OPEN ACCESS

African Journal of **Microbiology Research**

28 February 2018
ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

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Vol. 12(8), pp. 181-185, 28 February, 2018
DOI: 10.5897/AJMR2018.8796
Article Number: CAA58C356157
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibacterial and anti-adherent effect of *Mimosa tenuiflora* and *Myrciaria cauliflora* on dental biofilm bacteria

Maria Regina Macêdo-Costa[1*], Carlus Alberto Oliveira dos Santos[2], Eduardo Roberto de Lucena[2], Maria do Socorro Vieira Pereira[2] and Kenio Costa de Lima[1]

[1]Departamento de Odontologia, Universidade Federal do Rio Grande do Norte, Av. Salgado Filho, 59056-000 - Natal, RN - Brasil.
[2]Departamento de Odontologia, Centro Universitário de João Pessoa - Unipê, Rodovia BR-230, KM 22, s/n - Água Fria, 58053-000, João Pessoa, PB - Brasil.

Recieved 9 January, 2018; Accepted 9 February, 2018

The use of plants in the prevention and treatment of oral infectious and as an antibiofilm agent continues to be valued in many parts of the world. The aim of study is to evaluate *in vitro* antimicrobial action of the plant extracts of barks *Mimosa tenuiflora* (Willd.), Poir. (jurema preta) and leaf and stem of *Myrciaria cauliflora* Berg. (jabuticabeira) against dental biofilm bactéria. The oral bacteria were used to determine the minimum inhibitory concentration (MIC) and minimum inhibitory concentration of adherence (MICA): *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus salivarius* and *Lactobacillus casei*. Each assay was carried out in duplicate and the positive control (0.12% chlorhexidine digluconate) was subjected to the same procedure. Results were analyzed by Student t test or Mann-Whitney test, with the level of significance set at 5%. The extract of *M. tenuiflora* showed inhibition halos ranging from 10 to 25 mm in diameter, presenting an average performance superior to chlorhexidine digluconate, being statistically significant only at the concentration of 1:128 (3.9 mg/mL). The leaf and stem extracts of *M. cauliflora* were found to have inhibition halos varying from 10 to 18 mm in diameter and presented a significantly lower average performance in relation to chlorhexidine digluconate in crude extract (500 mg/mL) concentration; 1:2 (250 mg/mL) and 1:4 (125 mg/mL) and crude extract (500 mg/mL), 1:2 (250 mg/mL), 1:4 (125 mg/mL) and 1:8 (62 mg/mL), respectively. All extracts studied were effective in the inhibition of adherence, especially the stem extract of *M. cauliflora* (1:64/ 7.81 mg/mL). Conclusively, the extracts of *M. tenuiflora* and *M. cauliflora* produced a significant bactericidal activity and *in vitro* anti-adherent effect on the bacteria forming the dental biofilm, which suggests the use of these substances as an alternative and economically viable means for the control of infections in dentistry.

Key words: Microbiology, phytotherapy, *Mimosa tenuiflora* (Willd.) Poir., *Myrciaria cauliflora* Berg.

INTRODUCTION

In oral environment, microorganisms are organized as biofilm. The formation of biofilm follows a regular pattern, involving the initial association of pathogens with salivary pellicle in enamel, followed by interbacterial participation in a process known as secondary colonization (Cavalcanti et al., 2014; Lobo et al., 2015).

Oral infectious diseases can be prevented by disruption of the biofilm using mechanical or chemical methods. Various antimicrobial agents are used in the chemical control of biofilms, such as cationic agents, chlorhexidine and cetylpyridinium chloride, which immediately bind to the bacterial surface, negatively charged, while the triclosan and phenolic compounds are non-ionic agents (Ocheng et al., 2014; Vargas et al., 2015; Costa et al., 2017). Chlorhexidine is the most common anti-biofilm agent used in dentistry (Araújo, 2015; Vieira et al., 2014). It acts in the general disruption of the cell membrane and in the specific inhibition of membrane enzymes, which inhibits glucose uptake by *Streptococcus mutans* and uses lactic acid for its metabolism, as well as reducing the proteolytic activity of *Porphyromonas gingivalis*. Its action is unquestionable, but chlorhexidine has negative effects such as gustatory interference, mottling of the tooth surface and restoration, which may even cause microbial resistance (Hajifattahi et al., 2016; Salleh et al., 2011). Increasing antimicrobial resistance, adverse effects and evolution of new species of disease-causing bacteria, have shown a need for finding new and safe antibacterial agents. Medicinal plants is an attractive source for new discoveries of antibacterial agents, given their molecular diversity (Majali et al., 2015; Hajifattahi et al., 2016; Al-Ayed et al., 2016; Alagl et al., 2017). In Brazil, there is approximately 25% of the 250,000 medicinal species cataloged by UNESCO, and a little more than eight thousand species are in the Brazilian semiarid region, facilitating the access and use of the healing potential of these plants in the treatment of diseases. These include the area of dentistry, specifically, diseases dependent on biofilm, such as caries and gingivitis. In recent years, some studies have been developed in Brazil in order to assess the popular use of vegetation in dentistry, making it possible to identify species with potential antimicrobial activity (Majali et al., 2015; Vieira et al., 2014; Sette - de -Souza et al., 2014). Several studies with plants of Caatinga in Northeastern Brazil, including Jurema preta (*M. tenuiflora*) and Jabuticabeira (*M. cauliflora*) show the presence of some secondary metabolites, such as tannins and phenolic compounds. Such substances have significant antimicrobial activity and may act in activating enzymes which are responsible for important pharmacological effects such as anti-inflammatory, antimicrobial, antioxidant, and others (Bezerra et al., 2011; Bona et al., 2014; Azevedo et al., 2015). According to de Oliveira at al. (2012), phenolic compounds are associated with various medicinal effects. The objective of this study was to evaluate the antibacterial activity and non-stick effect of plant extracts of barks *M. tenuiflora* (Willd.) Poir. (jurema preta) and leaf and stem of *M. cauliflora* Berg. (jabuticabeira) in the control of microorganisms of biofilm related to tooth decay, the most prevalent chronic disease.

MATERIALS AND METHODS

Preparation of the *M. tenuiflora* (Willd.) Poir and *M. cauliflora* Berg. Extract

Barks of *M. tenuiflora* (Willd.) Poir and leaf and stem of *M. cauliflora* Berg. were collected in the town of Teixeira, Paraíba.

The crude extract was prepared at the Laboratory of Chemical and Biological Sciences, Federal University of Campina Grande (UFCG), Center for Health and Rural Technology (CSTR). A voucher specimen of the plant was deposited at the Dárdano de Andrade Lima Herbarium, Regional University of Cariri (URCA), Crato, Ceará (Registration No. 4016).

After collection, the *M. tenuiflora* (willd.) poir. (barks) and *M. cauliflora* Berg. (leaf and stem) samples were desiccated in an oven in circulating air at an average temperature of 45°C and then ground to powder in a mechanical grinder. The dry and ground material was macerated with 2 L of 95% ethanol for 72 h. The resulting crude *M. tenuiflora* (willd.) poir. and *M. cauliflora* Berg. extract was concentrated in a rotary evaporator under reduced pressure at a temperature that did not exceed 40°C.

Determination of minimum inhibitory concentration

Antibacterial activity was evaluated *in vitro* according to the method of Bauer et al. (1966) for the determination of minimum inhibitory concentration (MIC). The following bacterial strains were cultured in brain heart infusion broth (BHI; Difco, Detroit, MI, USA) at 37°C for 18 to 20 h under microaerophilic conditions: *Streptococcus mitis* (ATCC 903), *S. mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 15300), *Streptococcus oralis* (ATCC 10557), *Streptococcus salivarius* (ATCC 7073), and *Lactobacillus casei* (ATCC 9595) (bacteria of the resident microbiota and dental caries).

Saline inoculated with each bacterial growth was spread across petri dishes containing Mueller-Hinton agar (Difco) and five standard holes measuring approximately 6 mm in diameter were punched in each plate. Next, 50 µL of the test substance (crude extract diluted in distilled water up to 1:512) was added to the holes and the plates were incubated at 37°C for 24 h under microaerophilic conditions.

Each assay was carried out in duplicate for each strain. The same procedure was used for positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA).

Inhibition halos (in mm) were measured with caliper (Digimess®, São Paulo, Brazil) and MIC was defined as the lowest concentration of the extract that was able to inhibit bacterial growth. The results were transferred to database and Kolmogorov-Smirnov and Levene tests were used. After that, data were analyzed by the Student t-test or Mann-Whitney test, with the level of significance set at 5%.

Determination of minimum inhibitory concentration of adherence

The minimum inhibitory concentration of adherence (MICA) of *M.*

*Corresponding author. E-mail: mariareginamacedo@yahoo.com.br. Tel: +55 83 99921-4856.

Table 1. Student t-test or Mann-Whitney U test (MIC *M. tenuiflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		*p*-value
CE (500) vs. CD	21.5	21.0	0.361	0.725
1:2 (250)	19.5	19.3	0.159	0.878
1:4 (125)	19.2	17.8	1.105	0.295
1:8 (62.5)	18.3	16.5	1.467	0.173
1:16 (31.25)	16.8	14.3	1.612	0.153
1:32 (15.65)	14.7	11.0	1.46	0.201
1: 64 (7.81)	13.5	6.3	2.469	0.055
1:128 (3.9)	12.0	5.0	3.09	0.025*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

Table 2.. Student t-test or Mann-Whitney U test (MIC *M. cauliflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		*p*-value
CE (500) vs. CD	16.3	21.0	-3.715	0.008*
1:2 (250)	13.7	19.3	-5.271	0.001*
1:4 (125)	9.2	17.8	-4.046	0.002*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

tenuiflora (willd.) poir. (Stem) and *M. cauliflora* Berg. (Leaf and Stem) extract was determined in the presence of 5% sucrose as described by Gerbara et al. (1996) using concentrations, corresponding to the crude extract and diluted up to 1:512. *S. mitis* (ATCC 903), *S. mutans* (ATCC 25175), *S. sanguinis* (ATCC 15300), *S. oralis* (ATCC 10557), and *S. salivarius* (ATCC 7073) were sub-cultured in Mueller-Hinton broth (Difco) at 37°C (Table 3). Subsequent, 1.8 mL of the sub-culture was transferred to hemolysis tubes and 0.2 mL of the different dilutions of the extract was added.

Adhesion of bacteria to tube after shaking was analyzed visually after 24 h. Each assay was carried out in duplicate for each selected strain. The same procedure was used for positive control (0.12% chlorhexidine digluconate; Periogard®, Colgate-Palmolive Company).

The MICA is defined as the lowest concentration of the extract in medium with sucrose which prevented adhesion to the glass tube.

