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Full Length Research Paper

Identification of *Basidiobolus* species from the common house gecko (*Hemidactylus frenatus*) and their association with isolates from human basidiobolomycosis

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The genus *Basidiobolus* contains large groups of terrestrial fungi including the etiological agents of human gastrointestinal basidiobolomycosis (GIB). This study aimed to identify *Basidiobolus* species from the common house gecko and to compare them with human GIB isolates. Gecko and human GIB samples were collected from Muhayil Aseer area, south Saudi Arabia (2017-2019). Isolation of fungi from the gut contents of geckos was performed using Sabouraud dextrose agar incubated aerobically at 30°C for five days. Suspected *Basidiobolus* species were tentatively identified using routine bench tests and phenotypes were authenticated by phylogenetic analysis of the large subunit ribosomal RNA gene. Isolates (n = 10) were found to have zygomycete-like phenotypic characteristics. In the 28S ribosomal RNA gene phylogenetic tree, the strains assembled in the subclade encompass *Basidiobolus* spp. along with previously reported isolates from human' GIB. The strains had a close resemblance with *Basidiobolus haptosporus* (99.97%) as well as with *B. haptosporus* var. *minor* (99.97%). One isolate (L3) falls within the subclade containing *B. haptosporus* strain NRRL28635. The recovery of similar isolates from both humans and gecko lizards in one geographic region is an important development toward knowing risk factors for GIB.

Key words: Zygomycetes, Entomophthoromycota, *Basidiobolus,* lizards.

INTRODUCTION

Basidiobolus species belong to the family Basidiobolaceae (phylum Entomophthoromycota*)* which includes a vast collection of terrestrial fungi that have been categorized in the phylum *Zygomycota* (Gryganskyi et al., 2013, 2012). Recently, *Zygomycota* was noticed as phylum particularly difficult to assess taxonomically and

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phylogenetically and recent molecular and morphological studies clearly rejected the previously assumed monophyly of the *Zygomycota* (Gryganskyi et al., 2013; Möckel et al., 2022; Spatafora et al., 2016).

Basidiobolus can cause infections in animals as well as humans. This infection is also called entomophthoromycosis. It is characterized by its granulomatous nature and the formation of hard, non-ulcerating subcutaneous masses on the limbs, chest, back, and buttocks. Systemic infection is rare. *Basidiobolus* is a true pathogen, causing infections in immunocompetent hosts. However, recent data on angioinvasive infections due to *Basidiobolus* in immunocompromised patients suggest that it is emerging as an opportunistic pathogen as well (Kohler et al., 2017; Ribes et al., 2000). Human infections due to *Basidiobolus* are reported mostly from Africa, South America, and tropical Asia. *Basidiobolus ranarum* is the etiologic agent of subcutaneous chronic zygomycosis in man. Cases of invasive zygomycotic infection caused by *B. ranarum* in young humans and their clinical and pathological features have been reviewed with highlight reported from different countries (Hussein et al., 2021; Nazir et al., 1997). This suggests that gene or genome duplication may be an important feature of *B. ranarum* evolution and suggests that *B. ranarum* may have mechanisms which favors their difference from other related taxa or possessing unique properties (Elya and De Fine Licht, 2021; Henk and Fisher, 2012).

The significance of exotic animals maintained in temperate regions is a potential carrier of pathogenic organisms such as *Basidiobolus* spp. (Feio et al., 1999; Maniania et al., 2008). Cutaneous lesions in amphibians, subcutaneous infection in horses, and gastrointestinal lesions in dogs have so far been reported (Carmo et al., 2020; Gugnani, 1999; Khan et al., 2001; Nazir et al., 1997). The digestive tracts of various amphibians and reptiles have been found to harbor members of the family Basidiobolaceae. Similarly, isolation of such fungi was successful from soil and litter, but most readily from the intestinal contents of reptiles, amphibians, and some warm-blooded animals (Chaturvedi et al., 1984; Speare and Thomas, 1985). *Basidiobolus* fungus was isolated from the dung of amphibians, reptiles, and insectivorous bats, as well as wood lice, plant debris, and soil. The habitat of *Basidiobolus haptosporus* is often related to the gamasid mite *Leptogamasus obesus* (de Aguiar et al., 1980). Human cases of entomophthoromycosis due to *B. haptosporus* were linked to surgical wounds (Sood et al., 1997), skin and subcutaneous tissue (Bittencourt et al., 1980; de Leon-Bojorge et al., 1988; Hung et al., 2020), and deep invasive mycosis (Dworzack et al., 1978). A gastrointestinal entomophthoromycosis case due to *B. haptosporus* was described (Anand et al., 2010; de Aguiar et al., 1980). Many cases of gastrointestinal zygomycosis caused by *B. haptosporus* could be expected as either misdiagnosed or passed undiagnosed. In Florida (USA), the occurrence of the fungus in the digestive tracts of many types of toads has been documented (Nelson et al., 2002). The common isolation of *B. haptosporus* DNA from sources related to the gamasid mite *Leptogamasus obesus*, and from non-soil samples seems not to be in line with its presumed ecosystem as an ubiquitous saprophytic soil fungi. This leads to another working hypothesis that a second host species is present in the life cycle of *B. haptosporus* (Werner et al., 2012).

Gecko lizard (*Hemidactylus frenatus*) is classified in the family Gekkonidae (infra-order *Gekkota*). It is a native species in Southeast Asia and is habitually seen in several Asian countries including Saudi Arabia. It is called the Asian House Gecko, or simply, House Lizard. It has been indicated that the house gecko has a substantial role in the epidemiology of salmonellosis and has an effect on general public health (Jimenez et al., 2015). However, there is no data on its role in transmitting or acting as a source for fungal infections such as basidiobolomycosis.

This study aimed to detect *Basidiobolus* spp. in gecko lizards from an area endemic to GIB in Aseer, Saudi Arabia. This study attempts to identify and establish risk factors related to GIB disease.

MATERIALS AND METHODS

Samples collection and study location

The material analyzed was from the gut contents of four gecko lizards (*H. frenatus*) captured during 2017 to 2019 from the Muhayil Aseer (Asir) area (N 18° 33' 5.7492", E 42° 2' 57.7248") south Saudi Arabia. Ethical approval was granted by the Ethics Committee, College of Medicine, King Khalid University. A small portion of the intestinal contents was collected from each gecko lizard and placed in sterile containers and immediately transported to the laboratory for processing.

Basidiobolus spp. (n = 6) which have been isolated from human GIB and identified before (Bshabshe et al., 2020) were included in the study. The strains were isolated given the laboratory codes along with DSM codes (as shown in Table 1).

Isolation of fungi

Sabouraud dextrose agar (SDA; Difco Inc.) was used for the original isolation of fungi and for subsequent sub-culturing. Inoculated plates were then incubated at 25°C for up to one week. The isolates recovered from infected tissues were examined macroscopically and microscopically. Small pieces of colonies (thickness, 2 mm; diameter, 2 mm) were placed on lactophenol cotton blue (2 mL phenol, 2 mL lactic acid, 4 mL glycerol, 2 mL H_2O) on a clean microscopic slide and examined microscopically.

Phenotypic identification

Identification of *Basidiobolus* spp. was based on the key of O'Donnell (1979) with the following morphological characters as

Table 1. *Basidiobolus* species isolated from gecko lizard guts and from human gastrointestinal basidiobolomycosis used in the study.

Laboratory (DSM*) code	Source	Accession number
L1 (DSM107663)	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256649
L3 (DSM 05995)	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256652
L4	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256646
L4G	Gecko lizard, Muhayil, Aseer region, Saudi Arabia (2017)	MH256647
Doza	Human GIB, Aseer region, Saudi Arabia (2013)	MH254938
9-4	Human GIB, Aseer region, Saudi Arabia (2014)	MH256645
F15-1	Human GIB, Aseer region, Saudi Arabia (2017)	MH256650
F43-5	Human GIB, Aseer region, Saudi Arabia (2016)	MH256651
V81 (DSM06014)	Human GIB, Aseer region, Saudi Arabia (2017)	MH256648
85-5	Human GIB, Aseer region, Saudi Arabia (2019)	N/A

*DSM, Deutsche Sammlung von. Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, 38124 Braunschweig, Germany; GIB, gastrointestinal basidiobolomycosis.

primary for identification of the genus: production of zygospores with or without smooth walls retaining short paired protuberances known as "beaks", and apical globose primary conidia that were forcefully discharged from the conidiophores, usually still connected to parts of the conidiophore commonly referred to as "skirts".

DNA sequence-based identification

DNA amplification and sequencing service was done by Macrogen Inc. (Seoul, Korea). Briefly, the primers LR0R 5' (ACCCGCTGAACTTAAGC) 3' and LR7 5' (TACTACCACCAAGATCT) 3' (Vilgalys and Gonzalez, 1990) were used for amplification of the partial large subunit ribosomal RNA (LSU) region and analyzed using the ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). DNA sequences of the strains evaluated were aligned with other reference fungal sequences available in the GenBank database using BLAST, and the alignments were inspected visually. The gaps generated were treated as missing data. The fungal DNA sequences were analyzed phylogenetically by the neighbor-joining method (Saitou and Nei, 1987) using MEGA software (Kumar et al., 2018). Verification for internal branches was calculated by using 100 bootstrapped data sets.

Data availability

Data used to support the findings of this study are included within the article and its supporting information files. The 28S rRNA sequences established in this study and used for phylogenetic analysis have been deposited in the GenBank database ([https://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers shown in Table 1.

RESULTS

Fungal isolates (n= 10) which have been isolated from the common house gecko as well as from human GIB between 2017 and 2019 were used for the analysis (Table 1). The organisms were fast growing with isotropic, pale colonies in the primary culture on SDA at 25°C. Within the first 3 days of incubation, flat membranous colonies with a smooth, glabrous, and waxy appearance developed. Older colonies (3 days old and older) became powdery in appearance, with short aerial mycelia, and developed radiating folds from the center of the colonies (Figure 1). Morphological characteristics conform to *Basidiobolus* species that are round, flat, waxy, glabrous, and radially folded colonies (Campbell et al., 2013; de Leon-Bojorge et al., 1988; O'Donnell, 1979). The tested strains were found to have phenotypic properties distinctive for members of the genus *Basidiobolus*. The 12 isolates from human cases of GIB and from geckos were identified as *Basidiobolus* spp. based on their macroscopic and microscopic features. Colonies were examined microscopically from day 3 to day 5 for aerobic SDA growth. Young colonies show colorless broad hyphae with few septa, with smooth, thick walls, and abundant large, spherical, darkly colored chlamydospores and zygospores are formed (Figure 1).

Comparison of the 28S rRNA sequences of the isolates with corresponding nucleotide sequences of representatives of the class *Basidiobolomycetes* confirmed that they belong to the genus *Basidiobolus*. Isolates L1, L4, and L4G were found to have identical 28S rRNA sequences. The strains formed a monophyletic clade in the 28S ribosomal RNA gene. They shared 99.971% similarity with *B. haptosporus* NRRL28635, 99.969% with *B. haptosporus* var. minor strain ATCC 16579, 99.925% with *B. ranarum* and 100% similarity with previously reported human isolates from cases of GIB (Figure 2). The high 28S rRNA gene sequence similarities to the representatives of the genus *Basidiobolus* (93.9 to 98.7%) showed by these isolates support their placement in this genus. Few nucleotide mismatches were found within the isolates. One isolate (L3) fell within the subclade encompassing *B.*

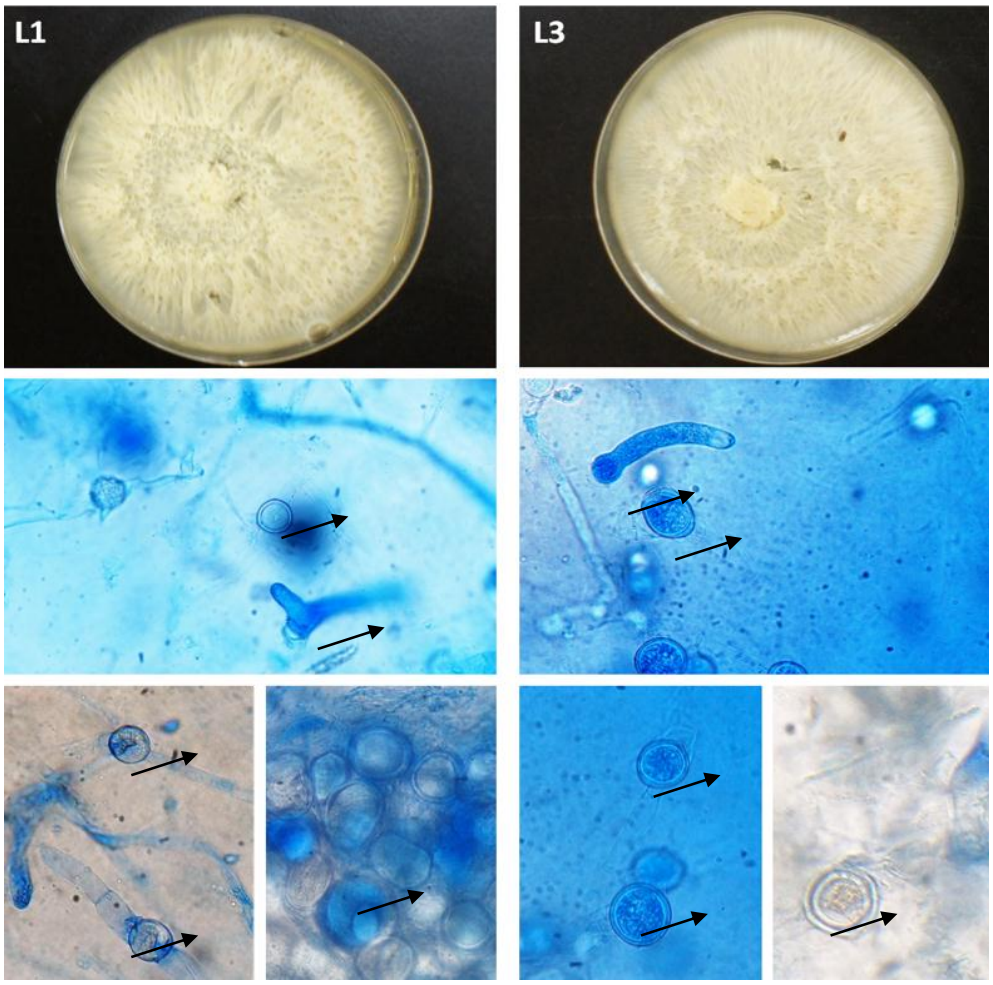

Figure 1. Colony morphology (upper row) and microscopic appearance (lower row) of some *Basidiobolus* strains. The pale membranous colonies with smooth, glabrous, waxy appearance which show radiating folds from the center of the colonies. Microscopically, the organisms show broad coenocytic hyphae with few septa together with abundant large, spherical, darkly colored chlamydospores and zygospores (Lactophenol Cotton Blue ×100).

haptosporus strain NRRL28635. High-stringency BLAST (http://www.ncbi.nlm.nih.gov/) analysis of the three sequences showed a close similarity of human isolates to those from the lizards.

