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Research Article

Phenotypic Characterization and Antibiotic Susceptibility Profile of Coagulase Positive Staphylococci from The External Surfaces of Hospital Cockroaches (*Periplaneta americana*)

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ABSTRACT

A study to phenotypically characterize and determine the antibiogram of coagulase positive Staphylococci (CoPS) from the external surfaces of hospital cockroaches (*Periplaneta americana*) was conducted using standard microbiological methods. Out of the 50 cockroaches collected from various hospitals in Uyo, sixty-two percent (n = 31) had coagulase positive Staphylococci which consisted of *Staphylococcus aureus* (44.0 %; n = 22) and *Staphylococcus intermedius* (18.0 %; n = 9). The CoPS isolates showed 100% resistance to Penicillin, Tetracycline, Clindamycin and 80.6% sensitivity to Amoxicillin-clavulanate. The CoPS showed multiple antibiotic resistances to ≥ 3 antibiotics, with 60 % exhibiting resistance to 6 antibiotics. Out of the 80 % (n = 31) of the multidrug resistant CoPS that were sensitive to Amoxicillin-clavulanate, none of them showed production of beta lactamase. The cockroaches bore multiple antibiotic resistant CoPS on their external surfaces and their contact can initiate contamination of patients' food. Pest control measures in hospital are hereby recommended to minimize cockroach related infections.

Keywords: *Periplaneta americana*, Coagulase positive, Staphylococci, Clindamycin, Amoxicillin-clavulanate, beta-lactamase

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INTRODUCTION

Cockroaches are the most common and globally present pest with over 3,500 species. They are persistent and troublesome to homes, restaurants, hospitals, warehouses, offices, and other structures with food processing areas. Their presence in almost all industrial yards could be attributed to their ability to successfully exploit the niches within human habitation (Gary and Durben, 2004). Cockroaches are known to feed omnivorously by destroying fabric and paper products. They are also noxious by indiscriminate deposition of faecal materials; release stain and odour to surfaces. (Gehad and Eman, 2011; Valles *et al.*, 1999). Cockroaches main survival strategies are due to their tendency to coexist in biological waste products such as sewers, septic tanks, garbage cans, poultry houses, and animal cages; their nocturnal ability and laying their eggs mostly outdoors and live as clones (Melton, 2012). They are mechanical vectors for pathogenic microorganisms through the use of their feet or other body parts and have been reported to cause serious allergies in hospital and homes when humans have close contact with contaminated surfaces by them (Donkor, 2019; Menasria *et*

al., 2014). Microorganisms generally associated with cockroaches and the diseases caused include *Aeromonas* spp (wounds and other infections, diarrhoea), *Alcaligenes faecalis* (Gastroenteritis, Urinary tract infection), *B. cereus* (Food Poisoning), *B. subtilis* (Conjunctivitis), *Campylobacter jejuni* (Enteritis), *Clostridium perfringens* (Food Poisoning, Gas Gangrene), *Enterobacter* spp. (Bacteraemia), *Enterococcus* spp (UTI and wound infections), *Escherichia coli* (Diarrhoea and wound infections), *Klebsiella* spp (Pneumonia and UTI), *Mycobacterium* (Leprosy), *Staphylococcus aureus* (wound, skin and internal organs infections), *Staphylococcus epidermis* (wound infections), *Streptococcus faecalis* (Pneumonia), *Serratia* spp (Food poisoning), *Salmonella typhi* (Typhoid) and *Streptococcus pyogenes* (Pneumonia) (Ifeanyi, 2015).

Staphylococcus spp. have been isolated from Cockroach (*Periplaneta americana*) and are responsible for the occurrence of nosocomial and community-acquired infections, food-borne diseases and food poisoning (Becker *et al.*, 2014). *Staphylococcus* species (especially *Staphylococcus aureus*) is the pathogenic agent primarily involved in nosocomial infections and transmitted by Cockroaches. They are part of

the human resident microbiota, commonly colonizing skin and mucosa of healthy people, being a versatile pathogen capable of causing a wide variety of human diseases. The genus *Staphylococcus* is one of the numerous pathogens that have undergone significant changes in antimicrobial susceptibility in recent decades (Cramton *et al.*, 1999). The ability of *S. aureus* to cause various infections and toxicity, results from the production of different extracellular and surface virulence factors with adhesive properties for a range of molecules (Cassat *et al.*, 2007). These microorganisms are Gram-positive cocci, catalase-positive, about 1.0 µm in diameter, immobile, non-endospore-forming and usually non-encapsulated. This organism both exist as coagulase negative and positive. There have been reports recently relating to the isolation of Coagulase positive *Staphylococcus* species (CoPSS) from cockroaches in the hospital mostly in developing countries and cases recorded in developed countries (Becker *et al.*, 2014). This research was however aimed at the determination of phenotypic characterization and antibiotic susceptibility profile of coagulase positive staphylococci (CoPS) from the external surfaces of hospital cockroaches (*Periplaneta americana*).