RESULTS

Determination of minimum inhibitory concentration

Hydroalcoholic extract of bark of M. tenuiflora

After data collection, the data were tabulated and analyzed statistically. Considering the extracts tested, the Student t-test was used at 5% significance level in the comparison between the hydroalcoholic extract of bark *M. tenuiflora* and 0.12% chlorhexidine gluconate (two independent groups), as shown in Table 1.

Hydroalcoholic extract of leaf M. cauliflora

At 5% significance level, the Student t-test was used to compare the hydroalcoholic extract of leaf *M. cauliflora* and 0.12% chlorhexidine gluconate as shown in Table 2.

Hydroalcoholic extract of stem M. cauliflora

At 5% significance level, the Student t-test was used to compare the hydroalcoholic extract of stem *M. cauliflora* and 0.12% chlorhexidine gluconate as shown in Table 3.

Determination of minimum inhibitory concentration of adherence

The test results of MICA extracts and 0.12% chlorhexidine gluconate are shown in Table 4.

DISCUSSION

Currently, there is an interest from the scientific community in medicinal plants with proven antimicrobial activity, especially with the increasing awareness of the side effects of traditional drugs. The undesirable effects of synthetic drugs and the difficulty in producing new drugs effective in microbial combat suggest that the use

Table 3.. Student t-test or Mann-Whitney U test (MIC *M. cauliflora* extract vs.chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine (mg/mL)	MIC – Mean		Statistic t	Significance
	Extract	Chlorhexidine		(*p*-value)
CE (500) vs. CD	16.8	21.0	-3.201	0.009*
1:2 (250)	15.0	19.3	-4.24	0.005*
1:4 (125)	12.7	17.8	-4.706	0.004*
1:8 (62.5)	10.5	16.5	-5.555	0.002*

CE: crude extract CD: chlorhexidine digluconate * (*p*-value < 0,05) (significant results).

Table 4. Determination of minimum inhibitory concentration of adherence of the extracts and 0.12% chlorhexidine gluconate

Dental biofilm bacteria	*M. tenuiflora* extract (MICA)	*M. cauliflora* extract stem (CIMA)	*M. cauliflora* extract leaf (CIMA)	Chlorhexidine digluconate
Streptococcus mitis	1:8	1:4	1:8	1:128
Streptococcus mutans	1:4	1:4	1:8	1:256
Streptococcus sanguinis	1:8	1:8	1:4	1:64
Streptococcus oralis	1:16	1:64	1:32	1:32
Streptococcus salivarius	1:8	1:16	1:16	1:64
Lactobacillus casei	0	0	0	1:64

of natural plant extracts in fighting the colonizing microorganisms of biofilm should be investigated (Upreti et al., 2012; Al-Ayed et al., 2016).

An initial selection of the antimicrobial potential should be done with the natural plant. The most common way to perform this study is through extract dilutions and test them on disks or wells made in petri dishes. These methods have scientific merit and are recognized to be of great value for studies of infectious diseases (Moreira et al., 2012; de Oliveira et al., 2013). In the present study, we used microorganisms which form biofilm and are resilient to antimicrobials. Pathogens were tested *in vitro* to determine the antimicrobial activity and non-stick of hydroalcoholic extracts from the bark of *M. tenuiflora* (Willd.) Poir. and leaf and stem of *M. cauliflora* Berg.

The results of the Student t-test showed that the hydroalcoholic extract from the bark of *M. tenuiflora* (Willd.) Poir had an average performance higher than 0.12% chlorhexidine gluconate. The data obtained in this test showed a great potential for *M. tenuiflora* (Willd.) Poir on microorganisms of the biofilm which confirms the results obtained in previous trials (Bezerra et al., 2009).

All strains proved to be sensitive to leaf of *M. cauliflora* extract, but the results of Student t test showed that the 0.12% chlorhexidine digluconate had an average performance significantly higher than the hydroalcoholic extract of *M. cauliflora* Berg leaf. The stem extract from *M. cauliflora* Berg. formed inhibition zones ranging from 10 to 20 mm, which agrees with studies by Bonn et al., (2014), where extracts ranged from 6.5 to 12.5 mm in various microbial species. All samples also showed sensitivity to stem of *M. cauliflora* extract at a dilution of 1:8 (62.5 mg/mL), but for *S. mitis*, *S. oralis* and *S. Salivarius*,the inhibition zone was 11 mm, while for *S. mutans*, *S. sanguinis* and *L. Casei*, it was 10 mm.

The data demonstrates the effectiveness of all extracts of *M. cauliflora*, showing the potential of these substances as antimicrobial agents. The bacteriostatic and bactericidal activity in the *M. cauliflora* extract may be related to the presence of the compounds of its main constituent class, tannins, and substances with significant antimicrobial activity. The formation process of the biofilm starts basically with the adherence of microorganisms to the tooth surface, further highlighting the importance of the non-stick ability of antimicrobials to act in the early stages of biofilm formation (Cavalcanti et al., 2014).

The results of these studies demonstrated the greater effectiveness of 0.12% chlorhexidine digluconate on the inhibition of adhesion on all tested strains, compared to that presented by the extract of *M. tenuiflora*. The *M. cauliflora* leaf extract and chlorhexidine digluconate inhibit the adhesion of *S. oralis* at the same concentration of 1:32 (15.65 mg/mL), while *M. cauliflora* stem extract showed greater effectiveness on inhibition of *Streptococcus oralis* at 1:64 (7.81 mg/mL).

The collected data demonstrate the high potential of antimicrobial and non-stick activity of the plant extracts, mainly *M. tenuiflora* on all tested microorganisms, suggesting the possibility of using this agent in concentrations which meet the MIC and MICA in the oral environment. Inhibition of glucan synthesis and its

bacteriostatic action enables these extracts to take effect in controlling biofilm buildup previously established and therefore prevent tooth decay and gingivitis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al- ayed MSZ, Asaad AM, Qureshi MA, Attia HG, AlMarrani AH. (2016). Antibacterial Activity of *Salvadora persica* L. (Miswak) Extracts against Multidrug Resistant Bacterial Clinical Isolates. Int. J. Biomat. pp. 1-5.

Alagl AS, Bedi S, Almas K (2017). Phytosolutions for Enterococcus faecalis in Endodontics: An Update. Oral Health and Dental Management.

Araújo DB, Gonçalves BEM, Martins GB, Lima MJP, Araújo MTB (2015). Saúde bucal: A importância dos enxaguatórios com antissépticos. Rev. Ciênc. Méd. Biol. 14 (1):88-93.

Azevedo TKB, Paes JB, Calegari L, Nascimento JWB (2015). *Mimosa tenuiflora* tannin quality for the production of tannin formaldehyde adhesive. Ciênc. Florest. 25(2):507-514.

Bezerra CDA, Fernandes Galvão Rodrigues F, Martins da Costa JG, Vieira Pereira A, Oliveira de Sousa E, Guedes Rodrigues O (2011). Abordagem fitoquímica, composição bromatológica e atividade antibacteriana de *Mimosa tenuiflora* (Wild) Poiret e *Piptadenia stipulacea* (Benth) Ducke. Acta Scientiarum. Biological Sciences.33(1).

Bona EAM, Pinto FGS, Fruet TK, Jorge TCM, Moura AC (2014). Comparison of methods for evaluation of antimicrobial activity and determination of minimum inhibitory concentration (mic) of aqueous and ethanol plant extracts. Arq. Inst. Biol. 81(3):218-225.

Cavalcanti YW, Bertolini MM, da Silva WJ, Del-Bel-Cury AA, Tenuta LM, Cury JA (2014). A three-species biofilm model for the evaluation of enamel and dentin demineralization. Biofouling. 30(5):579-588.

Costa PS, Souza EB, Brito EH, Fontenelle RO (2017). Antimicrobial activity and therapeutic potential of the genus Lippia sensu lato (Verbenaceae). Hoehnea. 44(2):158-171.

de Oliveira GP, Torrens AW, Bartholomay P, Barreira D (2013). Tuberculosis in Brazil: last ten years analysis–2001–2010. The Brazilian J. Infect. Dis. 17(2):218-233.

de Oliveira LM, Cardoso ND, Fernandez LG, de Abreu Roque MR, de Santana JR, Pelacani CR, de Castro RD (2012). The effect of growing conditions on phenolic compounds and antimicrobial activity of Myracrodruon urundeuva Fr. Allemão. Afr. J. Biotechnol.11(41):9770-9775.

Hajifattahi F, Maravej – Salehi E, Taheri M, ArashMahboubi, Kamalinejad M (2016). Antibacterial effect of hydroalcoholic extract of *Punica granatum* Linn. Petal on common oral microorganisms. Inter. J. Biomat. pp. 1-6.

Lobo PL, Marques LA, Gurgel MF, Soares JL, da Cruz Fonseca SG, de Moraes ME (2015). Atividade Farmacológica do Óleo Essencial de Lippia sidoides em Odontologia: Uma Revisão de Literatura. Saúde e Pesquisa 8(2):373-378.

Majali IS, Oran SA, Khleifat KLA, Qaralleh H, Rayyan WA, Althunibat OY (2015). Assessment of the antibacterial effects of *Moringa peregrina* extracts. Afr. J. Microbiol. Res. 9(51):2410-2414.

Moreira MSC, Cavalcanti YW, Almeida LFD, Padilha WWN. (2012). Antifungal activity of commercial antimicrobial solutions and natural products from *Rosmarinus officinalis* (rosemary). Int. J. Dent.11(1):38-42.

Ocheng F, Bwang F, Joloba M, Borg-Karlson AK, Gustafsson A, Obua C (2014). Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. J. Ethnopharmac. 155:852-855.

Salleh WM, Ahmad F, Yen KH, Sirat HM (2011). Chemical compositions, antioxidant and antimicrobial activities of essential oils of Piper caninum Blume. Int. J. Mol. Sci. 12(11):7720-7731.

Sette-de-Souza PH, do Rego Carneiro SE, Macedo-Costa MR, Borges SB, da Rocha Medeiros A, de Oliveira Fernandes TU, Amaro-da-Silva LC, Lopes HJ, Lima KC (2014). Antibacterial activity and phytochemical screening of extracts of *Lippia alba* (Mill). NE Brown. Afr. J. Microbiol. Res. 8(29):2783-2787.

Uprety Y, Asselin H, Dhakal A, Julien N (2012). Traditional use of medicinal plants in the boreal forest of Canada: Review and perspectives. J. Ethnobiol. Ethnomed. 8(1):7.

Vargas LAS, LLiyan S, Segura PEC, Silva YB, Méndez LG (2015). Etiology and microbiology of periodontal diseases: A review. Afr. J. Microbiol. Res. 9(48):2300-2306.

Vieira DRP, Amaral FMM, Marciel MCG, Nascimento FRF, Libério SA, Rodrigues VP (2014). Plant species used in dental diseases: ethnopharmacology aspects and antimicrobial activity evaluation. J. Ethnopharmac. 155:1441-1449.