DISCUSSION

The present study aimed to isolate and identify *Basidiobolus* fungi from the common house gecko. The study tried to link gecko as a potential risk of human GIB since house gecko is a contaminating agent for people in rural houses in Aseer region. This was done by comparing gecko isolates with previously reported human GIB isolates. The tested strains isolated from geckos have similar phenotypic properties distinctive for members of the genus *Basidiobolus* (Campbell et al., 2013; de Leon-Bojorge et al., 1988; O'Donnell, 1979).

The 10 isolates were identified as *Basidiobolus* spp. based on their macroscopic and microscopic features. Our initial phenotypic comparison of *Basidiobolus* spp. with those isolated from humans did not allow for solid conclusion on their close similarity, since growth and morphological traits alone are not always conclusive at the species level (O'Donnell, 1979). Application of DNA sequence analysis, using 28S rRNA gene was more decisive. This was useful in the present study supporting our hypothesis, as human and lizard isolates clustered in one clade related to but readily distinguishable from *B. haptosporus* and *B. haptosporus* var. *minor* (Figure 2). The results show that *Basidiobolus* isolates from humans and geckos from Aseer region have identical partial 28S rRNA sequences that distinguish them from representatives of closely related taxa, notably *B. haptosporus* and *B. haptosporus* var. *minor*.

The species of *Basidiobolus* have been identified, in

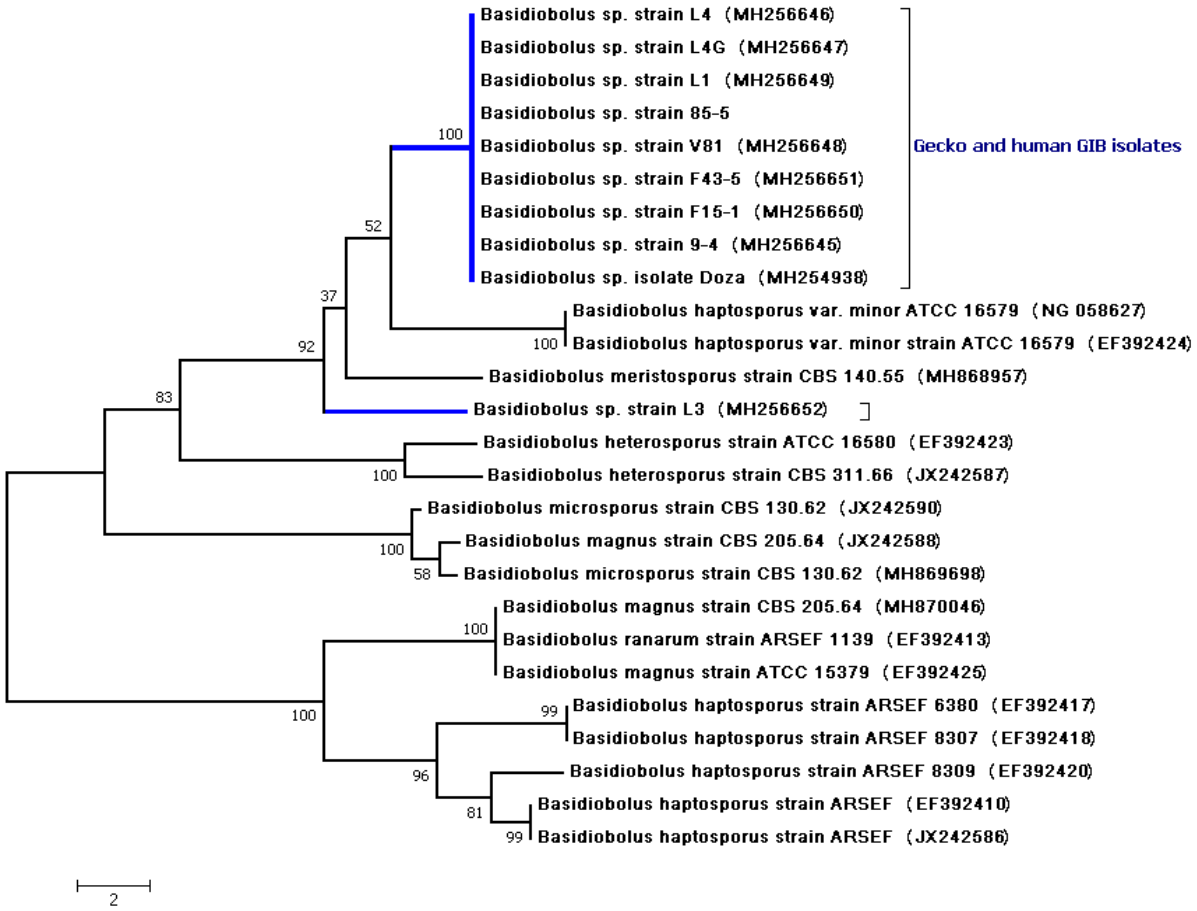

Figure 2. Neighbor-joining tree of aligned 28S large subunit ribosomal RNA genes of our strains from gecko lizards (L1, L3, L4, L4G) and five strains from humans (9-4, Doza, V81, F43-5, F15-1, 85-5) in relationship to closely related species. The numbers at the nodes indicate the levels of bootstrap support based on a neighbor-joining analysis of 100 resampled datasets. The bottom left bar represents 2 substitutions per nucleotide.

some records, as separate species including *B. ranarum*, *B. meristosporus*, and *B. haptosporus*, nevertheless, recent research on their antigens, restriction analysis of rDNA, and isoenzyme banding show that all *Basidiobolus* isolates that are pathogenic to humans be members of a single species, *B. ranarum* (Gugnani, 1999; Omar Takrouni et al., 2019). Similarly, antigenic analysis, isoenzyme banding and restriction enzyme analysis show that all human pathogens belong to *B. ranarum*. In two studies, *B. ranarum* was isolated in South India (Khan et al., 2001; Patro et al., 2019). Many articles have specified that the house gecko had a role in the epidemiology of salmonellosis and had an impact on public health (Jimenez et al., 2015). However, little evidence is available for its role in transmitting or representing a source for fungal infections, for example, basidiobolomycosis. A recent study showed that feces from South African reptiles often have *Basidiobolus* spp., indicating that they can add to the distribution of this fungus (Claussen and Schmidt, 2019).

B. haptosporus has been found in association with the gamasid mite *Leptogamasus obesus* (Werner et al., 2012). Gastrointestinal entomophthoromycosis owing to *B. haptosporus* were rare (Werner et al., 2012) and found to be exposed by surgical wounds (Sood et al., 1997), having skin tissue linkage (Anand et al., 2010; Bittencourt et al., 1980; Hung et al., 2020), or systemic mycosis (van den Berk et al., 2006). It is obvious that many gastrointestinal zygomycetes caused by *B. haptosporus* are misdiagnosed or undiagnosed.

Conclusion

Most of the isolates were identical to *B. haptosporus*-like fungi, including previous isolates from human GIB. The study suggests that the house gecko is a potential source of infection. The fungus is abundant in the lizard's guts and lizards live in close association with humans. Work is ongoing to further screen more lizard samples and their environmental habitat including water resources and soil in the GIB endemic area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Resistance profile of Staphylococcus strains and detection of the *Mec A*, *Van A* and *Van B* genes in private hospitals in Benin

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The present study aims to identify the emergence of Methicillin-resistant *Staphylococcus aureus, S. aureus* resistant to vancomycin and to investigate the presence of *Mec A*, *Van A* and *Van B* genes among *Staphylococcus* strains isolated from hospital environment. For each type of sample, surface of beds, recyclable material, boxes of rodar, floor and door slats, 53 samples were taken. So, a total of 265 samples were collected by swabbing (except boxes of rodar) in a private clinic in southern Benin. Bacteriological analysis was performed using the conventional method followed by DNA extraction with the Quiagen kit. The resistance genes *Mec A*, *Van A* and *Van B* were sought using specific primers. 215 samples were culture positive with 155 strains (62%) of coagulase negative (CNS) staphylococci and 95 strains (38%) of *S. aureus*. The majority of strains were resistant to gentamycin and clindamycin. These 155 strains were carried the *Mec A* gene and 10 strains carried the *Van A* and *Van B* gene. The study reveals the presence of resistant *Staphylococci* carrying the *Mec A* gene, which could be responsible for nosocomial infections in patients. Hygiene must be improved to limit the spread of these germs and protect patients.

Key words: Nosocomial infections, Staphylococcus, resistance antimicrobial *Mec A, Van A* and *Van B* gene.

INTRODUCTION

Nosocomial infections constitute a public health problem due to their frequency, their seriousness and also their socio-economic cost which represents a considerable burden for patients and for the health system (Mohamed, 2018). In 2009, the World Health Organization (WHO) estimated that 1.4 million people were sick as a result of infections contracted in hospitals. In developed countries, these infections affect 5-10% of patients. The prevalence of nosocomial infections (NI) is 4.5% in the USA, 10.5% in Canada,

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6.7%, in France and 6.2% in Belgium (Kakupa et al., 2016). Africa is not left out; the prevention of nosocomial infections is increasing every year. The highest prevalence rate is estimated at around 25% in Africa (Samou, 2005). In developing countries, nosocomial infections are estimated to be the third most common cause of death (Murni et al., 2013). The three bacteria most frequently responsible for nosocomial infections are *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a respective prevalence of 24.7, 18.99 and 10% (FHPH, 2015).

S. aureus is a Gram-positive bacterium responsible for nosocomial and community infections in humans (Saeed et al., 2020). It is considered to be an opportunistic pathogen responsible for high morbidity and mortality (Angela et al., 2015). In healthcare settings, this pathogen can contaminate furniture, clothing, and equipment around colonized or infected patients, which function as sources or reservoirs (Angela et al., 2015). In this context, Murray et al. (1996) suggested that health workers use adequate hygiene techniques, aimed at preventing cross infection with *S. aureus* among equipment, professionals and patients (Murray et al., 1996). Siobhan and Lucy have stressed the urgent need to alert and inform health workers of the potential risk of hospital infection due mainly to the lack of hygiene and poor sterilization of s devices medical (Karsten et al., 2018). *S. aureus* has a diversity of resistance genes and is able to acquire resistance to most antibiotics, making *S. aureus* a "superbug ".

Vancomycin is the treatment of choice for MRSA infection. During s recent years *S. aureus* resistant to methicillin (MRSA) has emerged as a frequent cause of infections in hospitals around the world (Marchese et al., 2009). This increase in resistance strains is a public health problem, in particular in nosocomial infections (Zaki et al., 2019). The effect of plasmid resistance in *Staphylococci* is demonstrated for β-lactamase-mediated resistance to penicillin. Resistance rates are greater than 60% in human isolates of *S. aureus* from the general population and greater than 90% in hospital cases, regardless of clinical setting. The mechanism of resistance to vancomycin in *S. aureus* is still unknown. This may be due to genes combination of resistance that vancomycin with *S. aureus.* These genes are seven to (*Van A*, B, C, D, E, G and L) and are usually transferred by the transposon Tn1546 (Karsten et al., 2018). In Benin, there is little data on *Staphylococcus* spp presenting *Mec-A* and *Van A* and *Van B* genes. The identification of the emergence of these resistant strains is however fundamental for the monitoring of hygiene and the prevention of nosocomial infections. The present study aims to identify the emergence of Methicillin-resistant *S. aureus, S. aureus* resistant to vancomycin and to investigate the presence of *Mec A*, *Van A* and *Van B* genes among *Staphylococcus* strains isolated from hospital environment.

