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# Bacterial contaminants of Date palm fruits *(Phoenix dactylifera)* sold in Kaduna, Nigeria, and their susceptibility to antibiotics

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## Abstract:

**Background:** Antibiotic resistance is a major challenge of antimicrobial therapy of infections today. Food-borne bacteria can serve as reservoir for transmission of antibiotic resistant strain. This study was aimed at determining the bacterial contaminants on dates palm fruits sold in Kaduna metropolis and to determine the antibiotic resistance pattern of the bacteria isolated.
**Methodology:** A total of 60 samples of Date palm fruits (*Phoenix dactylifera* L.) were collected randomly from five major markets in Kaduna metropolis. Total bacterial and total coliform counts were determined using pour plate method. Gram, staining, microscopy and biochemical tests were carried out to isolate the bacterial contaminants. Antibiotic susceptibility test for commonly prescribed antibiotics was also carried out through agar diffusion method and the percentage antibiotic resistance determined. Data were analysed using descriptive statistics on Microsoft Excel.
**Results:** The results showed that the mean total bacterial count range was $4\times10^5$-$2.7\times10^6$. The mean coliform count range was $8\times10^3$-$1.2\times10^6$. The following bacteria were isolated; *Staphylococcus aureus* (35.7%), *Streptococcus* spp (21.4%), *Escherichia coli* (12.5%), *Bacillus subtilis* (8.9%), *Enterobacter* spp (8.9%), *Proteus mirabilis* (7.1%) and *Salmonella* Typhi (5.4%). Gentamicin (43.2%) and cotrimoxazole (47.4%) were the most active against Gram-positive and Gram-negative isolates respectively. The bacterial isolates showed high level of antibiotic resistance with 57.1% being multidrug resistant, 10.7% extensively resistant and 5.4% were resistant to all the antibiotics tested.
**Conclusion:** The high level of contamination and antibiotic resistance observed in this study is alarming and of public health concern, as Date fruit is highly consumed in this part of the country. Thorough washing of Date fruits with clean water before eating is recommended.

**Keywords:** Date palm fruits, Resistance, Antibiotics, Bacteria

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# Contaminants bactériens des fruits du palmier dattier (*Phoenix dactylifera*) vendus à Kaduna, Nigeria, et leur sensibilité aux antibiotiques

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## Résumé:

**Contexte:** La résistance aux antibiotiques est aujourd'hui un défi majeur de la thérapie antimicrobienne des infections. Les bactéries d'origine alimentaire peuvent servir de réservoir pour la transmission de souches résistantes aux antibiotiques. Cette étude visait à déterminer les contaminants bactériens sur les fruits du palmier dattier vendus dans la métropole de Kaduna et à déterminer le schéma de résistance aux antibiotiques des bactéries isolées.
**Méthodologie:** Au total, 60 échantillons de fruits de palmier dattier (*Phoenix dactylifera* L.) ont été prélevés au hasard dans cinq grands marchés de la métropole de Kaduna. Le nombre total de bactéries et de coliformes totaux a été déterminé à l'aide de la méthode de la plaque de coulée. Des tests Gram, coloration, microscopie et biochimiques ont été effectués pour isoler les contaminants bactériens. Un test de sensibilité aux antibiotiques pour les antibiotiques couramment prescrits a également été effectué par la méthode de diffusion sur gélose et le pourcentage de résistance aux antibiotiques a été déterminé. Les données ont été analysées à l'aide de statistiques descriptives sur Microsoft Excel.
**Résultats:** Les résultats ont montré que la plage de numération bactérienne totale moyenne était de $4\times10^5$ à $2,7\times10^6$. La plage de numération moyenne des coliformes était de $8\times10^3$-$1,2\times10^6$. Les bactéries suivantes ont été isolées; *Staphylococcus aureus* (35,7%), *Streptococcus* spp (21,4%), *Escherichia coli* (12,5%), *Bacillus subtilis* (8,9%), *Enterobacter* spp (8,9%), *Proteus mirabilis* (7,1%) et *Salmonella* Typhi (5,4%). La gentamicine (43,2%) et le cotrimoxazole (47,4%) étaient les plus actifs contre les isolats Gram-positifs et Gram-négatifs, respectivement. Les isolats bactériens ont montré un haut niveau de résistance aux antibiotiques avec 57,1% de multirésistance, 10,7% de résistance extensive et 5,4% de résistance à tous les antibiotiques testés.
**Conclusion:** Le niveau élevé de contamination et de résistance aux antibiotiques observé dans cette étude est alarmant et préoccupant pour la santé publique, car les dattes sont très consommées dans cette partie du pays. Il est recommandé de laver soigneusement les dattes à l'eau claire avant de les manger.