MATERIALS AND METHODS

Study Area: Uyo is a city in South-Southern Nigeria and is the capital of Akwa Ibom State. Akwa Ibom State shares boundaries with Abia, Cross River and Rivers States. The population in Uyo is estimated to be about 451,128. Uyo is located between latitudes 5° 02' 37" North and longitudes 7° 54' 06" East.

Duration of Study: The research was conducted from November, 2019 to March, 2020.

Study Design: This is a cross-sectional descriptive study

Sample Collection: Fifty (N=50) cockroaches were obtained from the following hospitals: University of Uyo Teaching Hospital (n=15), Saint Luke's Hospital Anua (n=15), Mercy Clinic (n=10) and Petallice Medical Hospital (n=10). The samples were obtained using sticky traps and hand catch methods (this was carefully done to avoid spilling the internal organs of the cockroaches). The samples were obtained from sticky traps placed at the different wards, medical laboratory, operating rooms and other sections of the hospitals over a period of 2-5 days. The samples were transferred to Department of Animal and Environment Studies, University of Uyo for specie identification using a dissecting microscope using Harwood and James, 1979 method as *Periplaneta americana*. The identified samples were transferred to University of Uyo Microbiology Laboratory for microbiological analysis of samples.

Preparation of Sample (washing-enrichment approach)

The samples from the hospitals studied were well labelled using a paper tape tag. Each sample was carefully placed inside a well labelled sterile universal container containing freshly prepared normal saline water; and swirled

appropriately and carefully for about 2 min to obtain the microorganisms on the external surface (exoskeleton) of the cockroaches. The samples were removed after 2 min, while the remaining saline water samples were used for microbiological analysis.

Isolation and Identification of Coagulase Positive *Staphylococcus* (CoPS): The normal saline samples obtained from each cockroach sample were inoculated on selective Mannitol salt Agar (MSA) and incubated for 18 - 24 h at 37°C and coagulase test was done to isolate *Staphylococcus* species using standard microbiological methods according to Holt *et al.*, (2005). *Staphylococcus aureus* and *S. intermedius* were the two species of CoPS isolated; the two species were differentiated by:

- S. aureus* fermented purple maltose agar with production of acid/acetoin. The acid brings about a change in colour from purple to red or any other related colour
- S. aureus* fermented mannitol anaerobically but *S. intermedius* could not.

Thereafter, the colonies were subcultured onto plates of Nutrient agar slant, incubated at 37°C for 24 h. Pure cultures of isolates were streaked onto nutrient agar slants, incubated at 37°C for 24 hr and stored in the refrigerator at 4°C for characterization and identification. All isolates were Gram stained and subjected to various biochemical tests using standard microbiology methods.

Determination of Phenotypic Characterization and Antibiotic Susceptibility Profile of CoPS: *In-vitro* antibiotic susceptibility of bacterial isolates was determined using Kirby-Bauer disc diffusion technique. Briefly, 10 µL of each bacterial isolate, prepared directly from a 18-hr old agar plate and adjusted to 0.5 McFarland Standard, was inoculated on each plate of Mueller Hilton Agar (MHA). Susceptibilities of CoPS isolates were tested against several types of antibiotic groups including Penicillin (10 U), Tetracycline (30 µg), Oxacillin (1 µg), Clindamycin (2 µg), Erythromycin (15 µg), Cefotaxime (30 µg) and Amoxicillin-clavulanate (20/10 µg) antibiotic agents. The antibiotic discs were aseptically placed on the surfaces of the culture plates with sterile forceps, and the plates were incubated at 37°C for 18 hr. Thereafter, inhibitory zones were observed and measured in millimeters (mm). The interpretation of the measurement as sensitive and resistant was determined based on the criteria of CLSI (2015).

Determination of Beta- Lactamase Production: Beta-Lactamase production was phenotypically evaluated using the Nitrocefin test sticks (Oxoid, UK) as described by Akinduti *et al.*, 2012.

RESULTS

Percentage of Coagulase positive *Staphylococci*, CoPS with respect to Sample Source are depicted in Table 1. Out of the 50 samples obtained from various hospitals, 31 of samples (62%) showed the presence of Coagulase positive *Staphylococci*.