METHODOLOGY

Study area and sample collection

The study was carried out in a private clinic located in Southern Benin. The samples were taken by swabbing the hospital environment (bed surfaces, door latches, floor, recyclable material, rodar box) according to the methodology described by Dougnon et al. (2019). The samples taken were kept free and immediately taken to the laboratory for bacteriological analyzes. The collected swabs were placed in coolers with ice packs (4°C) before being transferred to Laboratory.

Bacteriological identification

3 ml of Mueller Hunton broth was added to each swab and incubated at 37°C (Dougnon et al., 2016). After 16 h of incubation, the broth was inoculated on Chapman Agar medium for 18 h for bacteriological analysis. After incubation, each type of colony was re-seeded to obtain a pure colony, followed by microscopic examination (fresh state and Gram stain). Biochemical tests were carried out in accordance with the results obtained with Gram stain. The search for catalase, free staphylocoagulase and DNase were performed (Dougnon et al., 2016).

Antibiotic sensitivity test

The resistance profile of each bacterial strain was established by performing antibiogram. Kirby Bauer Disc diffusion method was used. The interpretation of the diameter inhibition zone was made according to the recommendations of the antibiogram committee of the French society of microbiology (CA-SFM/EUCAST, 2018). The choice of anti-antibiotic discs was inspired by the recommendations of the French Society of Microbiology. The antibiotics used for the sensitivity tests of cocci strains are: Oxacilline (OXA; 1 µg), Fosfomycin (FO; 50 µg), Gentamycin (GEN; 10 µg), Vancomycin (VAN; 30 µg), Chloramphenycol (C; 25 µg), Clindamycin (CD; 10 µg), Pristinomycin (RP; 15 µg), Erythromycin (E; 15 µg) and Ampicillin (AMP; 2 µg).

DNA extraction

Extraction and purification of genomic DNA was performed from 500 µl aliquots of each, which were transferred to Eppendorf tubes and centrifuged for 10 min. The sediment was used for genomic DNA extraction with the DNeasy Blood and at 5,000 xg Tissue kit (Qiagen, Germany), according to the manufacturer's instructions. Purified DNA samples were then stored at 20°C for testing for resistance genes.

Detection of Mec A, Van A and Van B genes

The polymerase chain reaction (PCR) mixture had a final volume of 25 kit DNA Taq Polymerase with Standard Taq Buffer added with 0.5 µl of each oligonucleotide, following the manufacturer's instructions. The primers (Table 1) used were synthesized by Inquaba biotec; amplifications were performed in PTC-200 Peltier Thermal Cycler (MJ Research). To do this, an initial denaturation was carried out at 94°C for 4 min followed by thirty cycles of denaturation at 94°C for 1 min, hybridization at 50°C for 1 min and elongation at 72°C for 1 min. Finally, a final extension was

Table 1. List and sequence of primers used.

Target gene	Primer	Sequence 5'-----3'	Reference
Mec A	MecAF	GTAGAAATGACTGAACGTCC	Shanmugakani et al. (2020)
	MecAR	GTTGCGATCAATGTTACCGT	
Van A	VanAF	GGGCTGTGAGGTCGGTTG	Saïdani et al. (2006)
	VanAR	TTCAGTACAATGCGCCCGTTA	
Van B	VanBF	TTGTCGGCGAAGTGGATCA	Saïdani et al. (2006)
	VanBR	AGCCTTTTTCCGGCTCGTT	

Table 2. Distribution of isolated bacteria according to the sampling site.

Samples	*S. aureus* (%)	CNS (%)
Bed surface	30/95 (31.58)	35/155 (22.58)
door latches	5/95 (5.26)	20/155 (12.90)
floor	40/95 (42.10)	35/155 (22.58)
recyclable material	10/95 (10.53)	5/155 (3.22)
rodar box	10/95 (10.53)	60/155 (38.71)

CNS : Coagulase Negative Staphylococcus; *S. aureus : Staphylococcus aureus*

carried out at 72°C for 5 min (Ramya et al., 2016). The multiplex PCR was carried out according to the method of Perez-Roth et al. (2002) using the Qiagen Amplification Kit. Following amplification, 10 µl of the reaction mixture was loaded onto an agarose gel at 1.5 % stained with 6 µl of ethidium bromide and electrophoresis to estimate the sizes of the amplification products with a molecular size standard scale of 100 bp (Velasco et al., 2005).

Statistical analysis

Data were inserted in Microsoft Excel 2013. The graphs obtained were obtained thanks to the analysis software GraphPad Prism 7.

RESULTS

Bacteriological identification

Out of 265 samples collected, 215 were positive, that is, a rate of 81.13%. Of the two hundred and fifteen positive samples, 250 strains were isolated including 62% CNS and 38% *S. aureus*. 38.71 and 22.58% of the isolated CNS were obtained from rodar and bed plate samples, respectively. Of the isolated *S. aureus*, 42.10% came from soil swabs and 31.51% from bed swabs (Table 2).

Almost all of the CNS strains isolated were resistant to Erythromycin (80.65%), Ampicillin (93.53%), and Pristinomycin (90.32%) while the majority were sensitive to Vancomycin (70.97%), Gentamycin (80.65%) and Oxacillin (90.32). Strong resistance of *S. aureus* strains to pristinomycin (78.95%), clindamycin (68.42%), ampicillin (68.42%), fosfomycin (63.16%) and erythromycin (57.89%) was noted (Table 3).

Molecular detection of Mec A and Van A Van B genes

The result of the PCR revealed that 78.78% of the coagulase positive staphylococcus strains possessed the *Mec A* gene and 100% of these same strains possessed the *Van A* and *Van B* genes. As for the strains of *S. aureus*, 21.21% had the *Mec A* gene, a total absence of the *Van A* and *Van B* genes (Figure 1).

DISCUSSION

The resurgence of multidrug-resistant bacteria in hospitals is a worldwide phenomenon using all bacterial species, but to varying degrees depending on the country and the department, depending on prescribing habits and hygiene practices. The frequency of nosocomial infections in developing countries remains high. The general objective of this study was to identify emerging strains of *staphylococcus* resistant to Vancomycin.

This study was carried out on a total of 53 biological samples from the hospital environment and some equipment used in patient care. These samples were collected in the inpatient department. Of the 53 biological samples analyzed, 43 samples presented at least one

Table 3. Percentage of resistance of bacterial species to the antibiotics tested.

Bacterial species	Antibiotics tested								
	E (%)	AMP (%)	VAN (%)	C (%)	CD (%)	FO (%)	RP (%)	GEN (%)	OXA (%)
CNS	125/155 (80.65)	145/155 (93.53)	45/155 (29.03)	50/155 (32.26)	130/155 (83.87)	95/155 (61.29)	140/155 (90.32)	30/155 (19.35)	15/155 (9.67)
S. aureus	55/95 (57.89)	65 /95 (68.42)	25/95 (26.32)	20/95 (21.02)	65/95 (68.42)	60/95 (63.16)	75/95 (78.95)	35/95 (36.84)	10/95 (10.53)

E: Erythromycin; AMP: Ampicillin; VAN: Vancomycin; C: Chloramphenycol; CD: Clindamycin; FO: Fosfomycin; RP: Pristinomycin; GEN: Gentamycin; OXA: Oxacilline; CNS: Coagulase Negative Staphylococcus; *S. aureus*: *Staphylococcus aureus.*

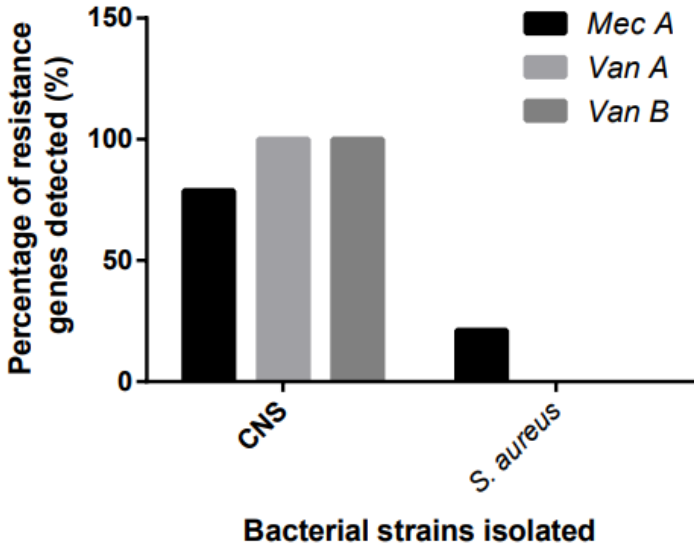

Figure 1. Percentage of resistance genes according to the bacterial species isolated.

microorganism. An overall prevalence of 81.13% was obtained in this study. This prevalence obtained is extended to that obtained in France and the United States, which were 40 and 30% respectively (Ito et al., 2012). The latter worked on the multidrug-resistant bacteria responsible for infections associated with hospital care.

The difference in the prevalence data obtained could be justified in part by the fact that their study was carried out on all multidrug-resistant bacteria in a hospital environment. Secondly, this result could also be justified by the level of hygiene in the two study areas. In fact, caregivers, patients and nurses respect hygiene measures more. 80% of the strains of *Staphylococcus* were resistant to clindamycin and resistant to gentamycin. Among the antibiotics used, vancomycin and oxacillin were the most active molecules. As a result, these antibiotics are the molecules of choice in the treatment of infections caused by these bacteria. This hitherto reassuring situation encourages continuous monitoring of the sensitivity of *Staphylococci* to glycopeptides.

31 strains out of the 50 bacteria isolated carried the *Mec A* gene and 2 strains carried the *Van A* and *Van B* gene. Presence of *Mec A* gene indicate the strains should be methicillin-resistant *S. aureus* (MRSA) (Bamigboye et al., 2018). Presence of *Van A and Van B* genes could explain resistance of Staphylococcin to Vancomycin (Karsten et al., 2018). Relatively different results have been obtained in similar works. Bamigboye et al. also investigated resistance genes in *S. aureus* isolated from 73 consecutive patients with infective conditions at Ladoke Akintola University of Technology Teaching Hospital (Nigeria) (Bamigboye et al., 2018). *Mec A* gene was detected in 5 (6.8%) isolates but *Van A* or *Van B* genes were absent. Aubaid et al.

(2020) found that 72/ 250 of *S. aureus* isolates from patients with different clinical cases whom admitted to Hospitals in Al Muthanna (Iraq), contained *Mec A* gene and five isolates contained *Van A* gene and only nine isolates contained *Van B* gene. The difference in the origin of the strains can explain the different results. Hygiene need to be improved to prevent nosocomial infections.

Conclusion

The problems caused by nosocomial infections are currently not sparing any country, but the daily struggle to maintain a requirement of cleanliness no longer simply depends on the means committed. Nosocomial infections are a real public health problem. The study carried out in a private hospital in southern Benin shows the prevalence of resistance genes in staphylococci. The most isolated strains were coagulase negative staphylococcus followed by *S. aureus*, most of which were all multidrug-resistant. It is important to promote good hygiene practices in order to reduce nosocomial infections in hospitals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Bacterial contamination and risk factors associated with naira notes circulating in Polytechnic Campus, Ado-Ekiti, Ekiti State Nigeria

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The abused Nigerian currency became an issue of concern recently, when the Central Bank of Nigeria (CBN) launched a nationwide enlightenment campaign aimed at educating the public on the proper handling of Naira notes. The study investigated the current bacterial contamination of Nigerian currency notes as well as the risk factors associated with it in Polytechnic Community Ado- Ekiti, Nigeria. A total of 32 samples of Naira notes, four pieces of each denomination of ₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, and ₦1000 were carefully collected from various locations on campus and subjected to standard methods for the isolation and identification of bacterial isolates. A total of 100 structured questionnaires were distributed at random to sample the opinions and views of the Polytechnic campus population on the use and mishandling of Naira notes. The findings revealed that all samples contain bacteria. The ₦50 notes had the highest bacterial contamination (18.7%), while the ₦5 notes had the lowest bacterial contaminant (7.5%). The most prevalent bacterial contaminants were *Escherichia coli* (78%), *Staphylococcus aureus* (66%), *Klebsiella* species (59%), *Micrococcus* species (31%), and *Pseudomonas aeruginosa* (16%). Bacteria contamination was higher in polymer notes than in paper notes. As a result, pathogenic bacteria were discovered on the surface of naira notes, making them useful candidates for food-borne pathogens and increasing the spread of food-borne disease. This result is critical in informing the public about the dangers of dirty currency notes to their health.

Key words: Bacteria, contamination, naira notes, risk-factor.

