



Evaluation of the Antagonistic Activity of Bacteria Isolated from Palm Wine (*Raphia vinifera*) on *Salmonella typhi* from Different Sources

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to evaluate the antagonistic effect of bacteria associated with palm wine from *Raphia vinifera* on *Salmonella typhi*, the etiological agent of typhoid fever.

Method: Isolation and identification of bacteria from palm wine and the isolation of *S. typhi* from different sources were done using standard microbiological techniques. The antagonistic assay of each isolated bacteria on *S. typhi* was also done using microbiological standard.

Results: The bacteria isolated from the palm wine used comprised six bacteria species namely; *Bacillus cereus*, *Micrococcus roseus*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Streptococcus lactis* and *Pseudomonas aeruginosa*. Out of the isolated bacteria, *Lactobacillus brevis* exerted the highest growth inhibitory effect on most of the *S. typhi* isolated from different sources and also the typed isolate (*Salmonella enterica* serovar *Typhi* ATCC 33458) used. The growth inhibition mediated by *L. brevis* is superior to that of all the conventional antibiotics used.

Conclusion: It is therefore suggested that *L. brevis* from palm wine (*R. vinifera*) could be used to treat cases of typhoid fever caused by *S. typhi*.

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1. INTRODUCTION

Palm wine is an alcoholic traditional beverage widely consumed in many parts of the world which are produced from the fermented sap of tropical plants of the *palmae* family, such as the oil palm (*Elaeis guineensis*), coconut palm (*Cocos nucifera*), raffia palm (*R. vinifera*) etc. Palm wine possesses antibacterial activity [1]. It is often infused with medicinal herbs to treat a wide variety of physical complaints especially in the treatment of skin rashes in children [2].

S. typhi is a Gram negative pathogenic bacterium that can be transmitted by ingestion of faecally contaminated food or water. Infection of *S. typhi* in human leads to the development of typhoid fever. The source of the pathogen is usually from direct or indirect contact with faeces of typhoid fever victims or of carriers, that is, healthy persons who harbour typhoid bacilli without presenting symptoms [3]. The high cost of commercially produced antimicrobial agents coupled with the increasing prevalence of multidrug resistant strains of bacteria adds urgency to the search for new antimicrobial agents [4].

Microorganisms such as *Lactobacillus* species exert strong antagonistic activity against many pathogenic microorganisms like *Shigella*, *E. coli*, by producing compounds; organic acids, diacetyl, hydrogen peroxide and bacteriocin or bacterial proteins during lactic fermentations.

Recently, little information is available on the antagonistic effect of bacteria isolated from palm wine (*R. vinifera*) on *S. typhi*.

Therefore, this work is designed to evaluate the antagonistic activity of bacteria isolated from palm wine (*R. vinifera*) on *S. typhi* from different sources.

2. MATERIALS AND METHODS

2.1 Sources of *S. typhi*

Clinical isolates of *Salmonella typhi* were obtained from Don Bosco Hospital, Akure, State Specialist Hospital, Akure and Federal medical center, Owo. Typed isolate; *Salmonella enteric* serovar *Typhi* (ATCC 33458) was obtained from National Institute of Medical Research (NIMER), Yaba Lagos. While the other *S. typhi* used were

isolated from raw beef, fresh crayfish, well water, stream water, poultry droppings and an healthy individual using standard microbiological methods [2].

2.2 Collection of Palm Wine Samples

Ten bottles of palm wine (*R. vinifera*) (750 ml) was purchased from palm wine tappers in two different locations namely; Ijare in Ondo state and Igbara-odo in Ekiti State of Nigeria, in sterile plastic bottles, well labelled and then immediately transported to the Microbiology laboratory, Federal University of Technology, Akure for further analyses.

