36(80.0%)	126(66.0%)
ERY	50(57.5%)	46(78.0%)	16(35.6%)	112(58.6%)
CHL	22(25.3%)	11(18.6%)	45(100%)	78(40.8%)
CXC	87(100.0%)	55(93.2%)	45(100%)	187(97.9%)
AUG	86(98.9%)	59(100%)	30(66.7%)	175(91.6%)
STR	47(54.0%)	57(96.6%)	45(100%)	149(78.0%)
AMX	85(97.7%)	57(96.6%)	32(71.1%)	174(91.1%)

DISCUSSION

In this study a total of 191 enterococci isolates were isolated from land snails. Their distribution follows the same trends with those isolated from higher animals. *E. faecalis* and *E. feacium* accounted for 76.44% of the total enterococci isolates from snail samples. This is similar to the report of Klein (2003) who reported the two *Enterococcus* species to be frequently isolated from the mammals. This trend however is different to livestock with fewer occurrences of *E. faecium* and *E. faecalis* (Franz *et al*., 1999). Land snails may have acquired the pathogen from the contaminated soil or food (which is largely of plant origin). Green plants which mainly consumed by snails have been reported to habour *Enterococcus* species (Kuhn *et al*., 2003). There is a possibility that land snails could be a reservoir of antimicrobial-resistant enterococci. Enterococci have been recovered from animal-based food samples like cheese, fish, sausages, minced beef and pork in spite of the heat treatment (Foulquie Moreno *et al*., 2006; Klein, 2003).

In all the species of *Enterococcus* isolated were found resistant to antibiotics of very high clinical importance (Salem-Bekhit *et al.,* 2012). *Enterococcus* spp. isolated were resistant to most antibiotics used as first line antibiotics in clinical practice. The antibiotic resistant pattern of the isolates however, were lower than the resistance of *Enterococcus* spp. isolated from fresh water tilapia fish (David *et al.,* 2010) and clinical samples (Salem-Bekhit *et al.,* 2012). Also of concern is the multiple antibiotic resistance notice among the isolates. Snails may be an important reservoir of antimicrobial-resistant enterococci (Seepersadsingh *et al.,* 2004). This could be an important public health risk to snail farmers, sellers and consumers alike.

This is a preliminary study on the isolation, characterization and antimicrobial pattern of enterococci. This study lends credence to the association of enterococci with land snails. Antimicrobial resistance of enterococci from snails was comparatively lower than those of clinical isolates however, snails should be considered as important potential source of resistant enterococci. The potential spread of antimicrobial-resistant pathogens from

snail to humans should be borne in mind and guide against.

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