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Vol. 12(8), pp. 186-191, 28 February, 2018
DOI: 10.5897/AJMR2017.8761
Article Number: 08D460056159
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation and phenotypic characterization of actinomycetes from Rabat neighborhood soil and their potential to produce bioactive compounds

Ayoubi Hasnaa[1], Mouslim Assia[1], Moujabbir Sara[1], Amine Soukaina[1], Azougar Iman[1], Mouslim Jamal[2] and Menggad Mohammed[1*]

[1]Laboratory of Physiopathology and Molecular Genetics, Faculty of Sciences Ben M'Sik, Hassan II University of Casablanca, Avenue Cdt Driss El Harti, PB 7955 Sidi Othman Casablanca, Morocco.
[2]Laboratory of Microbiology, Faculty of Sciences Ben M'Sik, Hassan II University of Casablanca, Avenue Cdt Driss El Harti, PB 7955 Sidi Othman Casablanca, Morocco.

Received 14, November, 2017; Accepted 8 February, 2018

This study aimed to examine the isolation of actinomycetes strains from unexplored Rabat neighbourhood soil followed by phenotypic diversity highlight to access their potential as novel bioactive compounds producers. Twenty nine actinomycetes strains were isolated from southeast of Rabat region. Morphological and cultural characterization, pigment production and antibacterial assay were carried out. All isolates (100%) were able to produce at least one non-diffusible pigment depending on the culture media. Twenty five (86.20%) isolates produce diffusible pigments, 6 (20.69%) produce melanoid diffusible pigments and 5 (17.24%) exhibited antibacterial activities. These results indicate an important potential of the actinomycetes isolated to produce polymorphic secondary metabolites.

Key words: Isolation, actinomycetes, diversity, pigment, bioactive compounds, antibacterial.

INTRODUCTION

The success of natural resources in drug discovery resides in their biodiversity to produce a huge structural diversity of natural compounds. Actinomycetes remain the most prolific producers of microbial-derived natural products, including anticancer, antifungal and antibacterial compounds. Prospection in this field is based on the structural diversity of molecules provided by combinatorial chemistry or natural organic compounds. Natural bioactive substances are screened mostly from plant and microorganisms (Newman and Cragg, 2012; Harvey, 2000).

Among the microorganisms, actinomycetes produce bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances (Okami and Hotta, 1988; Sanglier et al., 1993; Masayuki et al., 1993; Demain, 1999; Hopwood secondary metabolism biosynthesis pathway. The highest score is assigned to *Streptomyces* genus (Bérdy,

* Corresponding author. E-mail: mengm106@yahoo.fr.

Table 1. Bacterial count present in soil and number of actinomycetes isolated.

Sample	Count of bacteria × 10^5 UFC/g of soil			Actinomycetes isolated
	Actinomycetes	Other bacteria	Total	
A	12	38	50	14
F	8	26	34	8
C	4	12	16	7
Total	24	76	100	29

(F, Forest soil; A, Agricultural soil and C, Cattle manure deposit).

1995) which is also the most frequently isolated from soil.

Actinomycetes intensive screening over the past several decades provided a wide variety of bioactive compounds. Even with the decrease of new compounds discovery in actinomycetes isolated from the studied accessible soils in the past two decades, huge unexplored regions in the world remain to be studied. In Morocco, only few locations where actinomyctes have been isolated and reported (Barakate et al., 2002;

Hanane et al., 2011; Boussaber et al., 2014).

The isolated strains were mostly attributed to *Streptomyces* genus. Nevertheless, diversity of actinomyctes is still underexplored in our country. In this work, we present phenotypic diversity of strains isolated from Rabat neighbourhood (Morocco) and their potential to provide sources of secondary metabolites.

MATERIALS AND METHODS

Soil sample, actinomycetes screening and storage

Soil samples were collected in winter (February) from three locations in Rabat neighbourhood region. These locations represent three habitats: Forest soil (F), Agricultural soil (A) and Cattle manure deposit soil (C). One sample of each habitat was taken from a depth of 15 cm after removing soil surface, placed in aluminium folium closed tightly and transported aseptically to the laboratory. 1 g of each sediment sample was heated 15 min at 70°C and used for actinomycetes isolation by standard method. 100 µl of diluted (1/2 x 10^{-3} and 10^{-4}) suspensions in NaCl (8.5 g/L) were plated onto (GLM) yeast extract malt extract agar (Kitouni et al., 2005) and starch casein agar (SCA**)** (Kuster and Williams, 1964) supplemented with filter (Millipore 0.45 µm) sterilized cycloheximide (50 µg/ ml). The plates were incubated at 30°C for 1 to 3 weeks. After incubation, total colonies were counted and actinomycetes typically dry, powdery colonies were selected. To get pure cultures, colonies were purified onto ISP2 agar (Shirling and Gottlieb, 1966). Presence of mycelium and spores was examined under light microscope (x1000). KOH test was used to confirm gram positive (Halebian et al., 1981). ISP2 plate and slants of pure cultures were kept at room temperature for short-term storage. For long-term storage, sediment of soil was added and mixed to ISP2 culture slants and stored at room temperature. A second long-term storage was made from a loop-full of the isolate culture dispersed in 20% glycerol and kept at -20°C.

Morphological, cultural and physiological characteristics

All the following determinations were performed by visual observation in the solid media of the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966):

(1) Colonies morphologies and aerial/substrate mycelium colors of the isolates were determined on ISP2 and ISP4.
(2) Production of diffusible pigments was tested in ISP1, ISP2 and ISP4.
(3) Production of melanin pigment was made in ISP6.

The plates and slant cultures were incubated at 30°C, and observations were recorded on the 7th, 14th and 21th days. Growth rate was estimated on ISP media and GLM visually according to the abundance of mycelium and colonies size.

Antibacterial bioassay

Bioassays of the isolated actinomycetes were performed by disk diffusion assay. Agar disk prepared from lawn culture of each actinomycete isolate on ISP2 medium plate after 7 days of incubation at 30°C. Prepared disks were then aseptically transferred to Mueller Hinton agar (Difco, Germany) plates previously lawn with fresh culture of test bacteria as clinical strains of *Escherichia coli, Klebsiella pneumonie*, *Enterobacter cloacae, Staphylococcus aureus, Escherichia coli* ATCC8739 and *Staphylococcus aureus* ATCC6538. Sterile disk of ISP2 was used as control. After 24 h incubation at 30°C, bioactivity was determined by measuring the diameter of inhibition zone (mm) (Valgas et al., 1981).

RESULTS AND DISCUSSION

Isolation of actinomycetes

Bacterial count in the three samples used showed an important decrease of total bacterial population or actinomycetes from sample A, F to C (Table 1) correlated with increase of their organic matter content (Keen et al., 2011; Moral et al., 2005). These results showed a relationship between organic matter present in these soil samples and bacterial load. High organic matter soil seems to be more favourable to fungi consistent with a reported study (Bailey et al., 2002). Among total flora counted in the three samples, actinomycetes represented 24% indicating a high percent than recently reported (Elbendary et al. 2018). Since we noticed that GLM medium was favourable to non actinomycetes bacteria growth, we used SCA medium agar and we got 5 additional isolates to reach a total of 29 isolates (Table

Table 2. Evaluation of growth and sporulation of all isolates on different media.

Medium	Growth	Sporulation
GLM	Good	Weak
ISP1	Weak	None
ISP2	Good	Good
ISP4	Moderate	Good
ISP6	Weak	None

1). All isolates showed typical characteristics of actinomycetes:

(1) Powdery and dry colony surface appearance on solid media;
(2) Colony inserted in agar medium;
(3) Gram positive in the used KOH test and;
(4) Presence of mycelium and spore as checked by microscopic observation.

Growth and sporulation assessment

On solid media, actinomycetes growth rate presented different degrees of culture density. Slant and Petri dish cultures of all isolates showed best growth on GLM and ISP2. Sporulation of aerial mycelium on solid media was reflected at its surface by the change of the color due to spore pigmentation with powdery aspect. Good sporulation of all isolates was observed on ISP2 agar and ISP4 agar media (Table 2) consistent with reported study (Algafari, 2014).

Non-diffusible and diffusible pigments production

Aerial and substrate mycelium color is an important criterion in identification of actinomycetes (Pridham, 1965; Waksman and Lechevalier, 1953). It reflects the colors of their intracellular pigments. All isolates on ISP2 and ISP4 agar media showed a significant diversity of colors (Table 3). Substrate mycelium on ISP2 showed more isolates with yellow colors shade as previously reported (Ceylan et al., 2008). However, percent of yellow colors shade decreased from 69.23% on ISP2 to 23.07% on ISP4 compared to Ceylan et al. (2008) studies in which 93.3% isolates exhibit yellow shades. In fact, the ratio of non-yellow shade isolates at least in one of the two media became 76.93% and showing more diversity. Consequently, this color diversity reflects the important potential of theses isolates for biosynthesis of different non-diffusible pigments. On the other hand, 25 (86.20%) of the 29 isolates were able to produce diffusible pigments at least in one of the used media: ISP1, ISP2 and ISP4 (Table 4). Production opportunity of these pigments is higher in ISP2 followed by ISP1. In ISP4, only 6 isolates had produced diffusible pigments. Production of melanoid diffusible pigments in ISP6 was detected in 6 isolates (22.22%). Unlike non-diffusible pigments, diffusible pigments with antibacterial activities could exhibit a zone of inhibition in antibacterial assay. This aspect makes it easy to screen for antimicrobial activities (Mamunur et al., 2014) than non-diffusible pigments like the case of prodigiosin (Darah et al., 2014). In the three media used, more isolates produce yellow diffusible pigments as reported in previous studies (Sathi et al., 2002; Franks et al., 2005; Selvameenal et al., 2009; Sasidharan et al., 2013; Indra et al., 2014). However, the profiles of theses isolates for diffusible pigments production in the four media ISP1, ISP2, ISP4 and ISP6 are different. These results suggest the presence of polymorphic biosynthesis processes of these molecules.

Antibacterial activity screening

Screening of the isolated actinomycetes grown on ISP2 medium for antibacterial activity indicated that 5 (17.24%) of the 29 isolates are able to inhibit growth of at least one test bacterium (Table 5). Even there is a large difference in published ratio of isolates exhibiting antibacterial activity (Chaudhary et al., 2013; Ganesan et al., 2017), the study result of 17.24% is closely related to ratio obtained by Parthasarathi et al. (2010) and Chaudhary et al. (2013). Correlation between isolates producing diffusible pigments and exhibiting antibacterial activities has been suggested by Mamunur et al. (2014) and Ceylan et al. (2008). Consistent with these previous reported studies, only MFB-10 was negative in diffusible pigment production test among the 5 isolates exhibiting antibacterial activities. The Values of inhibition zone size showed that Gram positive test bacteria are globally more sensitive to the antibacterial compounds synthesised by these isolates. In Gram negative test bacteria, *E. coli* ATCC8739 was the most resistant to all compounds. MFB-27 and MFB-28 isolates produces the most effective compounds against Gram positive test bacteria. However in this antibacterial assay, isolates were grown on ISP2 medium. Their growth on other media could allow

Table 3. Colors of aerial and substrate mycelia of isolates on ISP2 and ISP4.