INTRODUCTION

Currency notes are used in day-to-day transactions and handled by people with varying environmental and personal hygiene conditions, providing a large surface area for pathogens to breed (Ofoedu et al., 2021). There are currently eight denominations of naira notes in use in Nigeria: ₦5, ₦10, ₦20, and ₦50, ₦100, ₦200, ₦500, and ₦1000 (Ahmed et al., 2010). The four lower denominations (₦5, ₦10, ₦20, and ₦50) are made of polymer substrate and are more commonly found in circulation, with a greater percentage of the Nigerian

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population engaging in daily cash transactions with them, whereas the four higher denominations (₦100, ₦200, ₦500 and ₦1000) are made of the paper substrate (Ogbuju et al., 2020). In a developing country like Nigeria, poor naira notes handling culture is the norm, and currency notes are abused indiscriminately. Various habits such as keeping currency notes in socks, shoes, and pockets, under the carpet or rugs, and squeezing them in the hand often introduce a number of microbes to the notes (Sharma and Sumbai, 2014). Methods such as wetting hands or fingers with saliva or contaminated water to lubricate the hand when counting money and handling currency notes with food-contaminated fingers may increase the contamination of currency notes. They may, however, increase the risk of infection from contaminated ones (Ahmed et al., 2010). Furthermore, contamination of currency notes can be traced back to dust, soil, water, and the microflora of handlers' bodies (hand, skin, etc) (Awe et al., 2010).

Many studies in various parts of Nigeria have reported microbial contamination of naira notes. The constant microbial contamination of currency notes is a public health concern because pathogenic microorganisms cause illness and has led to high mortality and morbidity. The current bacterial contamination of naira notes, as well as the associated risk factor, is of critical public health importance. It will provide information on potential pathogens found in currency notes from selected areas.

MATERIALS AND METHODS

Study area

The study was conducted in Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria. The school has a student population of 15,000 and is situated at latitude 7.6056°N and longitude 5.2886°E. Ekiti State shares borders with other states like Osun, Kwara, Kogi, and Ondo.

Collection of sample

A total of 32 samples of naira notes consisting of 4 pieces of each eight denomination of naira notes (₦5, ₦10, ₦20, ₦50, ₦100, ₦200, ₦500, ₦1000) were randomly collected from traders, civil servants, and students within the four faculties in the Federal Polytechnic Ado-Ekiti between January and March, 2021. One thousand naira denomination was obtained by exchange. Each currency note was collected directly into an individual sterile polythene bag, labelled accordingly, and taken to the laboratory immediately for analysis. Sampled coins are no longer useful because they are no longer in circulation among Nigerians.

Bacterial isolation and identification

Each of the naira notes was aseptically transferred into a sterile beaker containing 10 ml of sterile buffered peptone water and allowed to stand for 20 min at a temperature range of 25 to 28°C. During the period, the beaker was repeatedly shaken to obtain the resultant test sample for inoculation. The samples was streaked using a standard wire loop onto already prepared MacConkey agar, Mannitol Salt agar and Blood agar which were aerobically incubated at 37°C for 24 h. Plates with mixed colonies were sub-cultured in order to get pure colonies. Pure cultures were streaked on nutrient agar slants and stored at 4°C for further analysis (Adamu et al., 2012).

Bacteria that developed were identified using colony appearance, haemolysis, hydrogen gas production, motility, spore staining, Gram's staining, and biochemical tests involving the Indole, Citrate utilization, Catalase, Coagulase, Urease, Oxidase, Motility and Lactose fermentation tests according to the standard procedures of Cheesbrough (2006) and Buller (2014).

Questionnaire survey

A total of 100 structured questionnaires were randomly issued to sample the opinions and views of the populace in Federal Polytechnic, Ado-Ekiti on the use and misuse of the naira notes. The work was required to collect demographic data such as age, gender, occupation, and education level. The questionnaires were anonymous, and prior to distribution, verbal consent was obtained. The questionnaires included questions on exposures to the risk factors associated with naira notes and preventive ways to minimize contamination.

Analysis

Data were compiled in a spreadsheet Microsoft Excel, Version 2013, and analyzed as appropriate using descriptive statistics. The P-valve ($p<0.05$) was considered statically significant.

RESULTS

Table 1 shows the identification of the bacteria. Out of the 32 samples of naira notes examined for bacteria, all samples harbor bacteria. The ₦50 notes showed 18.7% of the highest contamination with bacteria, while ₦5 notes yielded a small bacterial contaminant with 7.5%. The percentage prevalence was highest for *Escherichia coli* (78%), followed by *Staphylococcus aureus* (66%), *Klebsiella* species (59%), *Micrococcus* species (31%), and *Pseudomonas aeruginosa* (16%) in descending order (Table 2). In generally, polymer notes (₦5, ₦10, ₦20, and ₦50) had higher bacteria significance than paper notes (₦100, ₦200, ₦500, and ₦1000), which had lower bacteria significance (Figure 1). Out of 100 questionnaires administered to the public, all were completed and returned. The demographic features of the sampled location are shown in Table 3, and the participants reside in Ekiti State, Nigeria. Table 4 shows that paper notes (68%) are more preferred than polymer notes (32%), and ₦50 was the most circulated among the individuals living within the sampled area. It also showed participants' opinions on the ways naira notes are kept, misused, contaminated, and preventive ways to minimize the contamination of naira notes.

DISCUSSION

The study shows that there is high occurrence of bacterial

Table 1. Cultural, morphological and biochemical characteristics of bacterial isolates.

Organisms	Colony appearance	GSR	Mo	H	H_2S	Spore	I	Ci	Ca	U	Coa	Ox	Lf
Staphylococcus aureus	Round and cluster in shape	+	-	+	-	-	-	+	+	+	+	-	-
Escherichia coli	They are flat, dry, pink colonies with a surrounding darker pink area of precipitated bile salts.	-	+	-	-	-	+	-	+	-	-	-	-
Micrococcus species	It produce yellow or red-pigmented colonies on blood agar	+	-	-	-	ND	-	-	+	+	-	+	-
Klebsiella species	It shows large dome shape, mucoid colony on maconkey agar	-	-	-	-	ND	-	+	+	+	-	-	-
Pseudomonas aeruginosa	flat, smooth, non-lactose fermenting colonies with irregular margin and slightly pigmented (greenish pigmentation	-	+	-	-	Non-sporing	-	+	+		-	+	-

GSR = Gram's stain reaction, Mo = motility, H = haemolysis, H_2S = hydrogen sulphide gas; Ci = citrate; Ca = catalase; I = indole; U = urease; Cog = coagulase; Ox = oxidase; Lf = lactose fermentation; + = positive; - = negative, ND = non-detectable.

Table 2. Prevalence of bacterial contamination on naira notes.

Organisms	5(₦) (n=4)	10(₦) (n=4)	20(₦) (n=4)	50(₦) (n=4)	100(₦) (n=4)	200(₦) (n=4)	500(₦) (n=4)	1000(₦) (n=4)	Total (n=32)
Staphylococcus aureus	2 (50)	2 (50)	4 (100)	4 (100)	3 (75)	1 (25)	2 (50)	3 (75)	21 (66)
Escherichia coli	2 (50)	4 (100)	3 (75)	4 (100)	4 (100)	3 (75)	2 (50)	3 (75)	25 (78)
Klebisella spp.	1 (25)	2 (50)	3 (75)	2 (50)	3 (75)	3 (75)	3 (75)	2 (50)	19 (59)
Micrococcus spp.	1 (25)	0 (0)	1 (25)	3 (75)	2 (50)	0 (0)	2 (50)	1 (25)	10 (31)
Pseudomonas aeriginosa	0 (0)	0 (0)	1 (25)	2 (50)	1 (25)	0 (0)	1 (25)	0 (0)	5 (16)
Total (%)	6 (7.5)	8 (10)	12 (15)	15 (18.7)	13 (16.3)	7 (8.8)	10 (12.5)	9 (11.2)	80 (100)

contamination on naira notes in circulation, which agrees with the findings of Usman et al. (2021) that confirmed bacteria are capable of surviving on naira notes when contaminated with pathogenic microbe during handling. The genera of bacteria isolated included *E. coli* (78%), *S. aureus* (66%), *Klebsiella* spp. (59%), *Micrococcus* spp. (31%), and *P. aeruginosa* (16%), similar to the findings reported in Adamu et al. (2012), Barua et al. (2019), and Kawo et al. (2009). The survival of pathogens on currency notes indicated that they represent a potential cause of food-borne diseases. The ₦50 note has the highest bacterial contamination (18.7%) since it frequently exchanges hands than the rest of the denominations. The ₦5 notes yielded a little bacterial contaminant (7.5%), probably attributable to its limited use, considering that in recent times, it is hard to find any commodities sold for ₦5 in Nigeria. The finding agrees with the report of Kawo et al. (2009) and Orababa et al. (2021).

Demographic characteristics of the sampled population showed that females participated more (62%), and the majority of the participants ranged within the ages of 14-33 groups (65%) which are students having one form of educational qualification or another, which agrees with Adamu et al. (2012). Polymer notes were more contaminated (51.37%) than paper notes because they are more often found in circulation and are usually engaged in daily cash transactions by a more significant percentage of people living in the surveyed area. Results of the questionnaire indicated that 68% of sampled opinions showed their interest in paper notes because the higher denominations are made with paper notes, considering the high poverty level in the country, which may influence their judgment. The study also reflects Nigeria's current poor economic situation, in which the naira has become so devalued that higher denominations are suddenly unavailable at all levels for daily transactions except for the wealthy. About 65% of the participants use wallets or purses to keep their money.

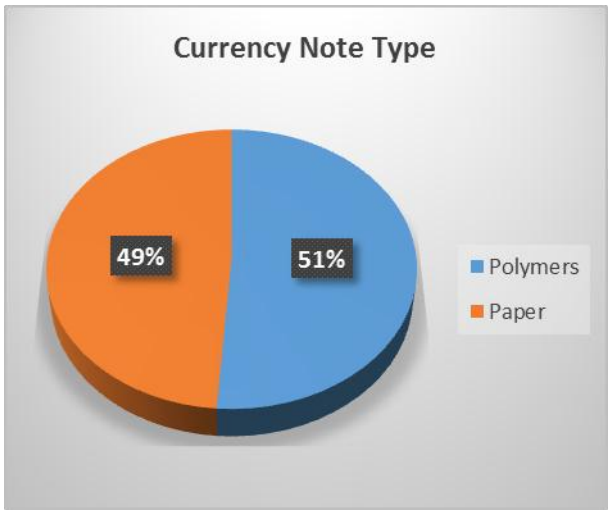

Figure 1. Level of bacterial contamination based on different note types.

Table 3. Demographic characteristics of the sampled population (n =100).

Variable	Numbers (%)
Gender	
Males	38
Females	62
Age group	
14-33	65
34-53	29
54-above	6
Education	
None	5
Primary/secondary	14
Tertiary	81
Occupation	
Students	62
Civil servant	17
Traders/Others	21

In comparison, the other 35% save their cash in underwear, pockets, and other means practiced by ignorant women living in rural areas. The participants shared their thoughts on how people abused naira notes at ceremonies by squeezing, tearing, and spraying them. In the sampled area, the most common risk factors associated with naira contamination are storing naira notes in moist places and keeping notes underbody surfaces. According to the survey results, the best ways to avoid naira contamination are to maintain good hand hygiene, avoid folding the notes, and avoid using wet naira notes during transactions.

Conclusion

The study has identified pathogenic bacteria on the surface area of naira note, which serves as useful

Table 4. Risk factors associated with Naira note contamination (n =100).

Risk factor	Numbers (%)
Preferred naira notes	
Paper notes	68
Polymer notes	32
Naira notes mostly circulated	
5	-
10	-
20	13
50	29
100	19
200	9
500	16
1000	14
Ways naira notes are kept	
Wallet/Purse	65
Underwear	5
Pocket	20
Others means	10
Ways to misuse naira notes	
Squeezing them	40
Tearing	22
Spraying at ceremonies	24
Writing on them	14
Ways naira notes is contaminated	
Squeezing & spraying during ceremonies	14
Keeping notes under body surfaces	21
Use of unclean water while counting	12
Storage of naira note in moist places	34
Use of saliva during counting	19
Ways to minimize the contamination of naira notes	
Avoid use of inks and other writing materials on naira notes	10
Ensure good hand hygiene	47
Avoid folding the notes	19
Avoid wet naira notes during transactions	17
Getting new notes from banks	7

candidates for food-borne pathogens and increases spreading of food-borne disease. This result is very important to show the public of the risk of dirty currency notes on their health. The Apex Bank should ensure continuous retrieval of mutilated notes from circulation. Regular public enlightenment on proper handling of the naira notes and risks associated with contaminated notes are needed in minimizing the contamination of organisms. A similar study should regularly remind the public of the risk of dirty currency notes on their health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Prevalence and multidrug-resistant ESBL-producing *E. coli* in urinary tract infection cases of HIV patients attending Federal Teaching Hospital, Abakaliki, Nigeria

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The study determined the frequency of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-PEc) in HIV-infected individuals with urinary tract infections (UTIs) attending Federal Teaching Hospital Abakaliki (FETHA II), and the responses of these bacterial pathogens to colistin, cephalosporins, aminoglycosides, fluoroquinolones, and ertapenem. Exactly 200 urine samples (mid-stream) were collected from HIV-infected individuals. Standard microbiological techniques were used to characterize bacterial isolates. Phenotypic screening for the production of ESBLs was done by double disc synergy test. Antibiotic susceptibility study was carried out by the Kirby-Bauer disc diffusion technique. Results showed the presence of ESBL-PEc in the urine samples of HIV-infected individuals. ESBL-PEc were highly resistant to gentamycin (85%), ofloxacin (75%), ciprofloxacin (75%), nalidixic acid (70%), tobramycin (65%), kanamycin (64.3%), and norfloxacin (60%), but susceptible to ertapenem (60%) and amikacin (57.1%). The ESBL-PEc isolates were multidrug-resistant. Average multiple antibiotic resistance indices (MARI) value of isolates was 0.8, further depicting misuse/abuse of these antibiotic classes in our study area. Thus, it is pertinent to carry out antibiotic susceptibility testing before the commencement of antibiotic therapy, especially in HIV-positive patients with UTIs so as to attain effective treatment regimens and reduce the incidence of antibiotic resistance in healthcare settings.

