



Antimicrobial resistance pattern of *Enterococcus* spp. isolated from Cockroaches from Ekiti State Teaching Hospital

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Abstract

Antimicrobial resistance pattern of *Enterococcus* spp isolated from cockroaches in different units/wards of Ekiti State Teaching Hospital was assessed using standard microbiological methods. Selected isolates were characterized by standard cultural and biochemical tests and they were tested with different antibiotics using disk diffusion method. Antibigram profiles show a relatively complete resistance (100%) of the isolates to cotrimoxazole, amoxicillin and cloxacillin. The least resistance was observed against gentamicin while few of the isolates were sensitive to erythromycin and tetracycline. It was observed that all the isolates across the wards/unit exhibited multiple resistances to the antibiotics used as majority showed 50% and above resistance to the antibiotics combinations. Eight selected isolates with multiple resistance harbored plasmids all of which were not less than 1.4 kbp in molecular weight. Hence, it showed that the gene coding for the isolates were located on the plasmid DNA while the remaining isolates which have no plasmid might showed gene coding for antibiotic resistance being located on chromosomal DNA. The results of the study suggest that cockroach infestation is a public health problem in Hospital.

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Introduction

Cockroaches are insects of the order Blattaria or Blattodea, of which about 30 species out of 4,500 total species are associated with human habitations. About four species are well known as pests. (Valles *et al.*, 1999). Among the best-known pest species are the American cockroach, *Periplaneta americana*, which is about 30 millimeters (1.2 inches) long, the German cockroach, *Blattella germanica*, about 15 millimeters (0.59 inches) long, the Asian cockroach, *Blattella asahinai*, also about 15 millimeters (0.59 inches) in length, and the Oriental cockroach, *Blatta orientalis*, about 25 millimeters (0.98 inches). Tropical cockroaches are often much bigger, and extinct cockroach relatives and 'roachoids' such as the Carboniferous *Archimylacris* and the Permian *Apthoroblattina* were not as large as the biggest modern species (Grimaldi, 1997; Pai and Cheng, 2004). The economic importance of cockroaches is one of the most commonly noted household pest insects (Rivault *et al.*, 2003). They feed on human and pet food, and can leave an offensive odour (Brenner *et al.*, 2007). They can also passively transport microbes on their body surfaces including those that are potentially dangerous to humans, particularly in environments such as hospitals (Elgderi *et al.*, 2006).

Enterococci are gram positive, facultative anaerobic, lactic acid cocci bacteria in the phylum Firmicutes, class Bacilli, order Lactobacillales, family Enterococcaceae and genus *Enterococcus* (Schleifer and Kilpper-Balz 1984). They are tolerant to a wide range of temperatures, pH and nutrient-poor environments (Lleo *et al.*, 1998, Heim *et al.*, 2002, Fisher and Phillips 2009). Enterococci are primarily commensals of the digestive tract of a wide range of animals including humans. *E. faecalis* and *E. faecium* are two of the most prevalent species and are of most medical and veterinary significance. Over the past few decades enterococci have emerged as the third most common nosocomial pathogens overall after *Escherichia coli* and *Staphylococcus aureus* (Arias and Murray, 2008). Specifically, they are the second most common bacteremia pathogen and third most common for urinary tract and surgical site infections (Hidron *et al.*, 2008).

This study aims at the isolation of *Enterococcus* species from cockroaches in different wards/units of Ekiti State Teaching Hospital and determines the resistance pattern of the isolates to commonly used antibiotics.

Materials and methods

Sample collection

A total of 120 cockroaches were collected from different wards of the Ekiti State Teaching Hospital between August and October 2012. The cockroaches were collected into sterile containers and were immediately transported to the laboratory.