Isolate	Mycelium color on ISP2		Mycelium color on ISP4	
	Aerial mycelium	Substrate mycelium	Aerial mycelium	Substrate mycelium
MFB-01	Gray	Beige	White	Gray
MFB-02	Light gray	Yellow orange	Off-white	Brown
MFB-03	Yellow	Yellow	Beige	Yellow
MFB-04	Gray	Yellow brown	Gray	White
MFB-05	White	Light yellow	White	Beige
MFB-06	White	Light yellow	Gray	Gray-Brown
MFB-07	Green bleue	Light green	Light green	Brown
MFB-08	Off-white	Brown chocolate	Gray	Brown
MFB-09	Pearl white	Dark yellow	Pearl white	Yellow
MFB-10	Off-white	Yellow-brown	White	Brown
MFB-11	White-gray	Yellow brown	Gray	Purple-red
MFB-12	White	Light yellow	White	Beige
MFB-13	Green	Light green	Green	Light green
MFB-14	White	Yellow-orange	Pearl white	Light yellow
MFB-15	Gray-green	Black	Gray-brun	Black
MFB-16	Beige	Light yellow	White	Yellow-brown
MFB-17	Off-white	Light yellow	White	Light yellow
MFB-18	White	Yellow-orange	Pearl white	Light yellow
MFB-19	Off-white	Yellow-orange	Pearl white	Brown
MFB-20	White gray	Brown	Gray	Purple-red
MFB-21	White gray	Brown chocolate	Gray	Purple-red
MFB-22	Light yellow	Yellow	Yellow	Dark yellow
MFB-23	Beige	Light yellow	Gray	Orange-red
MFB-24	Gray	Brown	Gray	Purple-red
MFB-25	Black	Black	Black	Black
MFB-26	White gray	Brown orange	Light gray	Gray-brown
MFB-27	White	Light yellow	Black	Gray-brown
MFB-28	White	Light yellow	Light white	Beige
MFB-29	White gray	Brown chocolate	Light gray	Dark brown

Table 4. Diffusible pigments of isolates according to the ISP culture media.

Isolate	Diffusible pigment			Melanin pigment
	ISP1	ISP2	ISP4	ISP6
MFB-01	-	-	-	-
MFB-02	Light yellow	Yellow brown	Dark yellow	-
MFB-03	ND	Light yellow	-	ND
MFB-04	-	-	-	-
MFB-05	-	-	-	-
MFB-06	Light yellow	Light yellow	-	+
MFB-07	Beige	Light green	-	+
MFB-08	-	Dark brown	-	-
MFB-09	Dark brown	Yellow orange	-	+
MFB-10	-	-	-	-
MFB-11	-	Dark yellow	-	-
MFB-12	Light yellow	Dark yellow	-	+
MFB-13	Light yellow	Light yellow	-	-
MFB-14	Dark yellow	Yellow orange	-	+

Table 4. Contd.

MFB-15	Yellow brown	Black	-	-
MFB-16	Yellow brown	Dark brown	-	-
MFB-17	Yellow brown	Dark brown	-	-
MFB-18	Light yellow	Yellow brown	-	-
MFB-19	Light yellow	Yellow brown	-	-
MFB-20	-	Light yellow	-	-
MFB-21	-	Light yellow	-	-
MFB-22	ND	Yellow orange	ND	ND
MFB-23	Beige	Dark yellow	-	-
MFB-24	-	Yellow brown	-	-
MFB-25	Yellow brown	Yellow brown	-	-
MFB-26	Dark brown	Light brown	Light yellow	+
MFB-27	-	Dark yellow	Light yellow	-
MFB-28	-	Yellow green	Light yellow	-
MFB-29	Beige	Dark brown	-	-

Table 5. Inhibition zone diameter of positive isolates on test bacteria.

Isolate	Test bacteria						
	***E. cloacae* (mm)**	***K. pneumonie* (mm)**	***E. coli* (mm)**	***E. coli* ATCC8739 (mm)**	***S. aureus* ATCC29213 (mm)**	***S. aureus* ATCC6538 (mm)**	***M. leutus* ATCC9341 (mm)**
MFB-27	11	10	10	0	25	27	40
MFB-28	10	0	9	0	23	24	35
MFB-10	0	0	0	0	14	10	11
MFB-16	0	12	0	0	0	0	0
MFB-07	12	0	0	0	0	0	0

expression of other bioactive secondary metabolites as exhibited in non-diffusible and diffusible pigments differential expressions which were culture media dependent.

Conclusion

In this assessment work, we showed a high diversity of the actinomycetes isolated and their potential to produce antibacterial compounds. Soil samples used were rich in actinomycetes. All the isolated actinomycetes were able to produce various non-diffusible pigments and most of them secreted various hydrosoluble pigments and/or hydrosoluble compounds with antibacterial activities depending on the media used. This finding suggests the presence of high opportunity if other different culture media could be used to express various bioactive molecules of interest by the isolated actinomycetes strains or further new isolates from this actinomycetes rich soil. More investigations are ongoing in our Laboratory on the pigment antiproliferative activities, molecular identification of isolates showing activities and for structural determination of the active secondary metabolites.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Special thanks to Pr BELHOUARI Abderrahman (Biology Department head) and colleagues for providing facilities to carry out this work.

REFERENCES

Algafari RN (2014). Assessment of profile depth, site of sampling, type of media and methods used for the isolation of actinomycetes. Int. J. Microbiol. Res. 6:553-558.

Bailey VL, Smith JL, Bolton H (2002). Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biol. Biochem. 34:997-1007.

Barakate M, Ouhdouch Y, Oufdou KH, Beaulieu C (2002). Characterization of rhizospheric soil strepomycetes from moroccan habitat and their antimicrobial activities. World J. Microbiol.

Biotechnol. 18:49-54.
Bérdy J (1995). Are actinomycetes exhausted as a source of secondary metabolites? Biotechnology 7-8:13-34.
Boussaber EA, EL Idrissi SBS, Meftah Kadmiri I, Hilali L, Hilali A (2014). Screening of actinomycete bacteria producing antifungal metabolites which could be used in biological control against a phytopathogenic fungus (*Rhizopus stolonifer*). Am. J. Biol. Life Sci. 2:84-89.
Ceylan O, Okmen G, Ugur A (2008). Isolation of soil Streptomyces as source antibiotics active against antibiotic-resistant bacteria. EurAsia J. BioSci. 2:73-82.
Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N (2013). Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). J. Adv. Pharm. Technol. Res. 4:118-123.
Darah I, The FN, Jain K, Sheh-Hong L (2014). Prodigiosin - an antibacterial red pigment produced by Serratia marcescens IBRL USM 84 associated with a marine sponge *Xestospongia testudinaria*. J. App. Pharma. Sci. 4:001-006.
Demain AL (1999). Pharmaceutically active secondary metabolites of microorganisms. Appl. Microbiol. Biotechnol. 52:455-63.
Elbendary AA, Hessain AM, El-Hariri a MD, Adel Seida A, Moussa IM, Mubarak AS, Kabli SA, Hemeg HA, El Jakee JA (2018). Isolation of antimicrobial producing Actinobacteria from soil samples. Saudi J. Biol. Sci. 25:44-46.
Franks A, Haywood P, Holmström C, Egan S, Kjelleberg S, Kumar N (2005). Isolation and structure elucidation of a novel yellow pigment from the marine bacterium *Pseudoalteromonas tunicata*. Molecules 10:1286-1291.
Ganesan P, Reegan AD, Antony David RH, Gandhi MR, Paulraj MG, Al-Dhabi NA, Ignacimuthu S (2017). Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India. Alexandria Med. J. 53:101-110.
Halebian S, Harris B, Finegold SM, Rolfe RD (1981). Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. J. Clin. Microbiol. 13:444-448.
Hamdali H, Virolle MJ, Von Jan M, Spröer C, Klenk HP, Ouhdouch Y (2011). Streptomyces youssoufiensis sp. nov., isolated from a Moroccan phosphate mine. Int. J. Syst. Evol. Microbiol. 61:1104-1108.
Harvey A (2000). Strategies for discovering drugs from previously unexplored natural products. Drug Discov.Today 5: 294-300.
Hopwood DA (2007). Therapeutic treasures from the deep. Nat. Chem. Biol. 3:457-458.
Indra AP, Umamaheswari S, Ranandkumar SG, Karthik C, Jayakrishna C (2014). Screening of yellow pigment producing bacterial isolates from various eco-climatic areas and analysis of the carotenoid produced by the isolate. J. Food Process. Technol. 5:292.
Keen YC, Jalloh MB, Ahmed OH, Sudin M, Besar NA (2011). Soil organic matter and related soil properties in forest, grassland and cultivated land use types. Int. J. Phys. Sci. 6:7410-7415.
Kitouni M, Boudemagh A, Oulmi L, Reghioua S, Boughachiche F, Zerizer H, Hamdiken H, Couble A, Mouniee D, Boulahrouf A, Boiron P (2005). Isolation of actinomycetes producing bioactive substances from water, soil and tree bark samples of the north–east of Algeria. J. Med. Mycol. 15:45-51.
Kuster E, Williams ST (1964). Selection of media for isolation of Streptomycetes. Nature 202:928-929.
Mamunur rashid MD, Fakruddin MD, Reaz MM, Fatema K, Alimuddin chowdhury MD (2014). Anti-bacterial activity of pigments isolated from pigment-forming soil bacteria. Br. J. Pharm. Res. 4:880-894.
Masayuki T, Rita RC, Russell TH (1993). Isolation and Diversity of Actinomycetes in the Chesapeake Bay. Appl. Environ. Microbiol. 59:997-1002.
Moral R, Moreno-Caselles J, Perez-Murcia MD, Perez-Espinosa A, Rufete B, Paredes C (2005). Characterisation of the organic matter pool in manures. Bioresour. Technol. 96(2):153-158.
Newman DJ, Cragg GM (2012). Natural Products as Sources of New Drugs over the Period 1981-2002. J. Nat. Prod. 75:311-335.
Okami Y, Hotta K (1988). Search and discovery of new antibiotics, *In* Goodfellow M., Williams S. T. and M. Mordarski (ed.), Actinomycetes in biotechnology. Academic Press, Inc., San Diego. pp. 33-67.
Parthasarathi S, Kim CJ, Kim PK, Sathya S, Manikandan M, Manikandan T, Karuppaiya B (2011). Taxonomic characterization and UV/VIS analysis of antagonistic marine actinomycete isolated from South West Coast of South Korea. Int. J. Med. Res. 1:99-105.
Pridham TG (1965). Color and Streptomycetes: Report of an International Workshop on Determination of Color of Streptomycetes. Appl. Microbiol. 13:43-61.
Sanglier JJ, Wellington EMH, Behal V, Feidler HP, Ghorbel RE, Finance C, Hacene M, Kamoun A, Kelly C, Mercer DK, Prinzis S, Tringo C (1993). Novel bioactive compounds from actinomycetes. Res. Microbiol. 144:661-663.
Shirling EB, Gottlieb D (1966). Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313-340.
Sasidharan P, Raja R, Karthik C, Sharma R, Indra AP (2013). Isolation and Characterization of yellow pigment producing *Exiguobacterium* sps.. J. Biochem. Technol. 4:632-635.
Sathi ZS, Sugimoto N, Khali MI, Gafur MA (2002). Isolation of yellowish antibiotic pigment 4-hydroxy Nitrobenzene from a strain of *Streptomyces*. Pak. J. Biol. Sci. 52:201-203.
Selvameenal L, Radhakrishnan M, Balagurunathan R (2009). Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening. Ind. J. Pharma. Sci. 71:499-504.
Valgas C, Machado de Souza S, Smânia EFA; Smânia AJr (2007) Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38:369-380.
Waksman SA, Lechevalier HA (1953). Guide to the Classification and Identification of the Actinomycetes and their Antibiotics. Edition: Baltimore: The Williams and Wilkins Co.