Key words: *Escherichia coli*, extended-spectrum beta-lactamase (ESBL), multidrug resistance, antibiotics, urinary tract infections (UTI), human immunodeficiency virus (HIV) patients.

INTRODUCTION

Urinary tract infection (UTI) is an infection occurring in any region of the urinary system such as ureters, bladder, kidneys, and urethra. UTI caused by pathogenic bacteria, especially ESBL-producing microorganisms has resulted in life-threatening sepsis (Tessema et al., 2020). Treatment of uncomplicated UTIs may be with

fluoroquinolones if the causative organism is sensitive to them, but increase in the resistant bacteria causing UTIs has caused limitations in their action in today's health sector (Marwa et al., 2015). Human immunodeficiency virus (HIV)-positive patients are liable to have opportunistic infections like UTIs, especially among people living in developing countries where proper healthcare facilities are limited (Foxman, 2002; Abongomera et al., 2021). Studies have shown high frequency (41 - 52%) of asymptomatic bacteriuria in HIV patients (Awolade et al., 2010; Ezechi et al., 2013), and the routine administration of trimethoprim-sulfamethoxazole prophylaxis among HIV-positive patients might increase the prevalence of multidrug-resistant (MDR) bacterial infections (Ezechi et al., 2013; Olaru et al., 2021). Extended-spectrum beta-lactamase (ESBLs) are a class of plasmid-mediated diverse, complex and rapidly evolving enzymes which can hydrolyze penicillins, monobactams, and broad-spectrum cephalosporins. Plasmids carrying gene-coding ESBLs often bear genes encoding resistance to various antibiotic classes; for instance, aminoglycosides and fluoroquinolones. Hence, getting antimicrobial agents against ESBL-producing microorganisms in healthcare is often difficult (Ruppe et al., 2015). Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-PEc) have been reported in non-hospitalized and hospitalized patients (Marwa et al., 2015). The spread of ESBLs and other forms of resistance to antibiotics is an international threat to the health sector. The misuse of antibiotics is one of the major factors causing an increase in resistance of antibiotics by bacteria in Abakaliki metropolis (Ozlem et al., 2007; Moses et al., 2018, 2020). Therefore, this study is designed to evaluate the frequency of ESBL-PEc isolated from urine samples of HIV-positive patients with UTIs attending Federal Teaching Hospital Abakaliki (FETHA), and the responses of these bacterial pathogens to selected members of four commonly used antibiotic classes (cephalosporins, aminoglycosides, fluoroquinolones, and carbapenem).

MATERIALS AND METHODS

Clinical samples collection

Two hundred mid-stream urine samples (129 from females and 71 from males) of HIV-positive patients attending FETHA II were used for this study between February and November, 2019. Urine samples were collected from both the medical ward and outpatient department of the hospital as clean catch urine with sterile 30 ml polystyrene universal containers. Collected samples were labeled appropriately and immediately transported in ice packs to the laboratory for bacteriological analysis within 30 and 60 min.

Ethical clearance

Ethical clearance for the study was approved by ethical clearance committee of FETHA (FETHA/REC/VOL 2/2019/233), Ebonyi State, Nigeria. Informed consent was also obtained from each patient before sample collection.

Isolation and phenotypic characterization of bacterial isolates from urine samples

Collected mid-stream urine samples were aseptically streaked on MacConkey agar. They were then incubated for 18 h at 37°C. At the completion of incubation, plates were observed for *E. coli* growth (red or pink colonies) on MacConkey agar, sub-cultured on eosin methylene blue (EMB) agar, and incubated for 24 h at 37°C. EMB agar plates were thereafter observed for *E. coli* growth (green metallic sheen appearance) after incubation. These suspected bacterial isolates were then characterized with standard microbiological methods; Gram-staining, motility, sugar fermentation, and other biochemical tests such as indole, methyl red, Voges-Proskauer, citrate, motility, and urease test. The pure isolates were further inoculated on nutrient agar slants, incubated for 24 h at 37°C, and stored in the refrigerator at 4°C for future use (Cheesebrough, 2010; Moses et al., 2018).

Detection of ESBL by double-disc synergy test (DDST)

The bacterial isolates that exhibited reduced susceptibility to any of the 2nd and 3rd generation cephalosporins were phenotypically confirmed for ESBL production using the double-disc synergy test (Iroha et al., 2010). DDST was performed as a standard disc diffusion assay on Mueller-Hinton (MH) agar (Oxoid, UK) plates in line with CLSI criteria (CLSI, 2015). The plates were incubated at 37°C for 18 to 24 h. ESBL production was suspected phenotypically when the zones of inhibition of the cephalosporins (cefotaxime 30 µg and ceftazidime 30 µg) increased in the presence of amoxicillin/clavulanic acid disk (20/10 µg). A≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins (cefotaxime and ceftazidime) tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirmed ESBL production phenotypically (CLSI, 2015; Iroha et al., 2010).

Antibiotic susceptibility test

Antibiotic susceptibility of the *E. coli* isolates was determined using the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute CLSI (CLSI, 2018). The isolates were sub-cultured on nutrient agar and incubated at 37°C for 24 h. Then the colonies of each of the isolate were adjusted to 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 cfu/ml), incubated for 10 min and inoculated onto Mueller-Hinton agar plates using sterile swab sticks. The surface of the medium was streaked in four directions while the plates were rotated approximately 60° to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for a few minutes. Standard antibiotic discs tested against the isolates were ceftazidime (CAZ) (30 µg), amikacin (AK) (30 µg), cefotaxime (CTX) (30 µg), tobramycin (Tob) (10 µg), gentamicin (NC) (10 µg), kanamycin (K) (5 µg), ciprofloxacin (CIP) (5 µg), ofloxacin (OFX) (5 µg), norfloxacin (NOR) (10 µg), cefotetan (CTT) (30 µg),

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Table 1. Frequency of ESBL-producing *E. coli* isolates in urine samples of HIV-positive patients in FETHA II.

Age	Female			Male		
	No. of samples screened	No. of *E. coli* isolated n (%)	No. of ESBL-producers	No. of samples screened	No. of *E. coli* isolated n (%)	No. of ESBL-producers
≤18	26	18 (69.2)	3	11	3 (27.3)	1
19 - 29	14	13 (92.9)	8	9	6 (66.7)	6
30 - 39	56	46 (82.1)	11	28	23 (82.1)	8
≥ 40	33	23 (69.7)	15	23	18 (78.3)	6
Total	129	100 (77.5)	37 (37%)	71	50 (70.4)	21 (42%)

Table 2. Percentage susceptibility frequency of ESBL-producing *E. coli* isolated from urine samples of HIV-positive patients to cephalosporins and ertapenem in FETHA II.

Sample	Antibiotics							
	CAZ	CTX	CTT	FEP	CRO	ATM	CXM	ETP
Resistance (%)	70	58	65	78	48	73	0	40
Susceptible (%)	30	42	35	22	52	27	100	60

CTX = Cefotaxime, ATM = Aztreonam, CTT = Cefotetan, CRO = Ceftriaxone, CXM = Cefuroxime, CAZ = Ceftazidime, ETP = Ertapenem, FEP = Cefepime.

cefepime (FEP) (30 µg), ceftriaxone (CRO) (30 µg), aztreonam (ATM) (30µg), cefuroxime (CXM) (30 µg), ertapenem (ETP) (10 µg), nalidixic acid (NA) (30 µg), and amoxicillin-clavulanic acid (AMC) (30 µg), (Oxoid, UK). The inhibitory zone diameter was interpreted as susceptible, intermediate or resistant according to the criteria of CLSI (CLSI, 2018; Moses et al., 2018). *E. coli* ATCC 25923 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains.

Multiple antibiotic resistance index determination

Multiple antibiotic resistance index (MARI) of the isolates was calculated as the number of antibiotics to which the isolates exhibited resistance (a), divided by the total number of antibiotics tested against the isolates (b) according to Moses et al. (2020). MARI = a/b.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 statistical software package (Moses et al., 2018). Comparison between categorical variables was calculated using Independent Samples T-test. Results were considered statistically significant if the p value was less than 0.05 ($p < 0.05$).

RESULTS

Results showed that 37 (37%) isolates were ESBL producers out of 100 *E. coli* isolates obtained from females, while 21 (42%) ESBL-PEc were obtained from the 50 *E. coli* isolates from males (Table 1). There was no statistically significant difference in the prevalence frequencies of *E. coli* in urine samples of male and female HIV-positive patients from FETHA II ($P < 0.05$). There was also no statistically significant difference in the prevalence frequencies of ESBL-producing *E. coli* in urine samples of male and female HIV-positive patients from FETHA II ($P < 0.05$).

Antibiotic susceptibility test results of ESBL-PEc isolates against cephalosporins and carbapenem (ertapenem) revealed that isolates were very resistant to cefepime (78%), aztreonam (73%), ceftazidime (70%), cefotetan (65%), and cefotaxime (58%), but susceptible to ceftriaxone (52%) and ertapenem (60%). All the isolates were completely susceptible to cefuroxime (100%) (Table 2). Antibiotic susceptibility test results of ESBL-PEc isolates against aminoglycosides and fluoroquinolones also revealed that isolates exhibited high resistance to gentamycin (85%), ofloxacin (75%), ciprofloxacin (75%), nalidixic acid (70%), tobramycin (65%), kanamycin (64.3%), and norfloxacin (60%) but susceptible to amikacin (42.9%) (Table 3).

The average MARI value of the ESBL-PEc isolates was 0.8.

DISCUSSION

Bacterial infections increase the rate of mortality and morbidity among HIV-infected patients because of cell-mediated and humoral immunity defects (Moremi et al., 2014). Many researchers have revealed that there is a relationship between HIV and opportunistic infections (Ya et al., 2008; Spach and Jackson, 2010). ESBL-producing *E. coli* (ESBL-PEc) usually associated with UTIs in HIV-

Table 3. Percentage susceptibility frequency of ESBL-producing *E. coli* isolated from urine samples of HIV-positive patients to aminoglycosides and fluoroquinolones in FETHA II.

Sample	Aminoglycosides				Fluoroquinolones			
	TOB	K	AK	CN	OFX	CIP	NA	NOR
Resistance (%)	65	64.3	42.9	85	75	75	70	60
Susceptible (%)	35	35.7	57.1	15	25	25	30	40

TOB = Tobramycin, K = Kanamycin, AK = Amikacin, CN = Gentamicin, OFX = Ofloxacin, CIP = Ciprofloxacin, NA = Nalidixic acid, NOR = Norfloxacin.

positive patients are growing in prevalence and causing serious burden in the health sector (Kemajou et al., 2016).

The present study was designed to determine the frequency of ESBL-producing *E. coli* in HIV-infected individuals attending Federal Teaching Hospital Abakaliki (FETHA II) with UTIs and the responses of these bacterial pathogens to cephalosporins, aminoglycosides, fluoroquinolones, and ertapenem. One hundred and fifty [150 (75%)] *E. coli* isolates (100 from females and 50 from males) were obtained from 200 mid-stream urine samples of HIV-positive patients. Interestingly, this study showed that cefuroxime was the most effective antibiotic against the *E. coli* isolates as all the isolates were completely susceptible (100%). Ertapenem and ceftriaxone were effective against ESBL-PEc isolates with susceptibility frequencies of 60 and 52%, respectively.

ESBL-PEc isolates in our study were also generally highly resistant to aminoglycoside and fluoroquinolones. However, amikacin was the most effective aminoglycoside antibiotic against the isolates as 57.1% of the isolates were susceptible. All ESBL-PEc isolates in this study were multidrug-resistant as they were resistant to at least three different antibiotic classes. Their multidrug resistance tendencies were further depicted by their average MARI value of 0.8. The frequency of *E. coli* in the urine samples of HIV-positive patients with UTIs (77.5% from females and 70.4% from males) is in concord with the work of Kemajou et al. (2016) who reported 141 (57.3%) *E*. *coli* isolates from 286 urine samples of HIV-positive patients (74.4% were from females while 25.6% were from males). The differences in the frequency of *E. coli* in the urine samples could be as a result of the larger number of urine samples from females (129) than males (71). The results of this study also agree with that conducted in Iran by Serkadis et al. (2014) who reported 19.3% ESBL-producing *E. coli* isolates from 250 urine samples of HIV-positive outpatients screened even though we recorded higher frequency (42% from males and 37% from females) values in our study. They also reported that *E. coli* was the most common uropathogenic bacteria in 114 (59.1%) of UTIs.