**Mots clés:** Fruits du palmier dattier, Résistance, Antibiotiques, Bactéries

## Introduction:

Date fruits are one-seeded fruits of date palm tree (*Phoenix dactylifera*), which belong to the family of Palmae (Arecaceae) plant. The common English names include dried dates and date palm. Date fruits are usually oblong, with varying sizes, shapes, colors, consistencies and quality. Date fruit is regarded to as a pivotal crop that is extensively farmed in the Middle East and Africa (1), and these regions are responsible for the exportation of date products worldwide (2). Date fruits are one of the most common fruits consumed by Nigerians. It is known as *Dabino* or *Dabinu* in Hausa language, and is mostly grown and cultivated in the northern part of the country.

Date fruits can be consumed fresh after harvest or can be semi-dried or totally dried for consumption. It is an important component of the diet in most of the hot arid and semi-arid regions of the world, and is known to contain carbohydrates, mostly in form of fructose and glucose (total sugars content is 35-88%), fats (0.2-0.4%), proteins (2.3-5.6%), fibers (6.4-11.5%), and minerals and vitamins (3,4). Other studies have shown that Date fruits contain many bioactive compounds such as anthocyanins, carotenoids, phenolics, sterols, procyanidins and flavonoids, which are thought to have beneficial effects on human health (5-8). They are also rich in polyphenols and functional dietary fiber that help to maintain the functions of the digestive tracts (9,10). The antimutagenic, antioxidant, anticarcinogenic and anti-inflammatory bioactivities of Date fruits have been attributed to the contribution of polyphenols (11).

Microbial contaminants have been isolated from Date fruits, including yeasts, molds, lactic acid bacteria and some potential pathogens such as *Staphylococcus aureus, Escherichia coli*, and *Aspergillus flavus/parasiticus* (12, 13). Bacteria growth can be facilitated by high moisture content of the Date flesh, but on the other hand, growth of molds become prominent when the Dates are dried and stored (14). The increasing rate of bacteria developing resistance to antibiotics globally is alarming, and antibiotic resistant bacterial strains have been isolated from ready-to-eat fruits and vegetables (15). This study therefore aimed to assess the bacteria contaminants in Dates fruits sold in Kaduna metropolis and to determine the antibiotic susceptibility of the isolates.

## Materials and method:

**Study setting and Date fruit samples collection:**

A total of 60 samples of Dates fruits (*Phoenix dactylifera* L.) each wrapped in polythene bags (as was being sold) were purchased randomly from five major locations in Kaduna metropolis, Kaduna State, Nigeria. The locations were Gonin gora, Central market, Sabo tasha, Ungwa Rimi and Kawo markets.

**Preparation of Date palm fruits for analysis:**

The samples of the Date fruits were cleaned and pitted. About 5g of the fruits were aseptically weighed into sterile stomacher bags and 45ml normal saline was added. Samples

were homogenized for 15 minutes and aliquots were used for microbiological analysis. One milli-liter of the aliquot from each sample was added into a tube containing 9ml of sterile normal saline, thoroughly shaken, and serially diluted up to $10^{-5}$.

**Determination of bacteria and coliform counts:**

A 0.1ml each of the serially diluted sample ($10^{-3}$ and $10^{-4}$) were spread over the surface of the nutrient agar plates (for viable bacteria count), and on MacConkey agar plates (for coliform count), with the aid of sterile glass spreader. This was done in duplicates for each dilution. The plates were incubated at $37^0$C for 24 hours, following which viable bacteria and coliform colonies were counted to determine the total bacterial and coliform counts, expressed as colony forming unit per millimeter (CFU/ml). Pure colonies from the total bacteria counts were isolated by sub-culturing on fresh nutrient agar plates. The pure cultures were preserved on nutrient agar slants for further studies.

**Identification of bacterial isolates:**

The culture plates were examined for colony morphology, and bacteria identified following Gram staining reaction, conventional biochemical tests and growth on selective media. The biochemical identification tests performed included catalase, coagulase, methyl red, Voges Proskauer, indole, triple sugar iron, oxidase and citrate utilization tests (16).