2.3 Microbiological Analysis

Ten millilitres (10 ml) of the samples were homogenised in 90 ml sterile peptone water solution to form the stock solution. One millilitre (1.0 ml) from the stock solution was pipetted and serially diluted to appropriate dilutions of 10^{-5} . Nutrient agar and de Man's Rogosa Sharpe agar (MRSA) were prepared according to manufacturers' guidelines and used for the culturing of total bacterial and lactic acid bacterial isolates respectively. Bacterial plates were incubated at 37°C for 24 hours and MRS agar plates were incubated anaerobically at 28°C for 24-48 hours. The pure isolates were obtained by repeated streaking on the freshly prepared microbiological media. Characterisation and identification of the isolated microorganisms were based on their cultural, morphological and biochemical tests [2].

2.4 Assessment of Antagonistic Activity of Microorganisms Isolated from Palm Wine on *S. typhi*

This was carried out using agar well diffusion method. *S. typhi* suspension (1.5×10^8 CFU/ml) was spread on solidified Mueller-Hinton agar using a sterile glass spreader. Two wells were made on each plate using a 6 mm sterile cork borer, 0.1 ml (100µl) of each microorganism isolated from palm wine was introduced into each well, while 0.1 ml (100 µl) of sterile distilled water was added to the second well in all cases. The plates were incubated at 37°C for 24 hours after which the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured using a transparent ruler [5].

2.5 Assessment of the Growth Inhibitory Activity of Some Antibiotics on *S. typhi* Isolates

Four different conventional antibiotics; chloramphenicol, amoxicillin, gentamycin and ciprofloxacin were used for this assay. About 0.1 ml of *S. typhi* containing 1.5×10^8 CFU/ml was spread on already prepared and solidified Muller-Hinton agar using sterile glass spreader. Five wells were made on each plate using sterile cork borer, each antibiotic was prepared to the concentration on conventional antibiotic sensitivity disk (30 µg for Chloramphenicol, 25 µg for Amoxycillin, 10 µg for Gentamycin and 10µg for Ciprofloxacin). Each of the antibiotics in solution (0.1 ml) was introduced into separate wells, one type per well, while sterile distilled water was added to the well at the centre. The plates were incubated at 37 °C for 24 hours after which the plates were observed for zones of inhibition. The diameter of the zone of inhibition was measured using a transparent ruler. The antibiotics served as positive control [6].

2.6 Statistical Analysis

The data obtained were analyzed. Data are presented as mean ± standard deviation. The significance of difference between different groups was tested using one-way analysis of variance (ANOVA) using SPSS Window 8 Version 23 software.

3. RESULTS

3.1 Total bacterial Counts from Palm Wine (*R. vinifera*)

The total bacterial counts from palm wine sample is represented (Table 1). The highest bacterial counts of 6.40 was observed in the sample purchased from Igbara-odo at point “g” when

cultured on nutrient agar and no growth was observed in samples purchased from Igbara-odo at point “a” and Ijare at point “b” when cultured on de Man’s Rogosa Sharpe agar (MRS).

3.2 Colony, Cell Morphological and Biochemical Characterisation of Bacterial Isolates from Palm Wine (*R. vinifera*) Samples

Table 2 shows the colonial, morphological and biochemical characterization of bacterial isolates from palm wine (*R. vinifera*).

3.3 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* from Stool

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from stool sample of an apparently healthy individual is represented (Fig. 1). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *S. typhi* from stool from an apparently healthy individual.

3.4 Inhibitory Activities of Isolated Bacteria and Antibiotics on *Salmonella enteric* Serovar *Typhi* ATCC33458

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *Salmonella enteric* serovar *Typhi* ATCC33458 is represented (Fig. 2). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *Salmonella enteric* serovar *Typhi* ATCC33458.