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Vol. 12(8), pp. 192-199, 28 February, 2018
DOI: 10.5897/AJMR2017.8679
Article Number: 2782BFD56161
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Pathological effects of staphylococcal species (*S. aureus*, *S. xylosus* and *S. lentus*) on some visceral organs (liver, kidney and bladder) of wistar rats following intraperitoneal inoculation

Onyebueke Ebere Adaeze[1]*, Onyemelukwe Ngozi Felicia[1], Achukwu Peter Uwadiegwu[1] and Onuba Augustine Chukwudum[2]

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
[2]Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria.

Received 16 August, 2017; Accepted 12 January, 2018

Following the intraperitoneal inoculation of 200 Wistar rats (96 males, 96 females and 8 controls) aged 2 to4 months with graded-doses of saline and peptone water broth cultures of *Staphylococcus aureus*, *Staphylococcus xylosus* and *Staphylococcus lentus* isolates obtained from urinary tract infection (UTI) in Enugu, Nigeria and typed down to species using API® Staph typing kit, the rats were observed for 72 h and were euthanized. The liver, kidney and bladder were harvested and processed histologically. Out of the 96 rats (48 males and 48 females) inoculated with peptone water broth cultures of *S. aureus* 834, *S. xylosus* 837, *S. aureus* 856 and *S. lentus* 853 strains, 12 (12.5%) died, with 8 (66.7%) from *S. xylosus* 837 and 4 (33.3%) from *S. aureus* 856 but no death from *S. aureus* 834 and *S. lentus* 853. More males 6(75%) died from *S. xylosus* 837 than females 2 (25%) while more females 4 (100%) died from *S. aureus* 856 than males 0 (0%). No death occurred from inoculation of another 96 rats with saline broth culture of the test strains. Evidence of necrosis of the liver parenchyma with infiltration of the inflammatory cells around the pericentral areas upon *S. aureus* inoculation was observed. *S. xylosus* and *S. lentus* showed no histological damage to the liver. In the kidney, *S. aureus* produced tubular casts, erosions and glomerular oedema. *S. xylosus* and *S. lentus* produced tubular casts, glomerular distortions and oedema. The bladder showed mild effect on the musculature with *S. aureus* and none for *S. xylosus* and *S. lentus*, respectively.

Key words: *Staphylococcus* species, liver, kidney, bladder, intraperitoneal inoculation, mortality rate and damage.

INTRODUCTION

Staphylococci are Gram-positive spherical cells, usually arranged in grapelike irregular clusters. The genus*Staphylococcus* has at least 40 species (Brooks et al., 2013) amongst which are *Staphylococcus aureus*, *Staphylococcus xylosus* and *Staphylococcus lentus*. Most are harmless and reside normally on the skin and mucous membranes of humans and other animals, found worldwide, they are small components of soil microbial flora (Madigan and Martinko, 2005).

Humans are the reservoir for staphylococci. The nose is the main site of colonization of *Staphylococcus aureus*

and approximately 30% of people are colonized at any one time. The skin especially of hospital personnel and patients is also a common site of *S. aureus* colonization. *S. aureus* is also found in the vagina of approximately 5% of women (Levinson, 2012). However, *S. aureus* is a cause of many pyogenic (pus producing) and toxin mediated diseases and a major cause of skin, soft tissue, bone, joint, lung, heart and kidney infections (Levinson, 2012). *S. aureus* have also been implicated in several literatures as a cause of urinary tract infection (UTI) (Oladeinde et al., 2011; Alemu et al., 2012; Amaeze et al., 2013; Pragash et al., 2014).

S. aureus, a coagulase positive *staphylococcus* is able to cause diseases due to possession of several virulence factors such as extacellular enzymes and toxins produced by strains of the organism which contributes to its invasiveness and pathogencity and this includes coagulase, haemolysins, leucocidin, staphylokinases, lipase, hyaluronidase, protein A, enterotoxins, and toxic shock syndrome toxin (Cheesbrough, 2010).

Although coagulase negative staphylococci (CoNS) are normal human microbiota, they sometimes cause infection often associated with implanted devices such as joint prosthesis, shunts and intravascular catheters especially in very young, old and immuno compromised patients (Brooks et al., 2013).

S. xylosus, a coagulase negative *Staphylococcus* has been associated with nasal dermatitis in gerbrils, pyelonephritis in humans, avian *Staphylococcosis,* bovine intermammary infection (Wikipedia the free encyclopaedia, 2013), and urinary tract infection (Bolaji et al., 2013). *S. xylosus* is able to cause disease due to possession of virulence factors such as haemolysin, protease, urease (Al-Mathkhury et al., 2008), and lipase (Mosbah et al., 2006). *S. xylosus* isolates from UTI have been reported to cause several pathological changes in mice (Al-Mathkhury et al., 2008). *S. lentus*, a coagulase negative *staphylococcus* has been isolated from urine (Bolaji et al., 2013) but not much is known concerning its pathogenicity.

S. aureus is the most reported of the staphylococcal species in UTI in clinical laboratories and in research works in the present study area and elsewhere while other CoNS encountered are usually disregarded as not able to cause UTI, are unreported and when reported they are most often not typed to species, lumped together as CoNS with very few cases where they are typed. The increasing use of medical devices such as shunts and heart valves can predispose to septicemia with any of the staphylococcal species (Levinson, 2012) and can lead to pyelonephritis while the use of urinary catheters predisposes to staphylococcal UTI.

There is paucity of documented works on the pathological effects of *S. aureus*, *S. xylosus* and *S. lentus* isolates from UTI on the visceral organs of experimental animals in the present study area which could help one to infer the possible effects of these staphylococcal species on the visceral organs of humans especially liver, kidney and bladder. This will help to substantiate the claims that these staphylococci cause UTI and show the need for our clinical laboratories to type them to species level and report on them in UTI cases.

This study therefore is aimed at investigating the pathological effects of *S. aureus, S. xylosus* and *S. lentus* isolates from UTI on the visceral organs (Liver, Kidney and bladder) of Wistar rats following intraperitoneal inoculation to determine which of these organs may be affected by them and the extent of damage that may be encountered.

MATERIALS AND METHODS

Isolation of staphylococcal species

After obtaining ethical clearance from the ethics committees of the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla and Enugu State University Teaching Hospital (ESUTH) Enugu, both in Enugu State, South East Nigeria and informed consents from the participants or their wards, 818 participants were investigated for staphylococcal UTI. Their urine samples were cultured onto blood agar and MacConkey agar plates, and the plates were incubated for 24 h at 37°C. At the end of the incubation period, the plates were examined macroscopically and samples showing uniform growth ≥ 10^5CFU/ml of urine were regarded as significant for UTI and subjected to further tests including gram reaction and standard biochemical tests. Isolates suspected to be *Staphylococcus* species (spp) were further tested using DNase test with deoxyribonuclease test agar (Fluka BioChemika), novobiocin (Oxoid) sensitivity test and '*Staph* speciation test' using the API® *Staph* test kit (Biomerieux France) while the identity of the staphylococcal spp were confirmed using the apiweb™ identification software (Biomerieux France) (Ochei and Kolhatkar, 2000; Bolaji et al., 2013). Out of the samples examined, 89 were positive for staphylococcal UTI. From the most commonly incriminated staphylococcal spp. in the UTI cases (*S. aureus*, *S. xylosus* and *S. lentus*), two strains of *S. aureus* and one strain each of *S. xylosus* and *S. lentus*, were used for the pathological study.

Study design

The study adopted an experimental design by intraperitoneal injection of the 4 test strains of *Staphylococus* (two strains of *S. aureus* and one strain each of *S. xylosus* and *S. lentus)* into albino Wistar rats. Methods for intraperitoneal injection were according to Janakat et al. (2002).

Animal procurement and housing

Two hundred laboratory reared Wistar rats (100 males and 100 females) obtained from the animal unit of the University of Nigeria,

*Corresponding author. E-mail: ebere.onyebueke@unn.edu.ng. Tel: +2348033500885.

Enugu campus were housed in the animal houses of the unit and were allowed to acclimatize for 14 days while feeding them with grower feed before inoculation.

Preparation of inoculums

Four strains of three staphylococcal species isolated from UTI in this study (*S. aureus*, 834, *S. xylosus* 837, *S. aureus* 856 and *S. lentus* 853) were used for the study. The strains of the Staphylococcal species used were previously confirmed in the Microbiology Laboratory of the Department of Medical Laboratory Sciences of the University of Nigeria Enugu Campus using standard microbiology biochemical tests, Novobicin® drug sensitivity test, API® *Staph* test Kit (Biomerieux France) ref no 20500 and the apiweb™ identification software (Biomerieux France) both for the identification of staphylococci, micrococcus and related genera. The isolates were maintained in nutrient agar slants in MacCartney bottles at 2 to 4°C in the Microbiology laboratory of the Department of Medical Laboratory Sciences of the University of Nigeria Enugu Campus. Prior to use, the strains were subculture onto fresh nutrient agar slants which were incubated aerobically for 24 h at 37°C. The colonies of each of the four strains of the *Staphylococcus* species were homogenized in sterile peptone water broth and sterile saline water broth respectively, and their turbidities adjusted to correspond to 0.5 MacFarlands turbidity standard (Equivalent to $1x10^8$ CFU/ml).

