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Full Length Research Paper

Biochemical, enterotoxigenicity and antibiogram profiles of *Staphylococcus aureus* isolated from intestines of snails

Efuntoye, M. O.*, Mabekoje, O. O. and Adekoya, F. A.

Department of Microbiology, Olabisi Onabanjo University, P. M. B. 2002, Ago-Iwoye, Nigeria.

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To evaluate the prevalence of enterotoxigenic *Staphylococcus aureus* in intestines of land snails and to determine the antibiogram of the isolates, samples for culture were obtained from ninety-nine different land snail species. Isolates of *S. aureus* were tested for enterotoxin production using the SET-RPLA and their antibiotic susceptibilities were determined by disc diffusion methods according to NCCLS standards. A total of 42 *S. aureus* isolates were recovered. Of these, 28 were enterotoxigenic. Antimicrobial susceptibility testing showed that all of the *S. aureus* isolates had 100% resistance to augmentin, cloxacillin, cefuroxime and amoxycillin-clavulanic acid and showed varying resistance to other antibiotics tested. The results showed that enterotoxigenic *S. aureus* could be found in snails and could show resistance to commonly used antibiotics. The presence of enterotoxigenic *S. aureus* underscores the need for thorough cooking of snails before consumption in order to prevent staphylococcal food poisoning.

Key words: Snails, *Staphylococcus aureus*, enterotoxin, antibiotic sensitivity.

INTRODUCTION

Snails belong to the group of invertebrate animals known as molluscs, the second largest group in the animal kingdom. The genera *Archachatina, Achatina* and *Limicolaria* are very well known edible land snails in Nigeria. The species of *Archachatina (Arch.)* and *Achatina (Ach.)* constitute the most conspicuous terrestrial molluscs in the southern part of Nigeria and are the most commercially important. They are eaten both in the rural and urban communities. There has been an upsurge in snail farming in different parts of the world as a result of an increase international trade in live snails and processed snail products. This upsurge result from studies on their nutritive value which showed the 'foot' (the part eaten by people) to be rich in essential acids (Creswell and Habibie, 1981). Snail meat is low in sodium, fat and cholesterol but contains high amount of iron and calcium, hence it is useful in the treatment of anaemia, hypertension and other fat-related ailments. In addition, the inedible parts are used in animal feed preparation, in pearl production and as tourist souvenir.

Bacteriological studies (Akinboade et al., 1980; Akpavie et al., 2000) showed that various bacteria which are potential pathogens inhabit different organs and tissues including lungs, haemocyanin, liver, kidney, crop and stomach of clinically healthy African giant land snails. However there is paucify of report on staphylococci inhabiting the intestine of snails. Staphylococcal food poisoning continue to be one of the leading reported cases of bacterial food-borne diseases all over the world and new food vehicles continue to be associated. This paper reports the incidence of enterotoxigenic *Staphylococcus aureus* isolated from snails intestines in Ago-Iwoye (a town located in southwestern Nigeria) and its environs and their antibiotic susceptibility profiles.

MATERIALS AND METHODS

One hundred and thirty seven samples of snail species comprising *Achatina achatina* (39), *Achatina fulica (*15), *Archachatina marginata* (74) and *Limicolaria* sp. (9) were purchased from local vendors along Ibadan-Ago-Iwoye road within a period of 10 weeks, packed in separate cellophane bags and transported to the

*Corresponding author. E-mail: mosolatoye@yahoo.com.

Table 1. Distribution pattern of Staphylococcus spp. and enterotoxigenic *S. aureus* in genera of snails.

Genera of snails analyzed	Identified isolates of *Staphylococcus* spp.*	Enterotoxigenic *S. aureus*** (Number)
A. marginata (74)	*S. aureus* (27) *S. saprophyticus*(4) *S. sciuri* (4) *S. gallinarum* (2) *S. lentus* (1) *S. salivarius* (2)	19 *(SEA)*
A. achatina (39)	*S. aureus* (7) *S. saprophyticus* (6)	4 *(*3 *of SEA and*1 *SEC)*
A. fulica (15) *Limicolaria* sp. (9)	*S. aureus* (8) *S. saprophyticus*(8)	5*(SEA)*