Table 1. Total bacterial counts from palm wine samples

| Locations | Total bacterial counts (CFU/ml) (10^5) | Lactic acid bacteria counts (CFU/ml) (10^5) |
|-----------|--|---|
| PWOa | 1.50±0.03 ^a | 0.00±0.00 ^a |
| PWlb | 1.40±0.02 ^a | 0.00±0.00 ^a |
| PWOc | 3.50±0.05 ^b | 2.90±0.13 ^b |
| PWld | 3.35±0.04 ^b | 2.90±0.11 ^b |
| PWOe | 5.90±0.02 ^{cd} | 3.80±0.02 ^c |
| PWlf | 5.85±0.02 ^c | 3.85±0.03 ^c |
| PWog | 6.40±0.05 ^d | 4.80±0.00 ^d |
| PWlh | 6.20±0.04 ^d | 4.90±0.03 ^d |
| PWoi | 5.70±0.06 ^c | 5.00±0.06 ^e |
| PWlj | 5.50±0.05 ^{bc} | 4.80±0.05 ^d |

Data are represented as mean ± standard errors with the same superscript down the column are not significantly different ($p \leq 0.05$). Keys: PWO= Palm wine from Igbara-odo; PWl = Palm wine from Ijare. a, b, c,j = Different points of purchase.

Table 2. Colony, morphological and biochemical characterisation of bacterial isolates from palm wine (*raphia vinifera*)

| Isolate No | Colony Morphology | Gram's Reaction | Catalase | Motility | Glucose | Fructose | Maltose | Lactose | Galactose | Oxidase | Citrate | Indole | Spore Forming | Methyl Red Test | Starch hydrolysis | Urease test | Probable identity |
|------------|-------------------|-----------------|----------|----------|---------|----------|---------|---------|-----------|---------|---------|--------|---------------|-----------------|-------------------|-------------|-----------------------------|
| 1 | CSR | +C | + | - | A | A | A | A | A | + | + | - | - | - | + | - | <i>Micrococcus roseus</i> |
| 2 | GOR | +R | + | + | A | A | A | AG | A | - | + | - | + | - | + | - | <i>Bacillus cereus</i> |
| 3 | RRE | +R | - | - | AG | A | AG | A | A | - | - | - | - | + | + | - | <i>Lactobacillus brevis</i> |
| 4 | LRC | +R | - | - | A | A | A | A | A | - | - | - | - | + | + | - | <i>L. plantarum</i> |
| 5 | SCS | +C | - | - | A | A | A | A | - | - | + | - | - | + | + | - | <i>Streptococcus lactis</i> |
| 6 | RFG | -R | + | + | - | - | A | A | - | + | - | - | - | - | - | - | <i>P. aeruginosa</i> |

Key: (+) = positive, (-) = negative, (A)= acid production, AG= Acid production with gas; A= Acid production, CSR= circular smooth, light red, GOR= Grey white irregular raised, RRE= Rods with round end, LRC= Long chain rod, creamy, SCS = Short chain, cocci, spherical, RFG= Rod, flagellate, green, R= Rod, C= cocci, NA= nutrient agar, MRSA= de Mann Rogosa and Sharpe (MRS) agar.