This study did not completely agree with the study of Inyang Etoh et al. (2009) in Calabar and Omoregie and Eghafona (2009) in Benin, Nigeria who reported a higher frequency of ESBL-producing *E. coli* in urine samples of females than males with UTIs. The outcome of higher UTI in females may be because of the predominance of *E. coli* in different populations and the proximity between vagina and anus, complex physiology during pregnancy, intake of contraceptives, and urethra in relation to personal hygiene (Thakur et al., 2013). Even though more urine samples were collected from females (129) than males (71), the high frequency of ESBL-producing *E. coli* (ESBL-PEc) reported among males (42%) in our study could be as a result of the high number of positive males with UTIs. Our study reported that the age between 19-39 years (82%) had the highest frequency of *E. coli* associated with UTI while the least *E. coli* frequency was in the age group < 18 (56%). This is in agreement with previous work (Thakur et al., 2013; Bomjan, 2005; Livermore and Hawkey, 2005; Prakash et al., 2009), where high *E. coli* frequencies were reported in urine between the age groups of 20 to 40 years. This could be due to the established report that the incidence of UTIs increases with sexual activity and age. However, our study disagreed with the work of Thakur et al. (2013) who previously reported high incidence of UTIs in old age males. This high incidence of *E. coli* in the urine samples of older men could be as a result of some circumstances such as diabetes, prostatitis, prolonged treatment with antibiotics, and weakened immune system (Thakur et al., 2013).

The isolates in this present study were generally resistant to cephalosporins, aminoglycosides, and fluoroquinolones. This is in agreement with the work of Perez et al. (2007) who indicated that 94% of *E. coli* isolates exhibited resistance to cephalosporins. This high resistance frequency might be as a result of ineffective antibiotic policy and the irrational use of cephalosporins in hospitals (Gonzalez-pedilla et al., 2015). High rate of *E. coli* resistance to cephalosporins is mainly due to the production of ESBLs; this was recorded from several countries (Bouchillon et al., 2004; Khanfar et al., 2009). However, self-medication practice, the use of antibiotics without doctor's prescription, easy availability of drugs from the pharmacy, and loop holes in guidelines in antimicrobial usage policies in developing countries such

as Nigeria may be a major contributing factor to antibiotic resistance (Moses et al., 2018).

Interestingly, isolates in this study were susceptible to cefuroxime (100%), ceftriaxone (52%), and ertapenem (60%); while they exhibited high resistance to cefepime (78%), aztreonam (73%), ceftazidime (70%), cefotetan (65%), and cefotaxime (58%). Our study partially agrees with the work of Serkadis et al. (2014) in Iran who reported that *E. coli* isolates from 87 HIV-positive outpatients were highly susceptible to nitrofurantoin, ceftazidime, cefoxitin, ceftriaxone, aztreonam, cefotaxime, and ciprofloxacin with resistance frequencies of 95.4, 94.3, 93.1, 91.95, 90.8, 89.6 and 72.4%, respectively. Our study showed that 37 (37%) isolates were ESBL producers out of the 100 *E. coli* isolates obtained from females, while 21 (42%) ESBL-PEc were obtained from the 50 isolates from males. This agrees with the study of Osazuwa et al. (2011) who reported ESBL-producing *E. coli* isolates in urine samples of HIV-infected. This study also concurs with the work of Iroha et al. (2017) who reported ESBL-producing *E. coli* in urine samples of HIV-positive individuals in Abakaliki, Nigeria.

Fluoroquinolones are widely used in the empirical therapy of UTI. They are the drug of choice for the treatment of infections due to ESBL-producing microbes (Pitout and Laupland, 2008). Nevertheless, a previous study reported a strong association between ESBL production and fluoroquinolone resistance (Frank et al., 2011). In our study, the resistance exhibited by ESBL-producing *E. coli* isolates to fluoroquinolones (ofloxacin, ciprofloxacin, norfloxacin, and nalidixic acid) and aminoglycosides (tobramycin, kanamycin, gentamicin, except amikacin) agrees with other studies that reported an increased resistance towards fluoroquinolones, aminoglycosides, and folate pathway inhibitors (Pitout and Laupland, 2008; Prakash et al., 2009). The average MARI value of the ESBL-producing *E. coli* isolates in this study was 0.8. Multiple antibiotic resistance index (MARI) of uropathogens is increasing worldwide. MARI usually varies according to geographic locations, but it is usually directly proportional to the misuse of antibiotics (Iroha et al., 2017).

Conclusion

This study has shown that *E. coli* is a causative agent of UTI in HIV-positive patients attending FETHA II. Results showed that *E. coli* isolates implicated in UTIs among HIV-positive patients are multidrug-resistant ESBL producers with co-resistances to aminoglycosides and fluoroquinolones. Cefuroxime, ceftriaxone, ertapenmen, and amikacin were the most active antibiotics against ESBL-PEc isolates. The increasing prevalence of ESBL-PEc among HIV-positive patients with UTIs poses serious challenges in healthcare settings. Therefore, it is imperative to carry out antibiotic susceptibility testing before the commencement of antibiotics therapy in HIV-positive patients with UTIs so as to ascertain the most current effective antibiotics. This will greatly help in achieving appropriate antibiotic therapy and curbing the increasing antibiotics resistance menace.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Comparative study of the phytochemical and antibacterial activity of leaf extracts of *Euphorbia heterophylla* and *Vitellaria paradoxa*

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A comparative study was carried out to determine the phytochemical components and *Euphorbia heterophylla* antibacterial activity as well as *Vitellaria paradoxa*leaf crude extracts on four enteric organisms, namely: *Proteus vulgaris, Salmonella* Typhi, *Shigella flexneri*, and *Escherichia coli.* The clinical isolates of the enteric organisms were subjected to test of antimicrobial susceptibility using technique of agar diffusion. Phytochemistry of the *E. heterophylla* crude extracts exposed the presence of more phenolics, phlobatannins, tannins and cardiac glycosides than *V. paradoxa*, which revealed the presence of more steroids. All crude *E. heterophylla* extracts produced high clear inhibition zones, compared to the *V. paradoxa* counterpart at concentration ranging from 50 to 200 mg/ml. *In vivo* antimicrobial assay discovered that the mice treated with the crude methanolic *E. heterophylla* extracts, after being infected with the test organisms, survived and showed no pathological effects as compared to the *V. paradoxa* counterpart, which showed 20% pathological effects. *E. heterophylla* crude extract could be a possible source for the diseases treatment associated with enteric organisms such as *P. vulgaris, S.* Typhi, *S. flexneri,* as well as *E. coli*. Additional studies should be directed towards isolation as well as characterisation of the active compound in the crude extracts.

Key words: *In vitro* activity, *in vivo* activity, *Euphorbia heterophylla*, *Vitellaria paradoxa*, Enteric bacteria.

INTRODUCTION

Plants, which are not only regarded as integral parts of the earth, have served as drugs sources as well as pharmaceuticals for man and other animals from immemorial time. They consist of 32% of the surface of the earth (Ghose, 2016). There are about half a million plants now growing on earth, which possess many therapeutic as well as pharmaceutical properties (Muller, 1973). According to a previous survey, about 25% of medicinal products as well as modern drugs are derived from plant secondary metabolites (Hamburger and Hostettmann, 1991). Essentially, most of these plants are said to be helpful in achieving stable health as well as most diseases treatment associated with the human race, and as such they are termed medicinal plants. Medicinal plants can be defined as natures' pharmacy for nearly 80% of people living in Africa (World Health Organisation,

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2002). A medicinal plant has been defined as any plant in which, one or more of its parts contain substances that can be used for therapeutic purpose or which acts as precursors for the useful drugs synthesis (Sofowora, 1982). Based on the cultural acceptability as well as fewer side effects, herbal medicine still remains the mainstay of 75 to 80% of the whole population for primary health care in Africa (Ghasi et al., 2000). In Nigeria, thousands of plant species are known to have medicinal values (Sofowora, 1982). Generally, the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Rios and Recios, 2005); but the current emergence scenario of multiple drug resistance to human pathogenic organisms, has required a search for new antimicrobial substances from plants.

Though, the plants ability to produce many phytochemicals that are used to perform significant biological functions is one of the many characteristics they possess as well as the medicinal values of these plants lie in the phytochemicals present in the plants and these phytochemical in turn produce certain physiological actions on the body of human (Lee et al., 1998; Afolabi et al., 2001; Doughari and Manzara, 2008). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well known that plants produce these chemicals to protect themselves and recent researches have demonstrated that they can also protect humans against diseases. These chemicals possess varied actions such as antioxidants, hormonal activity, stimulatory activities, antimicrobial effects, antidiarrhoreal effects, anti histamic effects, anti diabetic effects, anti malarial effects and anti carcinogenic effect. In most cases because of the these bioactive components presence or phytochemicals in plants, plants may be considered as possible candidates for developing new antimicrobial drugs or alternative treatments of numerous ailments caused by microorganisms resistant to most available synthetic drugs. Two effective medicinal plants that are gradually gaining grounds in the developing countries because of their medicinal benefits are *Vitelleria paradoxa* as well as *Euphorbia heterophylla*.

E. heterophylla is one of the several plants found in the field. *E. heterophylla* grows in distressed localities, as a cultivation weed and waste land, in gardens and along roadsides, from sea-level up to 3000 m altitude (Mosango, 2008). *E. heterophylla* belongs to the family of Euphorbiaceae. It is referred to as Mexican fire plant, milk weed as well as spurge weed in English, and commonly called *Nono-kunchiya* in Hausa, *Egele* in Ibo and *Adimeru* in Yoruba, Nigeria (Okeniyi et al., 2012). All parts of *E. heterophylla* comprised latex (Mosango, 2008). *E. heterophylla* is used widely in traditional African medicine and elsewhere in tropical countries. In general, this plant is regarded as a purgative, antiasthmatic, anti-inflammatory as well as an arbotifacient (Erden et al., 1999; Falodun et al., 2006). It has also been reported to be oxytocic (Unekwe et al., 2006). The dried leaves butanol extract exhibited marked inhibitory action on the *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* growth at 100 mg/ml (Mosango, 2008). An extract of the aerial parts showed antiplasmodial activity. A leaf extract showed significant nematicidal activity against *Meloidogyne graminicola* (Mosango, 2008).

V. paradoxa, on the other hand, is generally regarded as a multipurpose as well as deciduous plant, found and used in Africa. The size of the mature tree of *V. paradoxa* differs from 7 to 25 m. This plant belongs to the family Sapotaceae and is said to produce Shea butter as its main product (Djekota et al., 2014). *V. paradoxa* formerly called *Butyrospermum parkii* (which means butter seed) could also be called *Butyrospermum paradoxa*. In Nigeria, it is locally known as '*emi-emi*' among the Yorubas, *'ka'danya'* among the Hausas and '*okwuma*' among the Igbos (Orwa et al., 2009). The plant is said to possess white latex. The shea tree grows naturally in the wild in the dry savannah belt of West Africa from Senegal in the west to Sudan in the east, and onto the Ethiopian highlands foothills.

The leaves of this plant are mostly used as medicine to treat stomach ache in children (Fobil et al., 2002). A decoction of young leaves is used as a vapour bath to treat headaches in some Africa countries such as Ghana, Nigeria. The leaves usually contain saponins, which produces lathers in water and such water can be used for washing and cleansing various organs of the body such as the eyes. Generally, these plants are used for the diseases treatment caused by enteric bacteria which are known to be associated with high morbidity and mortality cases among the populace.

Enteric bacteria are Gram negative bacteria that are associated with gastrointestinal flora or disease (Murray, 1994). Enterics can be found in numerous natural habitats, not just in the intestinal tract. Most enterics are motile by peritrichous flagella; two major exceptions that lack peritrichous flagella, are *Shigella* as well as *Klebsiella* (AL-Ouqaili, 2013). Numerous enteric organisms are anaerobic in nature, a trait which allows them to thrive in the environment of the gut, and most produce energy by feeding on sugars as well as changing them into lactic acid. Some of the enterics can live in the gut without causing health problems in individuals of good health, whereas others almost always cause infection signs, including diarrhoea, vomiting, and related symptoms (Murray, 1994). Based on this health related threats posed by the enteric organisms, many individuals tend to abuse the use of available synthetic drugs, thereby encouraging these enteric organisms to develop resistance to these drugs. It is consequently imperative to seek other alternative remedy sources to numerous enteric diseases as well as determine the differences between them in this perspective, which is the main aim of this study.

METHODOLOGY

Collection and identification of the plant materials

Both plants leaves of fresh samples (namely *E. heterophylla* and *V. paradoxa*) were collected from Garatu, in a village called Anguwan noma. Anguwan noma in Garatu lies on Longitude 6.44°N, and Latitude 9.4°E. These plants' materials were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification and processing. The voucher specimens of these plants' material have been deposited in the herbarium with the deposition numbers such as: NIPRD/P/001/961 and NIPRD/H/6865 for *V. paradoxa* and *E. heterophylla*, respectively.

Drying procedure

Both plants leaves were washed thoroughly, air dried at room temperature (28°C) and ground into coarse powder using a sterile pestle as well as mortar. The dried leaves were further ground into a fine powder using an electric blender. This was done to improve the extracting solvent penetration, therefore facilitating the active components release (Iyamabo, 1991).

Extraction

One hundred grams of each ground leaves, were softened successively for three days (with occasional shaking) using cold maceration technique. 1000 ml each of distilled water, methanol, chloroform and petroleum ether were used as extraction solvents, respectively. The macerated samples were sieved with Muslin cloth as well as evaporated to dryness using a steam bath. The dried extracts were weighed and stored in sterile sample bottles and kept in the refrigerator for further studies (Iyamabo, 1991).