Isolation of *Enterococcus* species

The surface and the internal components of the cockroaches were inoculated into Nutrient Agar (Oxoid, England) for enrichment purposes, followed by incubation at 37°C for 24 hours. Re-inoculation was made from the enrichment broth to Bile Esculin Agar (Oxoid, England) and incubated at 37°C for 24 - 48 hours. Characteristic black colonies on Bile Esculin Agar were considered as *Enterococcus* species.

Biochemical characterisation

Suspected colonies were subjected to gram's reaction, catalase, growth in 6.5% NaCl, Voges Proskauer, aesculin hydrolysis and sugars utilization tests.

Antimicrobial susceptibility test

Antimicrobial susceptibility of the isolates was tested using multi-discs diffusion method. The following antibiotics were tested for its efficacy against the isolates; Augmentin (30 µg), Amoxycillin (30 µg), Erythromycin (5 µg), Gentamycin (10 µg), Tetracycline (10 µg), Cloxacillin (5 µg), Chloramphenicol (30 µg) and Cotrimoxazole (25 µg).

Plasmid profile analysis

Isolation of plasmid DNA of the isolated *Enterococcus* spp were determined based on the methods of Ausubel *et al.* (1995). Plasmid profile analyses of the isolated strains were determined using 0.8 % agarose gel electrophoresis as described by Akingbade *et al.* 2012.

Results

A total of 80 isolates of *Enterococcus* spp isolated from cockroaches from different wards at the Ekiti State Teaching hospital were selected and subjected to various physiological and biochemical tests. The summary of the biochemical characterization is shown in Table 1.

Table 2 shows the summary antibiotics susceptibility pattern of *Enterococcus* spp isolated from Cockroaches in the different areas of the Ekiti State Teaching Hospital. The high susceptibility of

Table 1: Biochemical characterisation of *Enterococcus* species isolated from Ekiti State Teaching Hospital

	Number of positive strains	Number of negative strains	Total number of test strains
Gram's reaction	80	0	80
Motility	0	50	80
Catalase	80	0	80
Oxidase	0	50	80
Growth in 6.5% NaCl	80	0	80
Voges Proskauer	80	0	80
Esculin hydrolysis	80	0	80
Sugar utilization (Production of gas and acids)			
Mannitol	80	0	80
Sorbitol	80	0	80
L-Arabinose	80	0	80
Lactose	80	0	80
Sucrose	76	4	80
D-Xylose	65	15	80
Glucose	80	0	80

Table 2: Antibiotics susceptibility pattern of *Enterococcus* species isolated from different locations in the hospital

	Number of susceptible strains					Number of resistant strains				
	MSW	FSW	LAB	EMG	OPD	MSW	FSW	LAB	EMG	OPD
GEN	8	9	8	6	5	2	1	2	4	5
COT	0	0	0	0	0	10	10	10	10	10
CHL	7	8	5	7	7	3	2	5	3	3
AUG	1	0	0	0	0	9	10	10	10	10
AMX	0	0	0	0	0	10	10	10	10	10
ERY	7	6	2	2	4	3	4	8	8	6
TET	7	4	5	7	6	3	6	5	3	4
CXC	0	0	0	0	0	10	10	10	10	10
Total	30	27	20	22	22	50	53	60	58	58
	(37.50)	(33.50)	(25.00)	(27.50)	(27.50)	(62.50)	(66.25)	(75.00)	(72.50)	(72.50)

Key: (MSW) Male surgical ward, (FSW) Female surgical ward, (LAB) Laboratory, (EMG) Emergency, (OPD) Outpatient department

(GEN) Gentamicin, (COT) Cotrimoxazole, (CHL) Chloramphenicol, (AUG) Augmentin / Amoxicillin-clavulante, (AMX) Amoxicillin, (ERY) Erythromycin, (TET) Tetracycline, (CXC) Cloxacillin

Note: Numbers in parentheses are percentages

strains was observed in gentamicin, chloramphenicol, erythromycin and tetracycline. The least resistance was exhibited against gentamicin. All the isolates exhibited complete resistance (100%) to amoxicillin and cloxacillin.