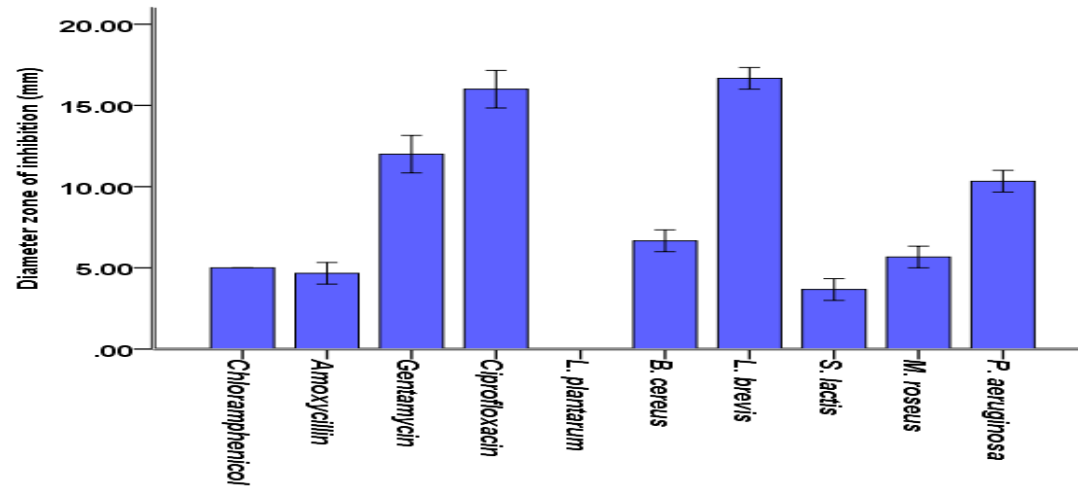


Fig. 1. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from stool sample of apparently healthy individual

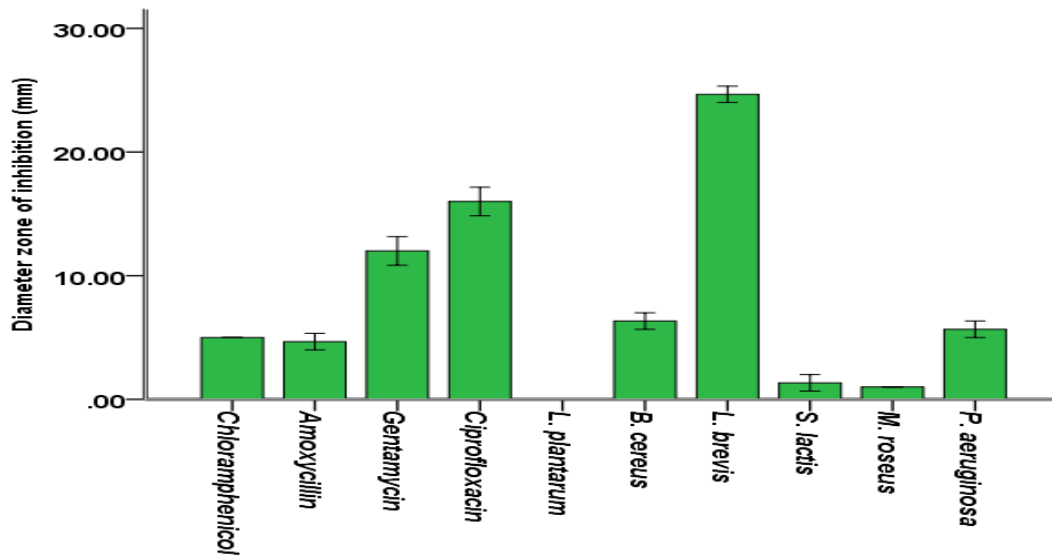


Fig. 2. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *Salmonella enteric serovar typhi* ATCC33458

3.5 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* Fresh Crayfish

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* from fresh crayfish is represented (Fig. 3). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *S. typhi* from fresh crayfish.

3.6 Inhibitory Activities of Isolated Bacteria and Antibiotics on Typhoid Fever Patient Stool

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from stool sample of typhoid fever patient at Don Bosco Hospital, Akure is represented (Fig. 4). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *S. typhi* isolated from stool sample of typhoid fever patients at Don Bosco Hospital, Akure.

3.7 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* from Poultry Dropping

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to

antibiotics on *S. typhi* isolated from Poultry dropping is represented (Fig. 5). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *S. typhi* isolated from poultry dropping.

3.8 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* from Raw Beef

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from raw beef (Fig. 6). Ciprofloxacin had the highest growth inhibitory activity compared to any other bacteria used in the inhibition of *S. typhi* isolated from raw beef.

3.9 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* Isolated from State Hospital Akure, Ondo State, Nigeria

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from State hospital Akure, Ondo State, Nigeria (Fig. 7). *L. brevis* had the highest growth inhibitory activity compared to any other antibiotics used in the inhibition of *S. typhi* isolated from *S. typhi* isolated from State hospital Akure, Ondo State, Nigeria.