Phytochemical screening

The crude extracts phytochemical screenings of both leaves were carried out to detect the presence or absence of some secondary metabolites. The methods by Harborne (1984) and Trease and Evans (1987) were employed.

Culture media

MacConkey, *Salmonella-Shigella* and nutrient agars were, respectively used as differential medium, selective medium and for susceptibility testing of the test organisms, respectively, as described by Idu and Igekele (2012).

Identification of the test organisms

The Multi drug resistant (MDR) test organisms (*Proteus vulgaris, Salmonella* Typhi, *Shigella flexneri* and *E. coli*) were gotten from the stock culture in the Microbiology Laboratory, General Hospital, Minna. The test organisms were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar tests as described by Cheesbrough (2010).

Antibacterial assay of the extracts

Well grown activated cultures were diluted serially in test tubes with normal saline until a cell concentration of 1.0×10^5 cfu/ml was gotten. The antibacterial assay of the crude extracts of both plants leaves was done using punch hole method defined by Idu et al. (2012). The plates were prepared by dispensing 20 ml of sterile molten nutrient agar into sterile Petri plates and allowed to set. A 4 mm cork borer was used to punch holes in the medium. Four holes were made in each agar, adequately spaced out after inoculation (which was carried out by aseptically streaking the inoculums on the surface of the media with a wire loop). About 0.2 ml of the different concentrations was introduced into each well. The Petri plates were incubated at a temperature of 37°C for 24 h, after which observed inhibition zones were measured as well as the results recorded in comparison with the effect of the standard antibiotic, known as Ciprofloxacin (5 µg) which was used as the control (Idu and Igekele, 2012). Only leaf extracts of each plant which showed high antibacterial activity and served as possible drug development source were used for the *in vivo* studies.

In vivo antibacterial activity of the crude extracts

Experimental animals

Mice within the age of 8-12 weeks with body weight from 18-22 g were picked up from Ibrahim Badamasi Babangida University Lapai. The mice were kept in standard cages with suitable food, water as well as under hygienic conditions for 2 weeks before inoculation (Canadian Council on Animal Care, 1997).

Challenge culture preparation (Preparation of Inoculum)

A loopful of the organisms was inoculated on *Salmonella-Shigella* agar to stimulate the test organisms. The test organisms were transferred further into test tubes containing 10 ml of sterilised nutrient broth and incubated at 37°C for 18-24 h. The activated culture was diluted serially in test tubes with normal saline until a cell concentration of 1.0×10^5 cfu/ml was gotten (Eman and Hoda, 2008).

Inoculation of test organisms and administration of plant extracts and antibiotic to albino mice

Division of the mice was into 15 sub-groups of 5 each. In each sub-group, a precise volume of an inoculum (approximately 1 ml of the infective dose of the inoculum) was introduced into each mouse intraperitoneally as approved by Eman et al. (2008). After the innoculation of the mice, each extract administration (namely *E. heterophylla* chloroform leaf, *E. heterophylla* aqueous leaf, *E. heterophylla* methanolic leaf, *V. paradoxa* chloroform leaf, *V. paradoxa* methanolic leaf and *V. paradoxa* aqueous leaf) and antibiotics namely Ciprofloxacin (5 µg) were done orally for 7 days (Itelima and Agina, 2014). The mice were closely observed daily and the mortality rate as well as other physical manifestations was recorded.

Observation of mortality rate, survival rate and other physical manifestations

The survival as well as mortality rate of the mice in the sub groups was calculated as numbers of the mice that died as well as survived during the experiment course in relation to all the mice that were used (Eman and Hoda, 2008). The animals were perceived to note the consistency, frequency as well as colour of their faecal waste. The mice were also perceived for any abnormalities and physical manifestations (for example loss of appetite, loss of weight

Table 1. Phytochemical constituents of the leaves of *Euphorbia heterophylla* and *Vitellaria paradoxa*.

Phytochemical compound	Leaf (*Euphorbia heterophylla*)				Leaf (*Vitellaria paradoxa*)			
	Chloroform	Methanol	Aqueous	Petroleum ether	Chloroform	Methanol	Aqueous	Petroleum ether
Carbohydrates	+	+	-	-	+	+	+	-
Starch	+	+	-	-	+	+	+	-
Cardiac glycosides	-	+	+	+	-	-	-	+
Saponins	+	+	+	+	+	+	+	+
Steroids	-	-	+	+	-	+	+	+
Alkaloids	-	+	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	+	-	-	+
Tannins	-	+	+	-	+	-	-	-
Phlobatannins	-	-	+	-	-	-	-	-

+= Presence of the phytochemical compound; - = Absence of the phytochemical compound.

and body weakness) during the experiment period (Itelima and Agina, 2014). The infected mice were killed using chloroform and were buried, to stop the spread of the infection associated with enteric pathogens in the environment at the end of the study (Itelima et al., 2014).

RESULTS AND DISCUSSION

The phytochemical components of the leaf extract of *E. heterophylla* were: starch, saponins, alkaloids, flavonoids and more of phenolics, phlobatannins, tannins and cardiac glycosides than its *V. paradoxa*, which revealed the presence of more steroids (Table 1).

The basis of the therapeutic activities of plants lies on the phytochemical components contained in these plants (Oyedum, 2015). This therefore implies that variations observed among the phytochemicals of two different plants will result to difference in their therapeutic abilities. This in turn is observed in this study, where the phytochemicals were found present at varying proportions among solvent extracts of the leaves of the various plants. From this study, more than 50% of *E. heterophylla* extracts (namely chloroform, methanol, aqueous and petroleum ether) possessed phytochemicals such as cardiac glycosides, phenolics, tannins and phlobatannins more than its *V. paradoxa* counterpart which revealed high contents of steroids, carbohydrates and starch among its extracts (Table 1). Onwuliri (2004) have also observed the presence of such constituents as phlobatannins, alkaloids, saponins, phenolics, tannins, cardiac glycosides among others in tropical plants growing in Nigeria, and some have been shown to exhibit varying biological activities. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993). Mensah et al., (2008) reported the importance of alkaloids, saponins and tannins in manufacturing various antibiotics used in treating common pathogenic strains and these phytochemicals have also been known to produce a definite physiological action on human body (Edeoga et al., 2005). The presence of cardiac glycosides indicates that they may act as good sedatives and have antispasmodic properties (Egunyomi et al., 2009). Schneider and Wolfhing (2004) have reported the therapeutic effects of some phytochemical constituents such as tannins and cardiac glycoside against cardiovascular disease and digestive problems. Therefore, the observation of these phytochemical constituents in different proportions, together with other phytochemicals found in these plants of study may be responsible for some of the observed antibacterial activity observed in this study.

Furthermore, this study showed great disparity in the results obtained from the antibacterial activities of the leaves of both *E. heterophylla* and *V. paradoxa*. The results revealed that the leaves of *E. heterophylla* showed high antibacterial activities than its *V. paradoxa* counterparts (Tables 2 to 9). This result could be attributable to the fact that high proportion of phytochemicals was observed in the leaves of *E. heterophylla.* This is based on the fact that the age and nature of the harvested leaves of *E. heterophylla* which is a shrub, as compared to its *V. paradoxa* counterpart which is a full grown tree, is said to affect the yield and quality of the bioactive components obtained, thereby giving rise to well concentrated and effective bioactive components. This observation conforms to the study of Calixto (2000), who reported that difference in the antibacterial activity could be due to differences in geographical location, season of plant, age of the plant, and method of extraction, all of which affect the yield and the active constituents of medicinal plants. Similarly, the difference observed in the antibacterial activities of both leaves that were studied, could be attributed to the

Table 2. Zones of inhibition of the leaf of *V. paradoxa* at 50 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
VPCL	1.33±0.33^{b}	0.33±0.03^{a}	0.67±0.033^{a}	0.00±0.00^{a}
VPML	5.67±0.33^{b}	4.67±0.33^{c}	5.67±0.33^{b}	5.00±0.58^{bc}
VPAL	0.67±0.33^{a}	0.00±0.00^{a}	3.33±0.33^{a}	2.33±0.33^{a}
VPPL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Control	18.00±0.58^{d}	17.33±0.67^{c}	17.00±0.58^{d}	14.33±0.67^{d}

Table 3. Zones of Inhibition of the leaf of *E. heterophylla* at 50 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCL	3.33±0.30^{bc}	3.00±0.58^{bc}	2.33±0.33^{bc}	2.00±0.58^{b}
EHML	6.00±0.58^{f}	5.67±0.67^{ef}	3.33±0.88^{bc}	5.67±0.88^{b}
EHAL	5.33±0.33^{def}	5.67±0.33^{ef}	4.00±0.58^{cd}	5.00±0.58^{b}
EHPL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Control	9.00±0.58^{g}	8.00±0.57^{g}	8.67±0.68^{g}	8.67±0.33^{e}

Table 4. Zones of inhibition of the lesf of *V.paradoxa*at 100 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
VPCL	4.67±0.33 ^{b}	3.33±0.90^{ab}	4.00±0.58^{b}	3.00±1.00^{ab}
VPML	7.00±0.58^{a}	5.33±0.88^{a}	6.67±0.33^{b}	6.00±0.58^{a}
VPAL	6.00±0.58^{b}	4.00±0.58^{a}	5.00±0.57^{a}	4.67±0.33^{a}
VPPL	2.67±0.33^{a}	2.00±0.58^{a}	2.33±0.33^{a}	1.67±0.67^{a}
CONTROL	24.67±0.33^{d}	23.00±0.00^{c}	24.00±0.58^{d}	20.00±0.58^{c}

Table 5. Zones of Inhibition of the leaf of *E. heterophylla* at 100 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCL	5.67±0.67^{bc}	5.00±0.58^{bc}	6.67±0.33^{cde}	6.33±0.33^{bc}
EHML	8.67±0.88^{defg}	8.33±0.33^{efgh}	8.00±0.58^{cde}	7.67±1.20^{cdef}
EHAL	8.33±0.33^{def}	6.33±0.90^{bcde}	7.000.60^{cde}	7.33±1.20^{cde}
EHPL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Control	15.00±0.60^{h}	13.33±0.90^{i}	13.33±1.45^{f}	12.33±1.45^{g}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet are significantly ($p < 0.05$). EHCL-Chloroform leaf extract of *Euphorbia heterophylla*; EHML-Methanolic leaf extract of *Euphorbia heterophylla*; EHAL-Aqueous leaf extract of *Euphorbia heterophylla*; EHPL-Petroleum ether leaf extract of *Euphorbia heterophylla;* EHCS-Chloroform stem extract of *Euphorbia heterophylla*; EHMS-Methanolic stem extract of *Euphorbia heterophylla* ; EHAS-Aqueous stem extract of *Euphorbia heterophylla;* EHPS-Petroleum ether stem extract of *Euphorbia heterophylla;* EHCR-Chloroform root extract of *Euphorbia heterophylla*; EHMR-Methanolic root extract of *Euphorbia heterophylla*; EHAR-Aqueous root extract of *Euphorbia heterophylla*; EHPR-Petroleum ether root extract of *Euphorbia heterophyll.*

different content of latex present in each plant; which is said to enhance the antibacterial potentials of various plants that contain it (Okeniyi et al., 2012; Oyedum, 2015). However, other studies have shown that *E. heterophylla* as a plant contains 0.77% of latex, of which the leaf contains 0.42% of the latex (Mosango, 2008). This

Table 6. Zones of inhibition of the leaf of *V. paradoxa* at 150 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCL	8.33±0.33^{c}	7.67±0.30^{c}	7.67±0.88^{bc}	7.33±0.90^{c}
EHML	11.33±0.60^{d}	10.33±0.33^{def}	10.33±0.88^{cdef}	9.67±0.90^{cd}
EHAL	10.33±1.33^{cd}	9.00±0.60^{cde}	9.33±1.45^{cde}	8.67±0.30^{c}
EHPL	4.00±0.58^{ab}	3.67±0.70^{b}	5.33±0.90^{b}	3.67±0.31^{b}
Control	20.00±0.60^{f}	18.67±0.70^{g}	19.33±0.33^{g}	19.00±0.58^{f}

Table 7. Zones of Inhibition of the leaf of *E. heterophylla* at 150 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
VPCL	9.00±0.60^{cd}	8.33±0.33^{bcd}	8.67±0.30^{cd}	8.00±0.57^{b}
VPML	11.00±0.58^{a}	10.00±0.58^{a}	10.00±0.58^{ab}	9.33±0.30^{ab}
VPAL	10.33±0.33^{a}	9.33±0.33^{a}	9.33±0.65^{a}	8.33±0.32^{a}
VPPL	4.33±0.33^{a}	3.33±0.30^{a}	3.33±0.33^{a}	3.00±0.60^{a}
Control	27.00±0.58^{c}	26.00±0.58^{d}	26.33±0.33^{d}	25.67±0.33^{d}

Table 8. Zones of Inhibition of theleaf of *V. paradoxa* at 200 mg.