Animal grouping and inoculation

The 200 Wistar rats (100 males and 100 females) were placed in 18 cages with the first 16 cages (1 to 16) containing 12 Wistar rats each while the last 2 cages (17 and 18) which served as controls contained 4 Wistar rats each. Virulence was assessed using graded doses of the *Staphylococcus* species (0.2, 0.5, 1.0, 0.0 control) ml /kg body weight of the rats in the experiment. Intraperitoneal inoculation (ip) of both the saline broth as well as the peptone water broth of the staphylococcal test species were conducted on the rats. The rats were weighed before inoculation. Cages 1 to 4 (Female rats) and cages 5 to 8 (male rats) contained 12 rats each (total = 96 rats). Rats in cages 1, 2, 3 and 4 (female rats) were inoculated intraperitoneally with peptone water broth culture of *S. aureus* 834, *S. xylosus* 837, *S. aureus* 856 and *S. lentus* 853 respectively. In each of the 4 cages, the rats were grouped into three groups of 4 rats each (A, B, C), and the 3 groups were inoculated with graded doses of the strains of the test staphylococcal species (A = 0.2 ml, B= 0.5 ml C= 1.0 ml) respectively. Same inoculation procedure was repeated for rats in cages 5, 6, 7 and 8 (male rats). The same type of grouping and inoculation procedure was performed for another set of rats in cages 9 to 12 (female rats) and cages 13 to 16 (male rats) which were inoculated intraperitoneally with graded doses of saline broth culture of the 4 strains of the test staphylococcal species. Cages 17 (female rats) and 18 (male rats) containing 4 rats each served as control and were not inoculated with any both culture.

General observation and sample collection

The animals were observed for 72 h (3days) post infection (dpi) while feeding them with their food and water. Subsequently, they were individually weighed and sacrificed. The visceral organs of the rats (liver, kidney and bladder) were harvested, preserved in formalin and sent quickly to the histology laboratory for tissue processing.

Histopathology

The formalin fixed visceral organs of the rats (liver, kidney and bladder) were processed histologically by paraffin wax embedding technique, stained with Erlirch's Haematoxylin and eosin stain, and mounted in DPX. The processed tissues were histologically examined.

RESULTS

The result of the present study showed that out the 96 rats (48 males and 48 females) inoculated intraperitoneally with graded doses (0.2, 0.5, 1.0) of the staphylococcal species in peptone water broth (*S. aureus* 834, *S. xylosus* 837, *S.aureus* 856 and *S. lentus* 853), 12 died showing a mortality rate of 12.5%. No death was recorded from intraperitoneal inoculation of the other 96 rats with saline broth of the four strains of *Staphylococcus* species tested. Of the 12 deaths encountered in peptone water broth inoculation of the staphylococcal strains, 8 (66.7%) came from *S. xylosus* 837 inoculation while 4 (33.3%) was from *S. aureus* 856 inoculation but no death was recorded from *S. aureus* 834 and *S. lentus* 853 peptone water broth inoculation.

Out of the 24 Wistar rats (12 males and 12 females) inoculated with graded doses of *S. xylosus* 837, 8 died with a mortality rate of 33.3%. Of this 8, males incurred more deaths (6 (75%)) than females (2 (25%)). Female verses male mortality ratio was 1:3. 24 Wistar rats were also inoculated with graded doses of *S. aureus* (856) of which four deaths occurred with a mortality rate of 14.7%. Deaths occurring only in females (4 (100%)) and non in males (0%) showing a female versus male ratio of 4:0.

For *S. xylosus* 837, of the eight deaths that occurred, none (0) occurred at a dose of 0.2 ml of the inoculum, 4 deaths occurred at 0.5 ml (2 females and 2 males) while 4 deaths occurred at 1.0 ml inoculum (males only). For *S. aureus* 856, out of the 4 deaths that occurred, none occurred at 0.2 ml and at 0.5 ml doses of the inoculum, but the four deaths occurred at 1.0 ml of the inoculum. This study was designed to assess the pathogenesis and virulence of *S. aureus*, *S. xylosus*, and *S. lentus* in the kidney, bladder (urinary system) and the liver following intraperitoneal inoculation of Wistar rats which produces some tissue alterations. The virulence was assessed using graded doses of the staphylococcal strains (0.2, 0.5, 1.0 and 0.0 control) ml/kg body weight of the rats in experiments A, B, C and D respectively, after 72 h. D served as control and as such was not inoculated.

In group 1 model of the experiment ABC using graded doses of *S. aureus* on the Wistar rats, the histology of the liver parenchyma remained intact (Plate 1). There was occasional mild infiltration of the inflammatory cells (Plate 2) around the peri-central areas of the liver. The kidney demonstrated a higher degree of toxigenic modification. There was presence of tubular casts in varying degree, tubular erosion with inflammatory cells (Plate 3) and

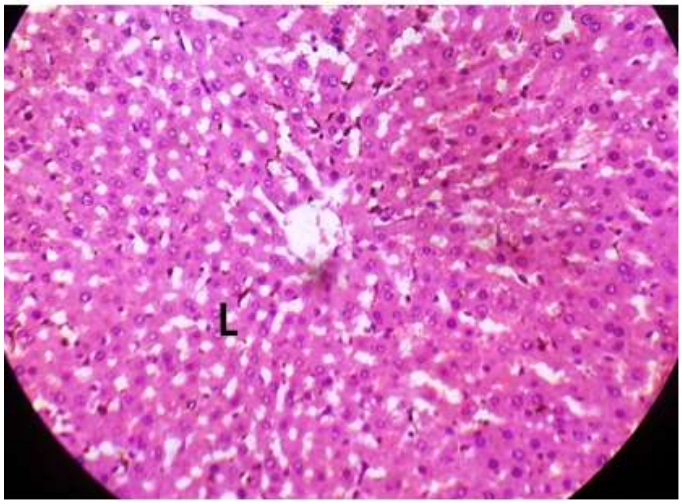

Plate 1. A piece of liver tissue of rat showing intact liver parenchyma(L) after inoculation with *S. aureus.* H/E x200.

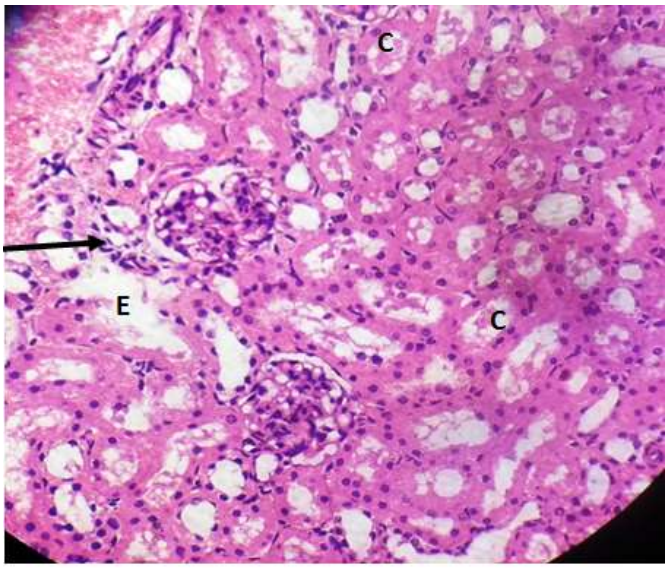

Plate 3. Kidney tissue from rat inoculated with *S. aureus* showing tubular casts (C) and tubular erosion (E) with inflammatory cells (arrow). H/E x200.

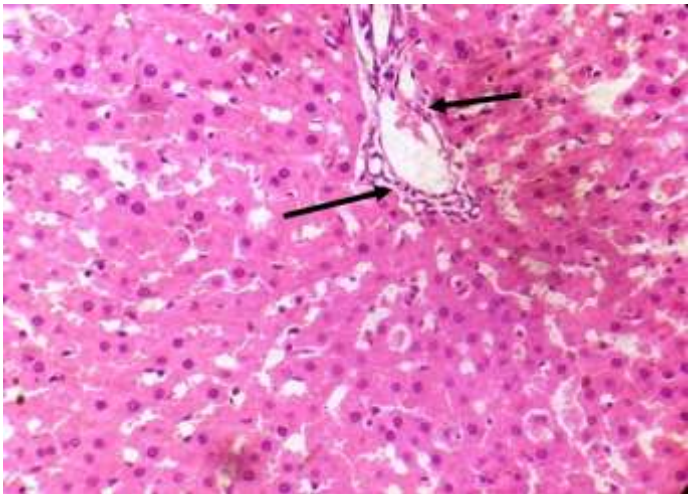

Plate 2. A piece of liver tissue of the experimental rats inoculated with *S. aureus*. Note the infiltration of inflammatory cells (arrows) around the pericentral area. H/E x200.

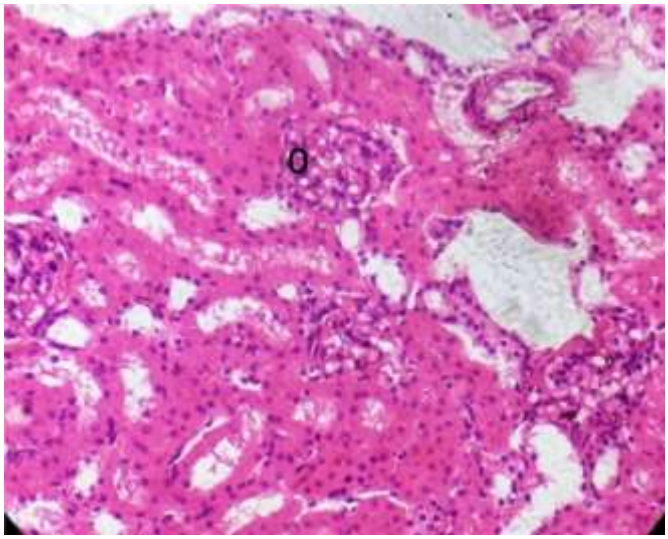

Plate 4. Demonstrates glomerular oedema (O) after *S. aureus* inoculation. H/E x200.

glomerular oedema (Plate 4) in high doses of the inoculum at the kidney.

The extent of cellular damage increased with more concentration of the inoculum. One remarkable feature of *S. aureus* is the tubular casts characterizing all values of the *S. aureus* inoculums in kidney tubules (Plate 5). The bladder upon inoculation with infective doses of 0.2 to 1.0 ml/kg body weight of *S. aureus* showed a mild effect on the musculature. The transitional epithelia of the bladder remained intact (Plate 6) with mild Infiltration of inflammatory cells which was in proportion of the infective doses of the inoculum.