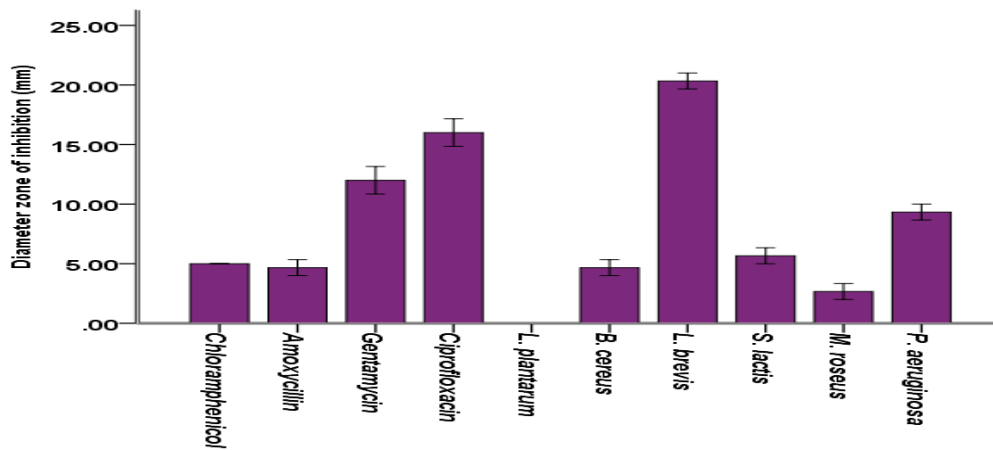


Fig. 3. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* fresh crayfish

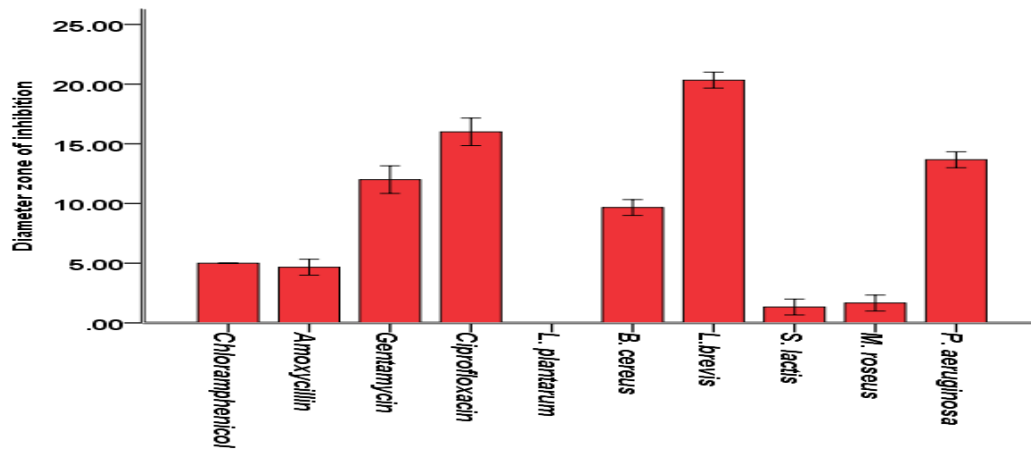


Fig. 4. Inhibitory activities of isolated bacteria from palm wine and antibiotics on typhoid fever patient stool at Don Bosco Hospital, Akure