Extract	*S.* Typhi	*S. flexneri*	*E. coli*	*P. vulgaris*
EHCL	9.33±0.66^{bc}	8.00±0.58^{bc}	8.00±1.16^{bc}	8.00±0.60^{bcd}
EHML	12.00±0.60^{e}	11.33±0.90^{e}	11.67±0.88^{d}	10.33±0.33^{e}
EHAL	10.67±0.70^{cde}	10.00±0.60^{de}	10.67±0.33^{cd}	9.67±1.20^{de}
EHPL	8.33±0.33^{b}	7.33±0.33^{b}	8.00±0.00^{b}	6.33±0.88^{b}
Control	26.00±0.60^{h}	25.00±0.70^{g}	25.33±0.33^{f}	24.33±0.33^{g}

Table 9. Zones of Inhibition of the leaf of *E. Heterophylla* at 200 mg.

Extracts	*S. typhi*	*S. flexneri*	*E. coli*	*P. vulgaris*
VPCL	10.33±0.33^{c}	7.67±1.20^{b}	9.67±0.33^{cd}	9.00±0.60^{bcd}
VPML	12.00±0.58^{a}	11.33±0.33^{a}	11.00±0.58^{a}	10.68±0.30^{a}
VPAL	11.67±0.33^{a}	11.00±0.58^{a}	9.67±0.65^{a}	10.00±0.56^{a}
VPPL	5.33±0.30^{a}	4.33±0.33^{a}	5.00±0.00^{a}	4.00±0.60^{a}
Control	30.00±0.58^{c}	29.67±0.33^{c}	29.33±0.67^{c}	28.00±0.58^{c}

Values are represented as Mean±Standard Error of Mean of triplicate determinations. Values along the column with different alphabet are significantly ($p < 0.05$). EHCL-Chloroform leaf extract of *Euphorbia heterophylla*; EHML-Methanolic leaf extract of *Euphorbia heterophylla*; EHAL-Aqueous leaf extract of *Euphorbia heterophylla*; EHPL-Petroleum ether leaf extract of *Euphorbia heterophylla;* EHCS-Chloroform stem extract of *Euphorbia heterophylla*; EHMS-Methanolic stem extract of *Euphorbia heterophylla* ; EHAS-Aqueous stem extract of *Euphorbia heterophylla;* EHPS-Petroleum ether stem extract of *Euphorbia heterophylla;* EHCR-Chloroform root extract of *Euphorbia heterophylla*; EHMR-Methanolic root extract of *Euphorbia heterophylla*; EHAR-Aqueous root extract of *Euphorbia heterophylla*; EHPR-Petroleum ether root extract of *Euphorbia heterophyll.*

therefore reveals that the percentage of latex in the leaf of *E. heterophylla* is far more than its *V. paradoxa* counterpart, which in turn enhanced its antibacterial activities on the various test organisms.

Figures 1 to 4 reveal that among the various groups of mice treated with the leaves of *E. heterophylla* and *V. paradox*a which were infected with *S. typhi, S. flexneri, E. coli* and *P. vulgaris*, all the infected mice groups

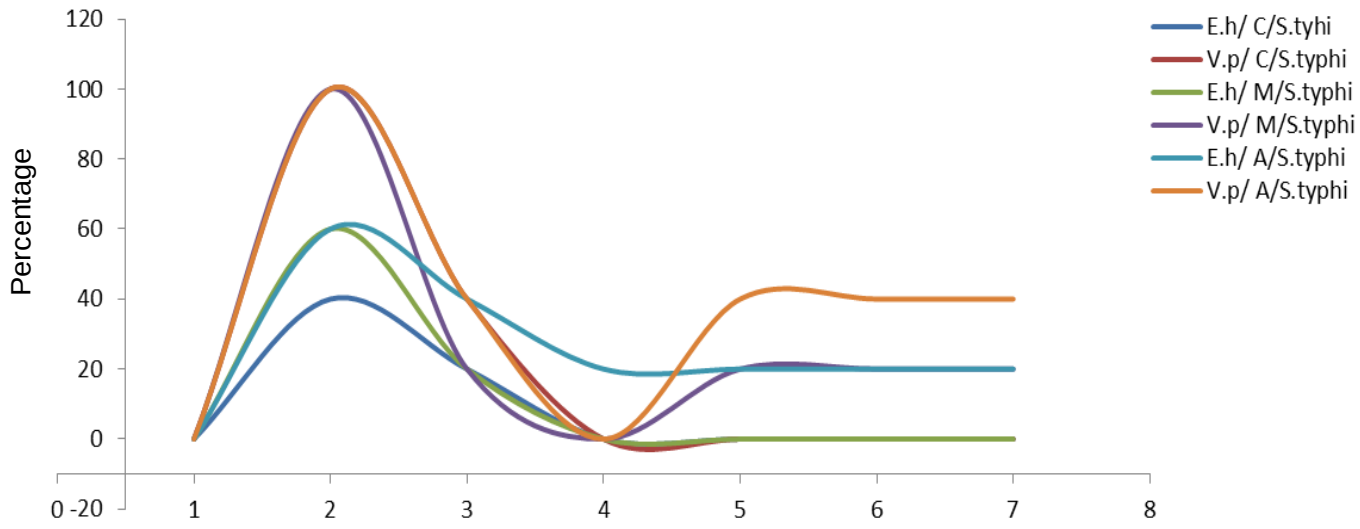

Figure 1. Pathological signs and symptoms of *S.* Typhi infected mice. 1= Mortality; 2=Watery diarrhorea(1-3days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/M=Methanolic leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa.*

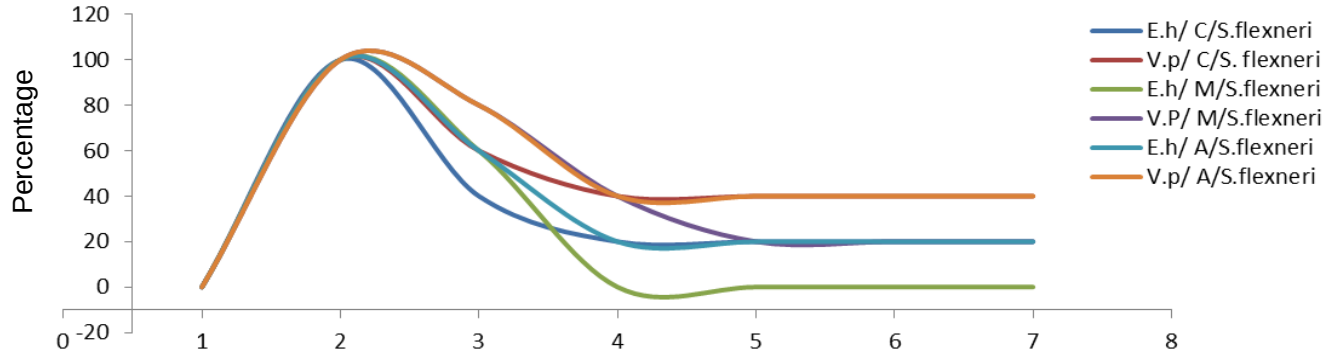

Figure 2. Pathological signs and symptoms of *S. flexneri* infected mice treated with various extract. 1= Mortality; 2=Watery diarrhorea(1-3days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/M=Methanolic leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa.*

treated with methanolic extracts of *E. heterophylla* showed 0% of pathological signs and symptoms (such as watery diarrhorea> 7 days, weight loss, loss of appetite and body weakness, while infected mice treated with chloroform and aqueous extracts of *E. heterophylla* showed 20% pathological signs and symptoms (such as watery diarrhorea> 7 days, weight loss, loss of appetite and body weakness) against the groups of mice treated with its *V. paradox*a counterpart, which showed 40% pathological signs and symptoms after the extracts were administered orally. This result could be attributed to the fact that bioactive components contained in the leaves of *E. heterophylla* are diverse and highly potent than those contained in the leaves of *V. paradox*a. This agrees with the result of Jayashree (2013).

Conclusion

The methanolic, chloroform and aqueous extracts of *E. heterophylla* contained diverse and efficient phytochemicals that were active against all test organisms at all the applied concentrations, namely: 50, 100, 150 and 200 mg/ml, indicating that *E. heterophylla* is potent and contains high yield of therapeutic properties against its *V. paradoxa* counterpart. However, the *in vivo*

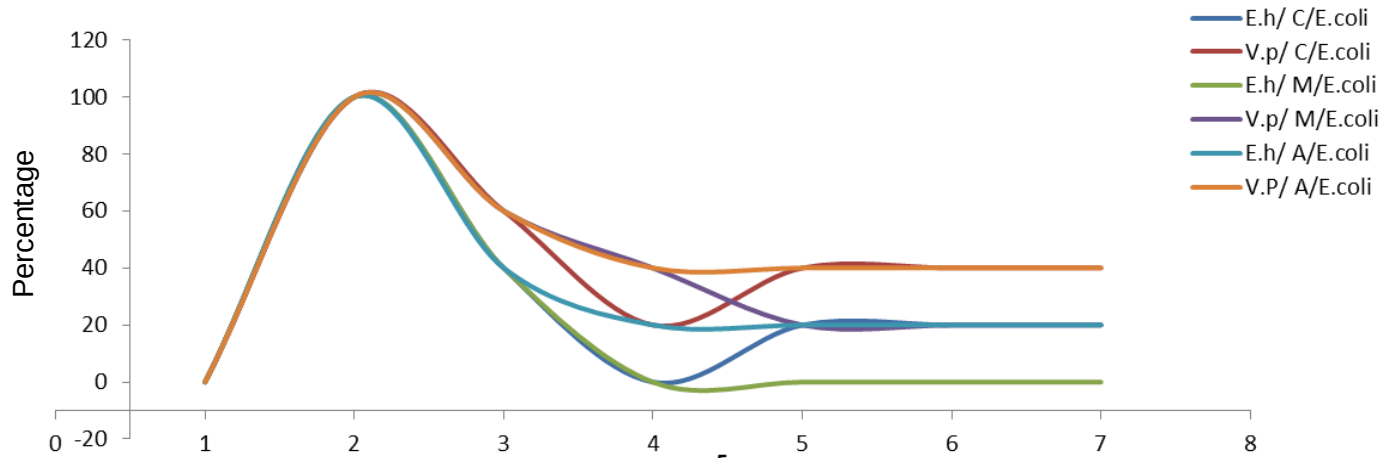

Figure 3. Pathological signs and symptoms of *E. coli* infected mice. 1= Mortality; 2=Watery diarrhorea(1-3 days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/M=Methanolic leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa*.

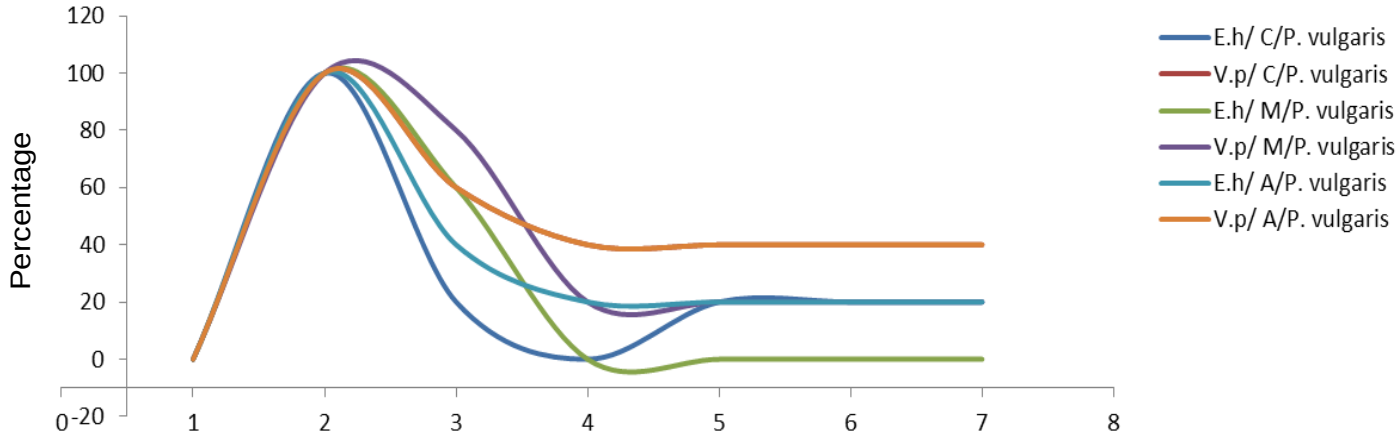

Figure 4. Pathological signs and symptoms of *Proteus vulgaris* infected mice. 1= Mortality; 2=Watery diarrhorea (1-3 days); 3=Watery diarrhorea (4-6 days); 4= Watery diarrhorea (> 7 days); 5= Weight loss; 6= Loss of appetite; 7=Body weakness; E.h/C=Chloroform leaf extract of *Euphorbia heterophylla*; V.p/C=Chloroform leaf extract of *Vitellaria paradoxa*; E.h/M= Methanolic leaf extract of *Euphorbia heterophylla*; V.p/M=Methanolic leaf extract of *Vitellaria paradoxa*; E.h/A= Aqueous leaf extract of *Euphorbia heterophylla*; V.p/A=Aqueous leaf extract of *Vitellaria paradoxa.*

studies revealed that only the methanolic leaf extract of *E. heterophylla* showed 0% pathological signs and symptoms against all the infected groups of mice treated. It is therefore recommended that in order to obtain goodyield of bioactive components from plants with effective eradication, preventive and therapeutic potentials, most especially against the emerging resistant antibiotic genes associated with most third world countries, alcohol based solvents should be utilized for extraction.

CONFLICT OF INTERESTS

The author has not declared any conflict of interest.

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