The multiple resistance pattern of *Enterococcus* spp isolated from cockroaches in different units of Ekiti State Teaching hospital is show in Table 3. Most of the isolates showed resistance to more than four antibiotics. The plasmid profile of selected *Enterococcus* spp isolates exhibited high percentage of multiple resistances across the wards/unit of Ekiti State Teaching hospital. Eight strains contained plasmid of which six contained more than one plasmid. Selected isolate Male surgical ward strain 1 and Outpatient Department strain 2 had no plasmid, while six (6) strains from the ten selected strains had two plasmids.

Discussion

Currently, enterococci are the third most common bacterial pathogens isolated from nosocomial bloodstream infections and the most common pathogen in surgical-site infections reported from intensive care units in the United States (Chatterjee *et al.*, 2007). Besides surgical-site infections and bacteremias, enterococci cause peritonitis, urinary tract infections, endocarditis, and a variety of device-related infections (Dautle *et al.*, 2002). The resistance pattern of *Enterococcus* spp isolated from cockroaches in

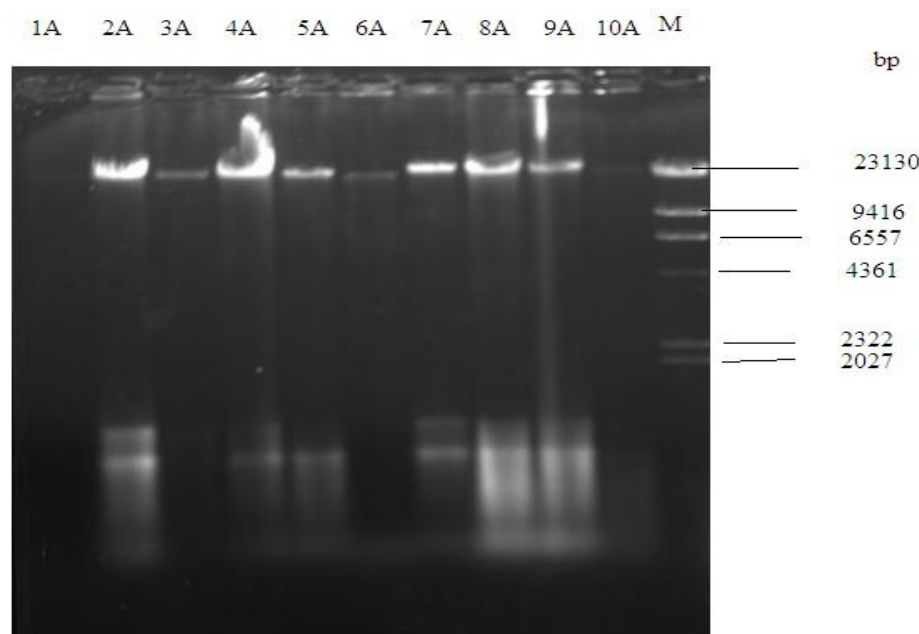
different wards/ units of Ekiti State Teaching Hospital in this study showed complete resistance (100%) against Cotrimoxazole, Amoxicillin and Cloxacillin. The least resistance was observed against gentamicin while erythromycin and tetracycline showed relative amount of antimicrobial activity against the isolates. The result of this study is similar to that of Aqeel *et al.* (2011) which showed high resistance of *Enterococcus* spp isolated from cockroaches to Erythromycin, Tetracycline and other commonly used antibiotics. The justifications for these higher incidences of infections with multi-resistant enterococci have partly been attributed to an increase in immuno-compromised and critically ill patients (Iredell and Lipman, 2005), also mainly due to exposure to these traditional antibiotics (Ammerlaan *et al.*, 2009). Enterococci are intrinsically resistant to low levels of aminoglycosides and to cephalosporines. In addition, enterococci are able to acquire resistance against a broad range of antibiotics belonging to different antibiotic classes through a combination of mutation and horizontal gene transfer (Kak and Chow, 2002).