In group II model of the experiment using graded doses of *S. xylosus* on the Wistar rats, the experiments E, F and G produced a relatively less degree of cell modification/alteration and damage. There was no visible alteration of the liver parenchyma. All hepatocytes of the rats' liver including those of the control rats were normal (Plate 7). There were tubular casts (Plate 8) in the kidney tubules. The glomerulus was mildly distorted with mild infiltration of inflammatory cells. Capsular oedema was noted in rats inoculated with high doses of *S. xylosus.* Tubular oedema was also seen in the stroma (Plate 9).

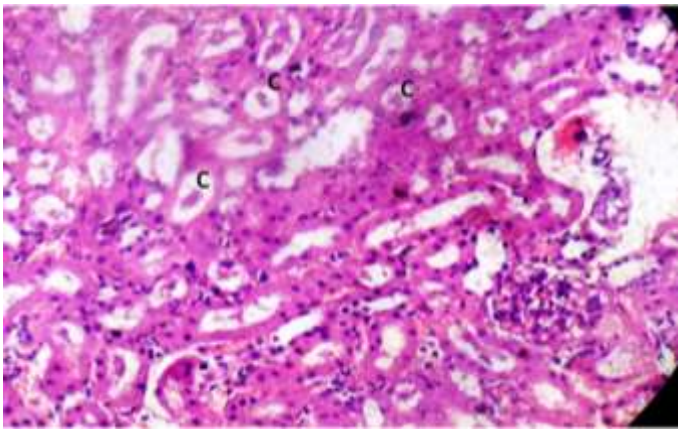

Plate 5. Kidney tubules in rats inoculated with *S. aureus*. Note the presence of tubular casts (C). H/E x200.

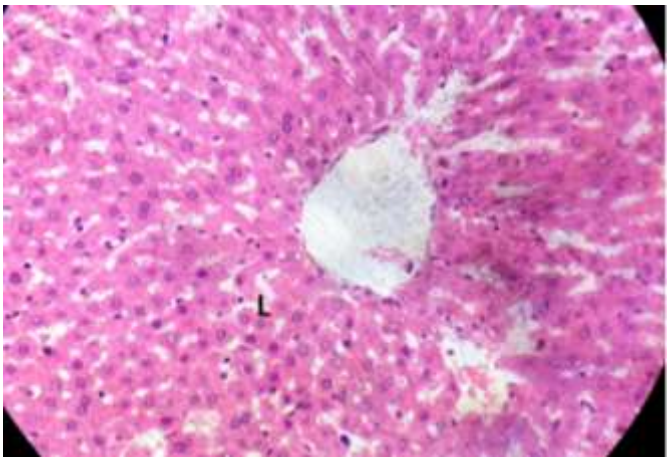

Plate 7. Showing intact liver parenchyma (L) following inoculation with *S. xylosus.* H/E x200.

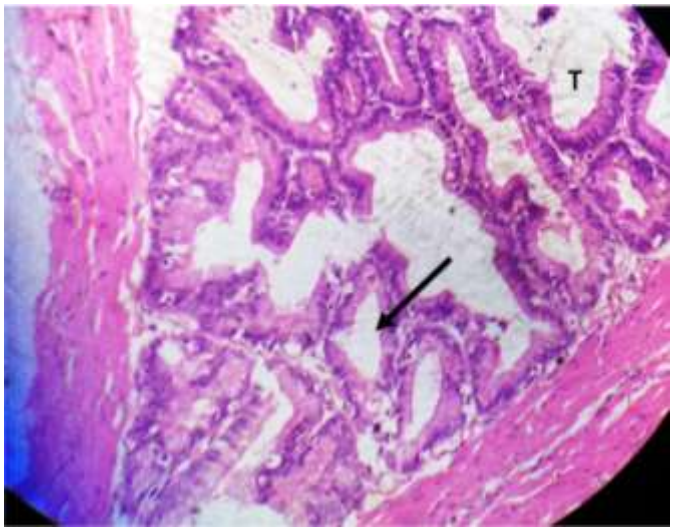

Plate 6. A piece of bladder tissue from rat following inoculation with *S. aureus*. The transitional epithelia (T) were intact with mild infiltration of inflammatory cells (arrow). H/E x200.

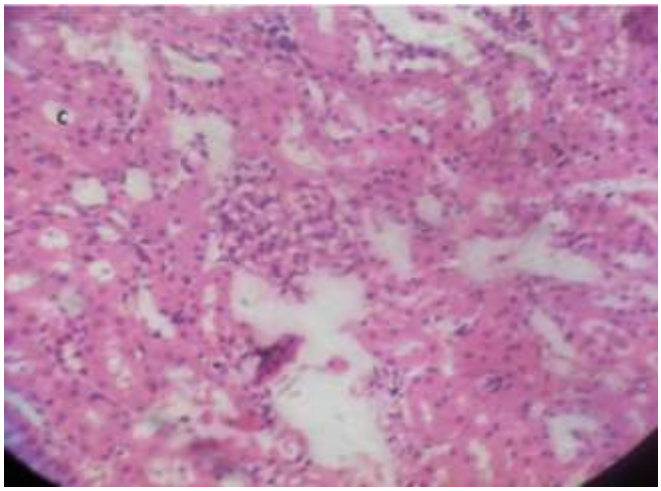

Plate 8. A piece of kidney tissue of rat inoculated with *S. xylosus*. Note the presence of tubular casts (C). H/E x200.

In the bladder, the infective dose *of S. xylosus* inoculum did not affect the musculature of the bladder. There were inflammatory cells in the mucosae and connective tissues of the bladder (Plate 10). The transitory epithelia were intact and well defined (Plate 11). Group III model of experimentation using infective doses of *S. lentus* had a similar effect in the liver sections of rats inoculated. The liver showed no toxigenic effect. The central canals were intact, radiated round by the liver sinusoids. All doses (0.2, 0.5 and 1.0) mg/kg body weight of the infective doses of *S. lentus* was produced into glomerular oedema, inflammatory cells infiltration and tubular casts in proportion to the inoculated dose (Plate 12). The bladder did not manifest any major damage but showed mild epithelial erosion with inflammatory cells.

DISCUSSION

Mortality rate for the Wistar rats in the present study was 12.5% and occurred from intraperitoneal inoculation of the rats with *S. xylosus* 837 and *S. aureus* 856 strains. *S. aureus* produces enzymes such as coagulase, haemolysins, leucocidin, staphylokinase, lipase, hyaluronidase, protein A and toxins such as enterotoxins and toxic shock syndrome toxin (Cheesbrough, 2010) which enables it to be invasive and pathogenic and these could be possessed by the *S. aureus* 856 test strains used in the present study which may have contributed to the 4 deaths recorded for it.

Moreover, *S. aureus* is a major cause of skin, soft tissue, bone, joint, lung, heart and kidney infections (Levinson, 2012), thus the rats may have suffered from generalized organ infections due to septicaemia

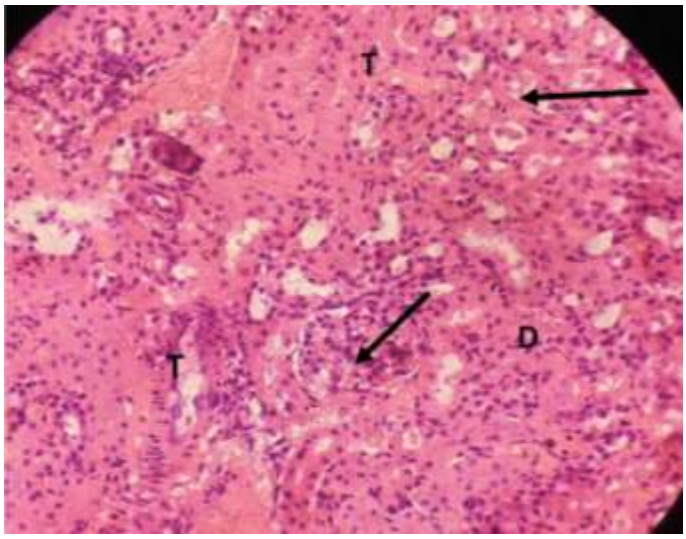

Plate 9. A piece of kidney tissue of rat inoculated with *S. xylosus* showing distortion of glomerulus (D); infiltration of inflammatory cells (arrows) and tubular oedema (T). H/E x200.

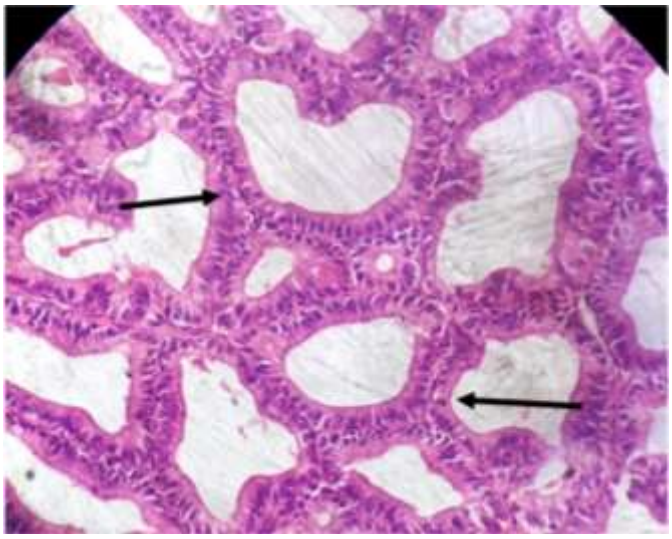

Plate 11. Bladder tissue showing normal epithelia (arrows) after inoculation with *S. xylosus*. H/E x200.

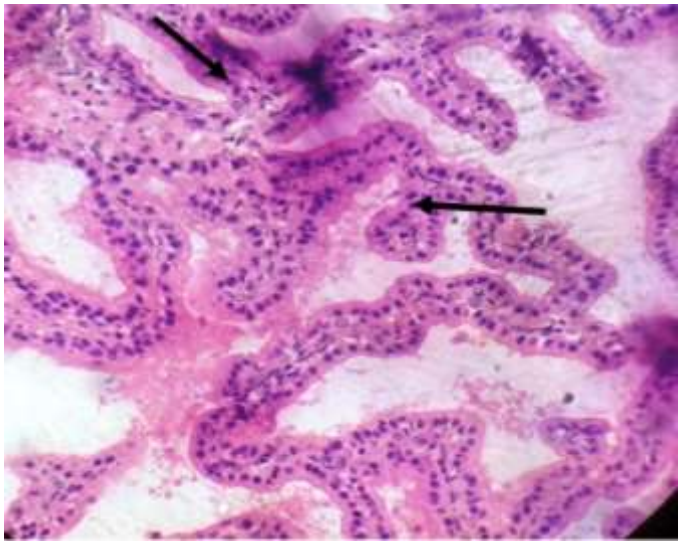

Plate 10. A piece of bladder tissue of rat showing inflammatory cells (arrows) in the mucosae and connective tissues following inoculation with *S. xylosus*. H/E x200.