The results of the CoPS (*Staph. aureus* and *Staph. intermedius*) revealed that there were more of former than the latter. Table 2 shows the *Staphylococcus* species diversities,

occurrence and frequency of occurrence of CoPS from the *Periplaneta americana* (Cockroach) samples. The result shows that of the 50 samples obtained, 22 samples showed the presence of *Staphylococcus aureus* recorded at 44 %, while 9 samples showed the presence of *Staphylococcus intermedius* which represented 18 %.

Table 1:
Percentage of Coagulase positive *Staphylococci* (CoPS) with respect to Sample Source

| S/N | Sample Source | No of Samples | Coagulase Positive <i>Staphylococci</i> | |
|--------------|---------------|---------------|---|------|
| | | | n | % |
| 1 | UUTH | 15 | 9 | 60.0 |
| 2 | SLHA | 15 | 10 | 66.7 |
| 3 | MC | 10 | 7 | 70.0 |
| 4 | PMC | 10 | 5 | 50.0 |
| Total | | 50 | 31 | 62.0 |

Table 2:
Species diversities, occurrence and frequency of occurrence of CoPS from the *Periplaneta americana* (Cockroach) samples

| S/N | Sample Source | No. of Samples | Coagulase Positive species | | | |
|--------------|---------------|----------------|------------------------------|-------------|-----------------------------------|-----------|
| | | | <i>Staphylococcus aureus</i> | | <i>Staphylococcus intermedius</i> | |
| | | | n | % | n | % |
| 1 | UUTH | 15 | 7 | 46.7 | 2 | 13.3 |
| 2 | SLHA | 15 | 9 | 60.0 | 1 | 6.7 |
| 3 | MC | 10 | 4 | 40.0 | 3 | 30.0 |
| 4 | PMC | 10 | 2 | 20.0 | 3 | 30.0 |
| Total | | 50 | 22 | 44.0 | 9 | 18 |

KEYS:

UUTH- University of Uyo Teaching Hospital ,
 SLHA- Saint Luke’s Hospital Anua
 MC- Mercy Clinic ,
 PMC- Petalice Medical Hospital

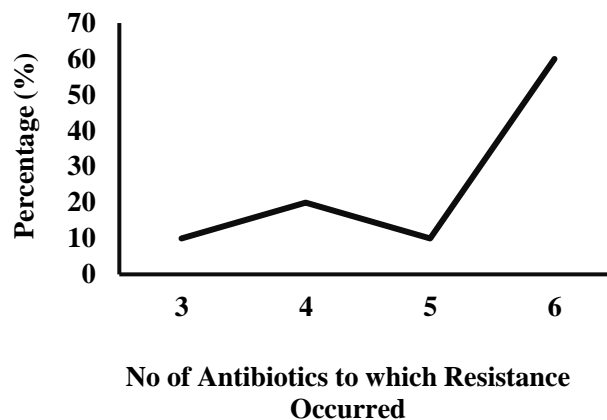
Table 3:
Antibiotic Susceptibility Profile of Coagulase Positive *Staphylococcus* species from cockroaches

| S/N | Antibiotics (disk content) | Susceptibility Pattern | | |
|-----|------------------------------------|------------------------|---------|----------|
| | | R | I | S |
| | | No (%) | No (%) | No (%) |
| 1 | Penicillin (10 U) | 31(100) | 0(0.0) | 0(0.0) |
| 2 | Tetracycline (30 µg) | 31(100) | 0(0.0) | 0(0.0) |
| 3 | Oxacillin (1 µg) | 15(48.4) | 1(3.2) | 15(48.4) |
| 4 | Clindamycin (2 µg) | 31(100) | 0(0.0) | 0(0.0) |
| 5 | Erythromycin (15 µg) | 21(67.7) | 4(12.9) | 6(19.4) |
| 6 | Cefotaxime (30 µg) | 21(67.7) | 6(19.4) | 4(12.9) |
| 7 | Amoxicillin-clavulanate (20/10 µg) | 6(19.4) | 0(0.0) | 25(80.6) |

The antibiotic profiles of coagulase positive *Staphylococcus* species from cockroaches are showed in Table 3. The CoPS showed 100 % resistant to Penicillin, Tetracycline and Clindamycin; while (67.7 %) exhibited resistance to Erythromycin and Cefotaxime. The CoPS exhibited 48.4 %

and 80.6 % sensitivity to Oxacillin and Amoxicillin-clavulanate, respectively. These CoPS from hospital cockroaches showed varied levels of multiple resistances to various conventional antibiotics.