Virtually all the isolates across the wards/unit exhibited multiple resistances to the antibiotics used as majority showed 50% and above resistance to the antibiotics combinations. Multiple drug resistance patterns were observed in all the isolates from hospitals and our observation agrees with report of Fortedor *et al.*, (1999). The occurrence of multi-drug resistant strains of *Enterococcus* spp

Table 3: Plasmid profile and molecular weight (Kbp) *Enterococcus* spp isolated from cockroaches in different Units / Ward of Ekiti State Teaching Hospital

S/N	Isolates	Organisms	Molecular weight of DNA (kbp)	Numbers of plasmids	Phenotypic resistance of Selected isolates
1	1A	Male surgical ward strain 1	NIL	0	GEN/COT/CHL/AUG/AMX/ERY/TET/CXC
2	2A	Male surgical ward strain 2	1.4, 1.6	2	GEN/COT/CHL/AUG/AMX/ERY/TET/CXC
3	3A	Female surgical ward strain 1	1.8	1	GEN/COT/CHL/AUG/AMX/ERY/TET/CXC
4	4A	Female surgical ward strain 2	1.4, 1.6	2	COT/CHL/AUG/AMX/ERY/TET/CXC
5	5A	Laboratory strain 1	1.4, 1.6	2	COT/CHL/AUG/AMX/ERY/TET/CXC
6	6A	Laboratory strain 2	1.4, 1.8	2	COT/CHL/AUG/AMX/ERY/TET/CXC
7	7A	Emergency unit strain 1	1.6, 1.8	2	COT/CHL/AUG/AMX/ERY/TET/CXC
8	8A	Emergency unit strain 2	1.6, 1.8	2	COT/CHL/AUG/AMX/ERY/TET/CXC
9	9A	Out-patient Department strain 1	1.8	1	COT/CHL/AUG/AMX/ERY/TET/CXC
10	10A	Out-patient Department strain 2	NIL	0	GEN/COT/CHL/AUG/AMX/ERY/CXC

Key: (GEN) Gentamicin, (COT) Cotrimoxazole, (CHL) Chloramphenicol, (AUG) Augmentin / Amoxicillin-clavulante, (AMX) Amoxicillin, (ERY) Erythromycin, (TET) Tetracycline, (CXC) Cloxacillin

**Figure 1: Photographic representation of plasmid profile of selected *Enterococcus* spp isolated from Cockroaches in different Units / Wards of Ekiti State Teaching Hospital**

from cockroaches in this study agrees with past reports (Fortedor *et al.*, 1999, Pai *et al.*, 2005; Jacobsen *et al.*, 2007).

The result of the plasmid profile analysis shown in table 3 revealed that 8 out of the selected 10 strains of *Enterococcus* spp exhibited the presence of at least one plasmid mediated antibiotics resistance. This conforms to the study of Piriyaama *et al.* (2003) that detected plasmids as a means of horizontal transfer of resistance genes among *Enterococcus* spp isolates. The different types of autonomous replicating extra-chromosomal genetic materials that possess the attributes to confer resistance have been documented (Weaver *et al.*, 2002, Top *et al.*, 2008, Akhtar *et al.*, 2009; Hegstad *et al.*, 2010).

The non-presence of plasmids in the two strains (designate 1A and designate 10A) showed that resistance gene could have been acquired vertically. Expectedly, the result of the presence of plasmids in isolated strains agrees with the study of Oleghe *et al.* (2011) which reported plasmids as the major source of acquiring

resistant strains to antibiotics in most pathogenic bacteria (Tenova, 2006).

Conclusively, this study shows infestation is a major public health problem. Proper sanitary policies must be instituted in the hospital vicinities to prevent the survival and multiplication of cockroaches and other crawling insects in the environment which could harbor many multiple drug resistant pathogens.

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