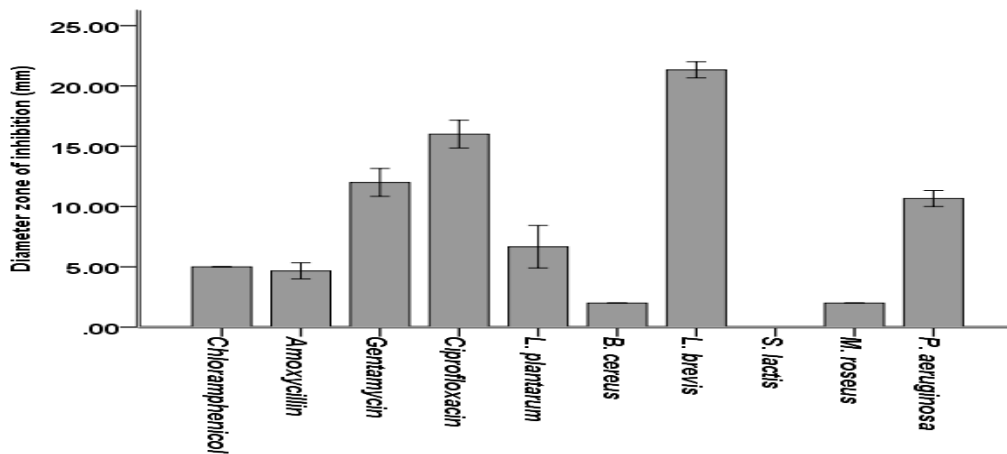


Fig. 5. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from poultry dropping