Varied numbers and percentage of the multiple antibiotic resistance (MAR) index were shown by Coagulase Positive *Staphylococcus* species as presented in Figure 1. The result revealed that CoPS exhibited multiple antibiotic resistance to at least 3 antibiotics, while 60 % of the CoPS exhibited resistance to 6 of the tested antibiotics



DISCUSSION

Staphylococcus aureus and *S. intermedius* were isolated from cockroaches obtained from the studied hospitals and this is in agreement with the findings of Pai *et al.* (2005), Pollianna *et al.* (2014) and Abdolmaleki *et al.* (2019) that separately reported *Staphylococcus* species were isolated from *P. americana* obtained from various hospitals. The presence of CoPS in the external surface of hospital cockroaches (*P. americana*) is of public health concern; due to its ability to serve as dangerous vectors for survival and spread of *S. aureus* especially the MRSA strains in the hospital environment (Dehkordi *et al.*, 2017). The threat associated with MRSA is recognized globally by World Health Organization (WHO, 2017; Adegoke and Okoh, 2013). Cockroaches as a potential vector for MRSA should be considered a priority for research and development due to threat it could pose.

Our findings specifically revealed a high prevalence of CoPS from the *Periplaneta americana* obtained from the four selected hospitals in Uyo. These findings are in line with earlier study conducted by Abdolmaleki *et al.* (2019) and were attributed to higher presence of the cockroach in hospital sewage and hospital remnant foods. The research findings also indicate a higher percentage of occurrences of *S. aureus* compared to *S. intermedius* due to their large presence in human sewage and hospital food. This is in conformity with works of (Ben *et al.*, 2017; Dehkordi *et al.*, 2017; Abdolmaleki *et al.*, 2019) that identified and reported most *Staphylococci* on the external surface of cockroach as *S. aureus*. The significance of having patients’ food contaminated with *S. aureus* is high because the bacteria produce heat stable enterotoxin A. Enterotoxin A can withstand temperature as high as 121°C for 10 min and cause staphylococcal food poisoning if consumed (Tsutsuura and Murata, 2012; Regenthal *et al.*, 2017).

Similar multiple drug resistance exhibited by *Staphylococcus* species were isolated by Abdolmaleki *et al.* (2019). However, these CoPS isolated from the research were 50 % sensitive to Oxacillin and 80 % sensitive to Amoxicillin-clavulanate, leaving a potential therapeutic benchmark within midclass antibiotics, in case of infection from these isolates. Improved susceptibility to amoxicillin-clavulanate in comparison with amoxicillin, in this study, reflected the potential protease inhibitory activity of the clavulanic acid on beta lactamase in the similitude of methicillin in methicillin sensitive *Staphylococcus aureus*. (Evans and Wittler, 2019). The high prevalence of *Periplaneta americana* CoPS resistance against human-based antibiotic showed potential exposure and transmission of resistant MRSA strains from infected patients hospitalized in hospitals and health care units to resident cockroaches of hospitals. High prevalence of antibiotic resistance reported in our study might also be attributed to the unauthorized and indiscriminate prescription of antibiotic agents in the hospitals (Dehkordi *et al.*, 2017; Abdolmaleki *et al.*, 2019).

Observed MAR index >0.2 in our report means that the isolate source (Cockroach) is high risk, most probably harbouring isolates previously subjected to high selective pressure (Suresh *et al.* 2000; Adegoke and Okoh, 2014; 2015; Adegoke *et al.*, 2016). Multiple antibiotic resistant *Staphylococci* harboured by the selected cockroaches in the four hospitals might reflect huge presence of potential pathogens liable to cause nosocomial infection with unpredictable prognosis (Abdolmaleki *et al.*, 2019).

In conclusion, cockroaches (*Periplaneta americana*) from some hospitals in Uyo harboured *Staphylococcus aureus* and *Staphylococcus intermedius*. These isolates were resistance to Penicillin, Tetracycline, Clindamycin, Erythromycin and Cefotaxime, but mainly sensitive to amoxicillin-clavulanate without production of beta lactamase. These multiple antibiotic resistant isolates on the external surface of the cockroaches can easily be transferred to patient food via contact causing difficult to treat nosocomial infection. From the research, it is recommended that strict insect control measures to reduce the proliferation of cockroaches and their contact with household items including food should be encouraged and hospital patients must ensure that their food is properly covered to prevent cockroach related nosocomial infection.

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