*Figure in parenthesis indicates the number of isolates obtained from the specific genera of snails.**Figure in parenthesis indicates the number of enterotoxin type isolates.

laboratory immediately. The shells were cracked and the fleshy portions were removed. The intestines were identified, dissected with the aid of a sterile scalpel blade and the intestinal contents transferred into a sterile universal bottle. The contents were streaked onto mannitol salt agar (MSA), nutrient agar (NA) and blood agar (BA) and inoculated plates were incubated at 37◦C for 24 h. After incubation, colonies exhibiting morphology resembling staphylococci characteristics were selected, purified and identified further using the conventional tests such as Gram staining, production of catalase. oxidase, coagulase, phosphatase, staphylokinase and DNAase and fermentation of sugars. The haemolytic character in the isolates was tested in blood agar plates (Barrow and Feltham, 1993).

Enterotoxin analysis

The "SET –RPLA" kit (Oxoid, Basingstoke, UK) that relied on rapid reversed agglutination to detect enterotoxin in *S. aureus* was used. Forty –two isolates of *S. aureus* were tested and results were interpreted in accordance with manufacturer's guidelines.

Antibiogram pattern

The isolates of *S. aureus* were subjected to antibiotic susceptibility test by the disc diffusion method (Bauer et al., 1966) using standard antibiotic discs including penicillin, tetracycline, cotrimoxazole, erythromycin, gentamicin, streptomycin, chloramphenicol, cloxacillin, cefuroxime and amoxycillin-clavulanate. All tests were performed on Mueller-Hinton agar (Oxoid, Basingstoke, U.K).

The resistance of the *S. aureus* isolates to some fluoroquinolones was also determined. The zone of inhibition was measured and the results were interpreted based on the guidelines of NCCLS (1999).

RESULTS

The species of the snails investigated carried at least one or two staphylococcal species in their intestine (Table 1). From the results, *S. aureus* appeared to be the predominant species in the intestine of *A. fulica* examined. The isolates of *S. aureus* were obtained from seven samples of *A. achatina* and from 27 samples of *Archachatina marginata*. In addition to *S. aureus,* coagulase negative species of staphylococci other than *Staphylococcus saprophyticus* namely *Staphylococcus sciuri*, *Staphylococcus lentus*, *Staphylococcus gallinarum* and *Staphylococcus salivarius* were isolated from *A. marginata*. All the *S. aureus* isolates produced DNAase, phosphatase and were mostly beta haemolytic. The isolates of *S. saprophyticus* were non- haemolytic and non-DNAase producers whereas other staphylococci encountered were alpha haemolytic. Three of the *S. sciuri* isolates produced DNAase and phosphatase. Twenty nine isolates (69.04%) of *S. aureus* produced staphylococcal enterotoxins (SE). Majority of the enterotoxin-producers were of SEA (93.1%). Only 2 isolates produced SEC and these isolates were recovered from *A. achatina* (Table 1).

The antibiogram pattern of staphylococcal isolates is shown in Table 2. The results show that resistance of the *S. aureus* isolates to chloramphenicol, streptomycin, gentamicin, erythromycin, tetracycline, cotrimoxazole and ampicillin were 61.1, 25.0, 13.9, 55.6, 72.2, 63.9 and 83.3%, respectively. Further, *S. aureus* isolates showed more than 80% resistance to the penicillins investigated. Apparently the penicillins are commonly used antibiotics in Nigeria. Majority of the isolates were sensitive to all the fluoroquinolones tested, except for moxifloxacin (Table 3).

DISCUSSION

The antibiogram pattern of *S. aureus* isolates is consistent with the earlier research investigations, although the sources of the isolates were varied (Ohehe, 1997;

Table 2. Susceptibility of *S. aureus* isolates to antibiotics.

***S. aureus* isolates from respective genera of snails**	**Antibiotcs tested* (No. of isolates sensitive)**							
	Chl	**Str**	**Gen**	**Ery**	**Tet**	**Cot**	**Amp**	**Pen**
Twenty four(*A. marginata*)	10	20	22	9	7	10	4	2
Four(*A. achatina*)	1	2	3	2	1	2	1	-
Eight (*A. fulica*)	3	5	6	5	2	1	1	-

*All the *S. aureus* isolates showed 100% resistance to augmentin, amoxycillin-clavulanate, cloxacillin and cefuroxime. Chl, chloramphenicol; Str, streptomycin; Gen, gentamicin; Ery,erythromycin; Tet, tetracycline; Cot, cotrimoxazole; Amp, ampicillin; Pen, penicillin.