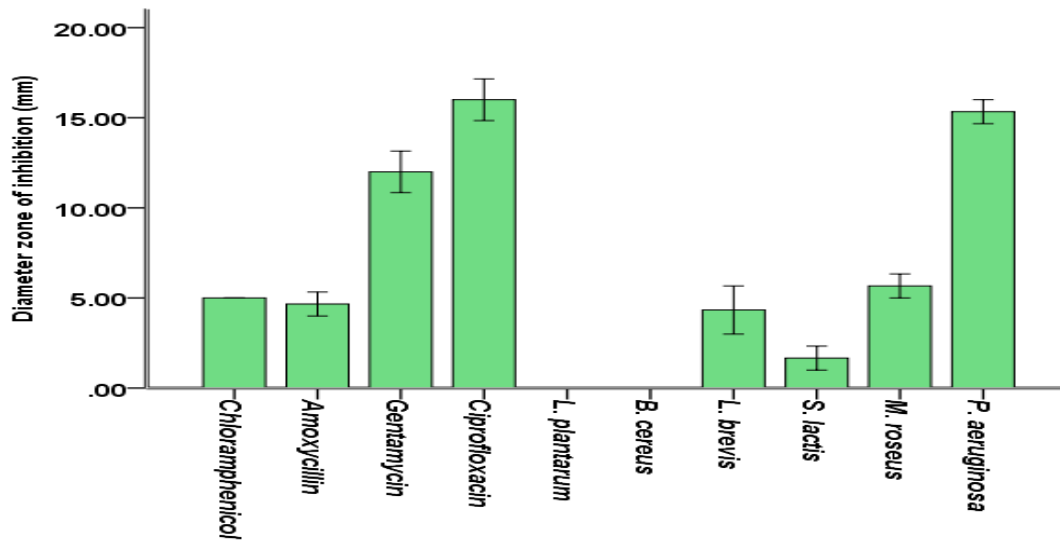


Fig. 6. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from raw beef

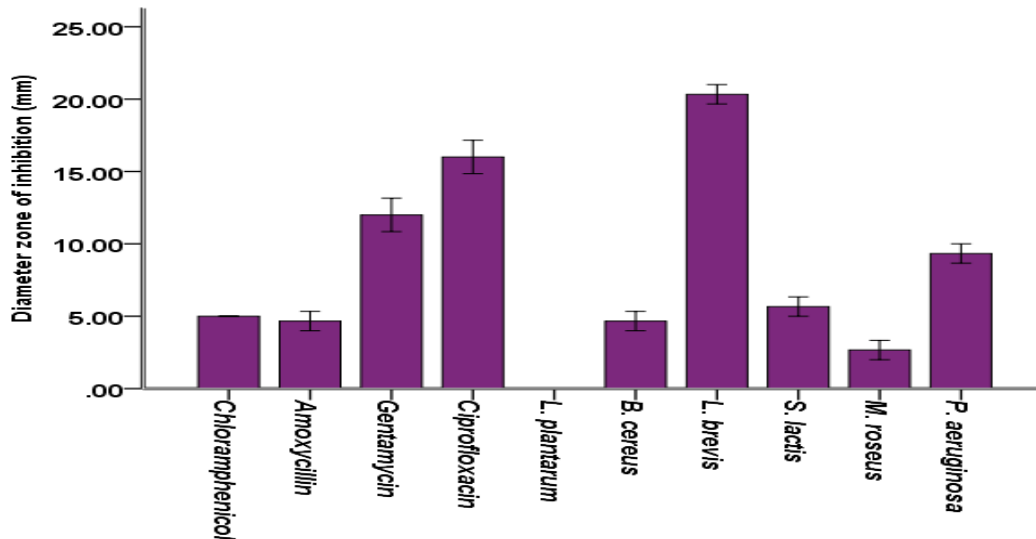


Fig. 7. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from State hospital Akure, Ondo State, Nigeria.

3.10 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* Isolated from Stream Water

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from stream water (Fig. 8). Ciprofloxacin had the highest growth inhibitory activity compared to any other bacteria used in the inhibition of *S. typhi* isolated from stream water.

3.11 Inhibitory Activities of Isolated Bacteria and Antibiotics on *S. typhi* Isolated from Well Water

The growth inhibitory activity of bacteria isolated from palm wine (*R. vinifera*) as compared to antibiotics on *S. typhi* isolated from well water is represented (Fig. 9). Ciprofloxacin had the highest growth inhibitory activity compared to any other bacteria used in the inhibition of *S. typhi* isolated from well water.