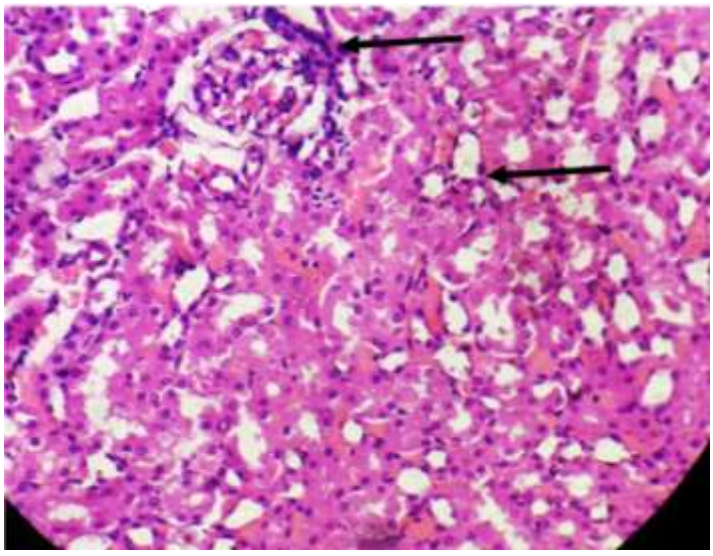

Plate 12. A piece of kidney tissue showing infiltration of inflammatory cells (arrows) after inoculation with *S. lentus*. H/E x200.

leading to their deaths. The possession of virulent factors such as haemolysin, protease, lipase, and urease by *S. xylosus* could have contributed to deaths of the 8 rats inoculated with *S. xylosus* 837.

S. aureus 834 and *S. lentus* 853 did not cause deaths to any of the test animals in the present study, probably they are less virulent than the other *Staphylococcus* strains tested. Moreover, most staphylococci are said to be harmless and reside normally on the skin and mucous membranes of humans and other animals (Madigan and Martinko, 2005) but could be opportunistic.

Of the 12 deaths that occurred in the present study, 8 (66.7%) came from *S. xylosus* 837 in peptone water broth inoculation while 4 (33.3%) was from *S. aureus* 856 peptone water broth inoculation, suggesting that *S. xylosus* 837 strain is more virulent than the *S. aureus* (856) strain. Of the eight deaths from *S. xylosus* 837 inoculation, males incurred more deaths (6 (75%)) than females (2 (25%)), so *S. xylosus* (837) strain seemed to be more virulent in male rats than in female rats. Of the 4 deaths from *S. aureus* (856) inoculation, all 4 deaths

occurred in female rats (100%) while none occurred in males (0%) suggesting that *S. aureus* 856 may be more invasive in females than in males.

For *S. xylosus* 837, mortality rate was equal at both 0.5 ml and at 1.0 ml inoculations (four deaths occurring at each of the doses) representing 50% each and no death at 0.2 ml inoculation. For *S. aureus* 856, all the four deaths occurred at the highest dose of the inoculum 1.0 ml while none occurred at 0.2 ml and at 0.5 ml dose inoculations. The findings of the study suggest that mortality rates increased with increasing doses of the staphylococcal test strains.

Baba-Moussa et al. (2010) reported that *S. aureus* isolates from UTI in their work produced at least one enterotoxin. 21.5% of the *S. aureus* isolates from Cotonou University Hospital (CUH) in Benin used for their study produced Panton Valantine Leucocidine (PVL) ($P < 0.01$). Six of 14 (43%) PVL-positive isolates were methicillin resistant. Genes encoding clumping factor B, elastin and laminin binding proteins were detected in almost all isolates (80%), hence they concluded that staphylococcal toxins and adhersion factors may be important in physiology of UTI. The finding of Baba-Moussa et al. (2010) could have also contributed to pathological changes seen in the Wistar rats inoculated with graded doses of *S. aureus* 856 in the present study where the visceral organs of rats examined showed toxigenic modifications on the kidneys of the rats with presence of tubular casts, tubular erosions and glomerular oedema in high doses of the inoculum, and extent of cellular damage increased with more concentration of the inoculum, with tubular casts characterizing all concentrations of the *S. aureus* 856 inoculums in the kidney tubules. The bladder of the rats showed a mild effect on musculature. The transitory epithelia of the bladder remained intact with mild infiltration of inflammatory cells which was in proportion of the infective doses of the inoculum.

The work of Al-Mathkhury et al. (2008) on pathological study on *S. xylosus* isolated from patients with urinary tract infections showed that all strains of *S. xylosus* isolates from UTI in their study were able to produce protease, haemolysin while urease activity was variable. Pathological changes were caused by the *S. xylosus* Isolates on the mice used in their study which was represented by glomerulus shrinkage, haemorrhage, congestion and infiltration of inflammatory cells in the kidney while the urinary bladder suffered hydrophobic degeneration, dekeratinization as well as infiltration of inflammatory cells.

Similarities exists between the findings of Al-Mathkhury et al. (2008), and the result of the present study which shows that inoculation of the Wistar rats with graded doses *of S. xylosus* 837 produced tubular casts in the kidney tubules, mild distortion of the glomerulus with mild infiltration of inflammatory cells, capsular oedema in rats inoculated with high doses of *S. xylosus* 837, and tubular oedema seen in the stroma while the bladder of the rats showed inflammatory cells on the mucosae and connective tissues.

All doses (0.2, 0.5 and 1.0) ml/kg body weight of the infective doses of *S. lentus* produced glomerular oedema, inflammatory cells infiltration and tubular casts in proportion to the inoculated dose in the kidney while the bladder of rats did not manifest any major damage but showed mild epithelial erosion with inflammatory cells. Hence, *S. lentus* showed pathological potentials.

Conclusion

The present study has shown pathogenic and virulent potentials of two of the staphylococcal strains investigated *S. xylosus* 837 and *S. aureus* 856 with mortality rates for both at 12.5%. *S. xylosus* 837 caused more deaths than *S. aureus* 856 suggesting higher virulent potential by *S. xylosus* 837. *S. xylosus* 837 showed higher sex mortality rates in males than in females while *S. aureus* 856 demonstrated higher sex mortality in females than in males. Mortality rates increased in both staphylococcal strains with increased doses of their inoculums. The two test strains *S. xylosus* 837 and *S. aureus* 856 demonstrated several pathological changes on the kidney and bladder of Wistar rats and these changes heightened with increased doses of the test strains inoculum. The liver of the rats showed occasional mild infiltration of the inflammatory cells around the pericentral areas for *S. aureus* 856, no visible alterations of the liver parenchyma or hepatocytes by *S. xylosus* 837 and no toxigenic effect on the liver by *S. lentus* 853.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the management of University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla and Enugu State University Teaching Hospital (ESUTH), Enugu, both in Enugu state, Nigeria, for granting ethical permission that enabled urine samples to be collected from their patients and other participants for this study. Thanks to all the participants who submitted their urine samples from which the staphylococcal isolates used for the study were obtained, and thanks to all who contributed in one way or the other to make this work a success.

REFERENCES

Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A (2012). Bacterial profile and drug susceptibility pattern of urinary tract

infection in pregnant women at university of Gondar Teaching Hospital North West Ethiopia. BMC Res Notes. 5:197.
Almathkuhury HJF, Flayyih MT, Abdullah AL-Ghrairy ZK (2008). Pathological study on *Staphylococcus xylosus* isolated from patients with urinary tract infections. J. Al-Nahrian University 11(2):123-130.
Amaeze NJ, Abah AU, Okoliegbe IN (2013). Prevalence and antibiotic susceptibility of uropathogens among patients attending University of Abuja. Int. J. Med. Med. Sci. 5(10):460-466.
Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Reigel P, Haikou N, Hounsou F, Montel H, Sanni A , Prevost G (2008). Virulence factors produced by strains of *staphylococcus aureus* isolated from urinary tract infections. J. Hosp. infect. 68(1):32-38.
Bolaji RO, Onaolapo JA, Ibrahim YKE and Igwe JC (2013). Incidence of uropathogens from asymptomatic bacteriuric pregnant women in Zaria, Nigeria. Niger. J. Pharm. Sci. 12(1):1-8.
Brooks GF, Caroll KC, Butel JS, Morse SA, Mietzner TA (2013). Jawetz, Melnic and Adelbergs Medical Microbiology 26th ed. Mc Graw-Hill companies Inc. USA. pp. 199-207.
Cheesbrough M (2010).*Staphlocococcus aureus*. District laboratory Practice in Tropical countries. Second edition update. Part 2 Cambridge low price ed. (Cambridge University Press South Africa). pp. 157-158.
Janakat S, Al-Merie H (2002). Optimazation of the dose and route of injection, and characterization of the time couse of carbon tetrachloride-induced hepatotoxicity in the rat. J. Pharmacol. Toxicol. Methods 48(1):41-44.
Levinson W (2012). Review of Medical Micobiology and Immunology, 12th ed. MacGrawHill companies inc. USA. 710pp.
Madigan M, Martinko J (2005). Brock Biology of Microorganisms (11th ed.) Prentice Hall ISBN 0-13-144329-1.
Mosbah H, Sayiri A, Bezzine S, Gargouri D (2006). Expression, purification and characterization of his tagged *Staphylococcus xylosus* lipase wild-type and its mutant Asp 290 Ala. Protein Express. Purif. 47(2):516-523.
Ochei J, Kolhatkar A (2000). Medical Laboratory Science Theory and Practice. Tata MacGraw- hill publishing company limited 7 West Patal Nagar New Delhi 110008, India. 1338p.
Oladeinde BH, Omoregie R, Olley M, Anunibe JA (2011). Urinary tract infection in a rural community of Nigeria. North Am. J. Med. Sci. 3(2):75-77.
Pragash DS, Giraja S, Shekar U, Rayupu V, Malathi (2014). Study of uropathogens among type II diabetic patients and their antimicrobial resistant pattern among rural south indian population. Sch. J. Appl. Med. Sci. 2(2B):589-591.
Wikipedia the free encyclopedia (2013). *Staphylococcus xylosus* http//en.wikipedia.org/w/index.php?title=Staphylococcus_xylosus&oldid=542552341 This page was last modified on 7 march 2013 at 09:34 and accessed on14/6/2013

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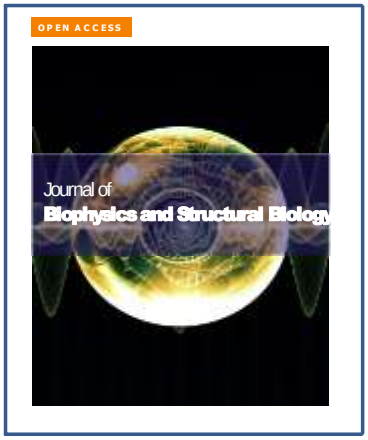

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