Table 3. Frequency of resistance of *S. aureus* to fluroquinolones.

Antibiotic	**Frequency of resistance (%)***
Nalidixic acid	4 (11.1)
Ciprofloxacin	3 (8.3)
Norfloxacin	2 (5.6)
Pefloxacin	2 (5.6)
Ofloxacin	1 (2.8)
Enoxacin	2 (5.6)
Moxifloxacin	- (0)

*Number of isolates tested for each antibiotic was 36.

Obiazi et al., 2007; Donkor et al., 2008). The isolates showed very high susceptibility to gentamicin and streptomycin with only five and nine isolates being resistant to the antibiotics respectively. This is not surprising since aminoglycoside antibiotics have been reported to be bactericidal (Koneman et al., 1992). There have been reports of high level of sensitivity of clinical isolates of *S. aureus* to gentamicin (Manie et al., 1998; Lilenbaum et al., 1998; Uwaezuoke and Aririatu, 2004; Ako-Nai et al., 2005; Gums, 2006).

Obiazi et al. (2007) reported a high susceptibility (80%) of skin isolates of *Staph. aureus* to gentamicin. However, clinical isolates of methicilin resistant *S. aureus* to which gentamicin had shown weak susceptibility have been reported (Bukharie and Abdelhadi, 2001; Panhotra et al., 2005; Matynia et al., 2005).

Obiazi et al. (2007) and Akortha and Ibadan (2008) also reported 28 and 50.2% susceptibility of their clinical isolates to gentamicin, respectively. The lower incidence of resistance to the fluoroquinolones is possibly due to the absence of selective pressure occasioned by widespread antimicrobial abuse involving these drugs. The fluoroquinolones are not readily available on the sale counters. Only two of the *S. aureus* isolates in this study exhibited resistance to methicillin.

The present study revealed that species of *Staphylococcus* inhabit the intestine of land snails in Ago-Iwoye environment. In earlier studies, *S. aureus,* among other bacteria were isolated from different organs of snails including lungs, kidney, crop, stomach and haemocyanin, whereas *Staphylococcus epidermidis* was recovered from foot and kidneys (Akinboade et al., 1980; Akpavie et al., 2000). These investigators could not isolate all the species of *Staphylococcus* recovered in this study from the intestine of snails. This may mean that the species of organism recovered is a function of the habitat where the snails are reared. It is suggested that the occurrence of these species of *Staphylococcus* in the snails could have arisen from contact with the soil and pollution with sewage in the environment of breeding and domestication (Buxon and Frazer, 1977). The presence of coagulase-negative staphylococci in some *A. marginata* samples is not surprising since these species are associated with farm and pet animals. Isolates of *S. sciuri* have been reported in goats, sheep, cattle, pigs, squirrels and wild birds while *Staphylococcus gallinarum* has been reported only in poultry. It is common practice in the environment of this study to find these animals. In most cases many of the animals are kept as free range animals and may shed these bacteria in the environment. Soils contaminated with the organisms in which environment the snails were obtained are possibly responsible for their presence in the snails investigated (Nagase et al., 2002; Lee, 2003; Baptiste et al., 2005; Voss et al., 2005).

It is of interest to observe that *Escherichia coli*, a regular inhabitant of intestinal tract of vertebrates was not recovered in the snails samples investigated. Although in an earlier study, few isolates of enterotoxigenic *E. coli* were isolated, the investigators could not establish that these isolates were normal inhabitant of the snails intestines (Akinboade et al., 1980). The results of the present study could suggest that *E. coli* is indeed not a normal flora of intestines of snails.

The presence of isolates of *S. aureus* positive for coagulase, DNAase, phosphatase and enterotoxin in snails pose the problem of a health risk to unsuspecting consumers. Enterotoxigenic *S. aureus* have been implicated in diarrhoea associated with children in Nigeria (Efuntoye and Adetosoye, 2003). It becomes desirable to adequately cook the snails prior to human consumption. The presence of *S. sciuri* and *S. gallinarum* also needs to be viewed with concern.

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