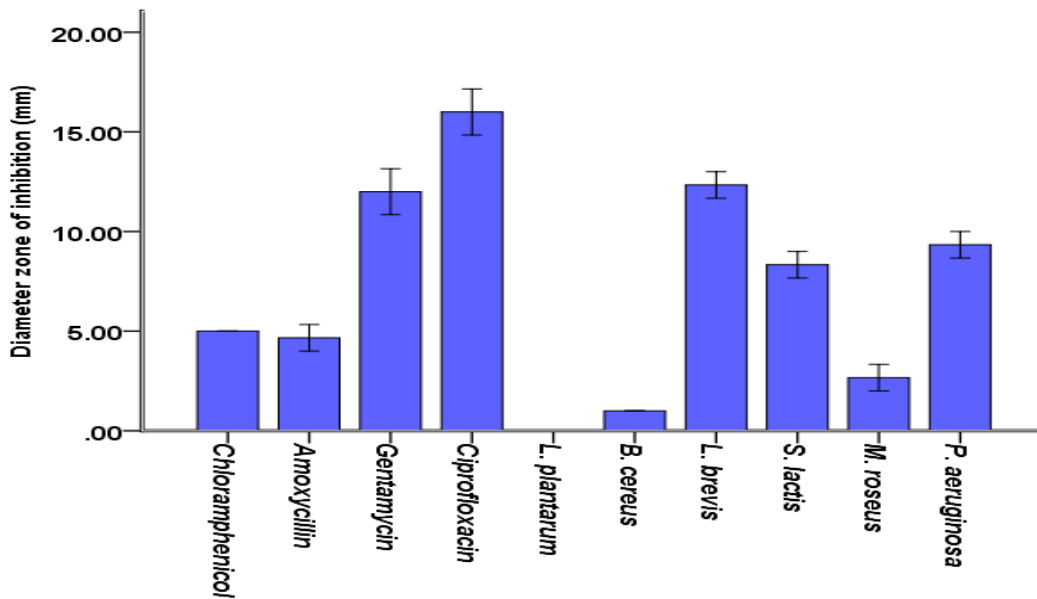


Fig. 8. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from stream water

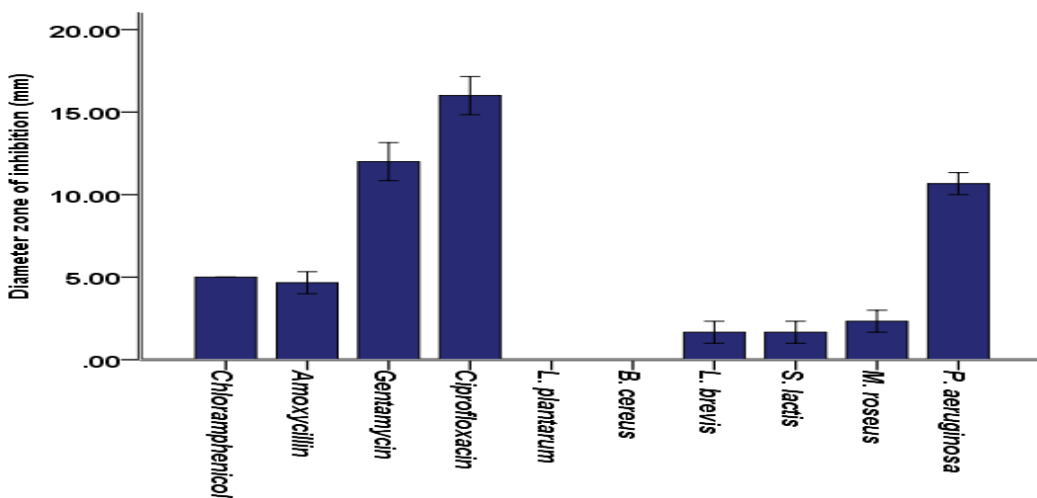


Fig. 9. Inhibitory activities of isolated bacteria from palm wine and antibiotics on *S. typhi* from well water

4. DISCUSSION

In this present study we evaluate the antagonistic activity of bacteria isolated from palm wine (*R. vinifera*) on *S. typhi* from Don Bosco Hospital, Akure, State Specialist Hospital, Akure, Federal medical center, Owo, Typed isolate; *Salmonella enteric* serovar *Typhi* (ATCC 33458), raw beef, fresh crayfish, poultry dropping, well water and stream water. Different microorganisms were isolated and characterized from palm wine

samples using standard microbiological and biochemical methods. The presence of bacteria like *Bacillus cereus*, *Micrococcus roseus*, *L. brevis*, *L. plantarum*, *Streptococcus lactis*, *Pseudomonas aeruginosa*, and also higher bacterial load in palm wine (*R. vinifera*) from sample in Igbara-odo in Ekiti State in Nigeria might be due to human activity, traditional tapping equipment like the machet used to make incisions on the palm tree, the collection gourd and unhygienic handling by the tapper and

exposure to air [7]. The report from this study is similar to the findings of Chandrasekar et al. [8] who isolated arrays of bacteria from palm wine.

The isolates used for this study showed that these bacteria especially *L. brevis* isolated from palm wines (*R. vinifera*) have exerted greater antagonistic effect on most of the *S. typhi* isolated from different sources. The growth inhibitory effects recorded may be attributed to the presence of metabolites such as organic acids, diacetyl, hydrogen peroxide produced by lactic acid bacteria [9] and the ability of those bacteria to produce bacteriocins which might be responsible for their antagonistic activity. This study is similar to the report of Abdel-Daim et al. [10] who reported an antagonistic effect of *Lactobacillus* species on the *S. typhi* in vivo.

5. CONCLUSION

It has been demonstrated in this study that some of the microflora from palm wine such as *Lactobacillus* sp., *Pseudomonas aeruginosa* produce metabolites that have growth inhibitory activity on *S. typhi*; the etiology of typhoid fever that is even higher than that mediated by conventional antibiotics. Therefore, *L. brevis* from palm wine could play an important role in treatment of typhoid fever.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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