**Antibiotic susceptibility test:**

The agar disc diffusion method was used for antibiotic susceptibility test according to the Clinical and Laboratory Standards Institute (17) guideline. A sub-culture of the bacterial isolate from nutrient agar slant was prepared on nutrient agar plate and incubated aerobically overnight. Colonies from the plate were emulsified aseptically in sterile saline to produce inoculum equivalent to 0.5 McFarland turbidity standard (~$1.5 \times 10^8$ CFU/ml). A sterile swab was dipped into the standardized inoculum and squeezed gently against the inside of the tube to remove excess fluids. The swab was streaked on sterile Mueller Hinton agar plate and allowed to dry for 5 minutes. Antibiotic discs were then placed on the surface of the agar using sterile forceps, and the plates were inverted carefully and incubated for 24 hours at 37° C. The diameter of zone of inhibition for each antibiotic was measured with a metric ruler and interpreted as sensitive, intermediate or resistant according to the interpretative chart of CLSI (17). The following antibiotic discs (Bio-Rad®, Ca, USA) were used; tetracycline (30µg), gentamicin (10µg), cefoxitin (30 µg), erythromycin (15µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), amoxicillin-clavulanate (30µg) and trimethoprim/sulfamethoxazole (1.25/23.75µg).

**Determination of multiple antibiotic resistance:**

The bacterial isolates resistant to at least one agent in three or more antimicrobial categories were classified as multidrug resistant (MDR) while extensively drug resistant (XDR) isolates were defined as those that were non-susceptible to all the antibiotic categories used except one or two. Pandrug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories used (18).

**Statistical analysis:**

The data was analysed using descriptive analysis through Microsoft excel.

## Results:

The total bacteria viable count from the samples of Date fruits from different sites in Kaduna metropolis varies from $4x10^5$ to $2.7x10^6$ CFU/ml while coliform count varies from $8\times10^3$ to $1.2\times10^6$ CFU/ml (Table 1). Samples collected from Ungwan rimi had the highest total bacteria count ($2.7\times10^6$ CFU/ml) and coliform counts ($1.2x10^6$) while Gonin gora had the least bacteria count ($4\times10^5$ CFU/ml).

A total number of 56 bacterial isolates; 19 (33.9%) Gram-positives and 37 (66.1%) Gram-negatives, were recovered on cultures, with *S. aureus* 20 (35.7%) being the most frequent bacteria isolates. Other isolated bacteria were *Streptococcus* spp*, E. coli, Bacillus subtilis, Proteus mirabilis, Enterobacter* spp and *Salmonella* Typhi. Sample collected from Ungwan Rimi market had the highest frequency of bacteria isolates (Table 2).

Table 1: Total viable bacteria and coliform counts of Date fruits from selected markets, Kaduna, Nigeria

| S/N | Market sample location | Total viable bacteria count (CFU/ml) | Total coliform count (CFU/ml) |
|---|---|---|---|
| 1 | Ungwa Rimi (U) | $2.7 \times 10^6$ | $1.2 \times 10^6$ |
| 2 | Gonin gora (G) | $4 \times 10^5$ | $5 \times 10^4$ |
| 3 | Central (C) | $5 \times 10^5$ | $6 \times 10^4$ |
| 4 | Kawo (K) | $7 \times 10^5$ | $1 \times 10^5$ |
| 5 | Sabon tasha (S) | $2.3 \times 10^6$ | $8 \times 10^3$ |

CFU/ml = Colony forming unit per milliliter

Table 2: Frequency of bacterial isolates of Date fruits from different market sample sites in Kaduna, Nigeria

| Bacteria isolates | Central | Ungwa Rimi | Sabo tasha | Gonin gora | Kawo | Total (%) |
|---|---|---|---|---|---|---|
| *Proteus mirabilis* | 1 | 0 | 3 | 0 | 0 | 4 (7.1) |
| *Staphylococcus aureus* | 5 | 3 | 3 | 5 | 4 | 20 (35.7) |
| *Streptococcus* spp | 2 | 5 | 1 | 2 | 2 | 12 (21.4) |
| *Bacillus subtilis* | 1 | 1 | 1 | 1 | 1 | 5 (8.9) |
| *Escherichia coli* | 1 | 1 | 2 | 1 | 2 | 7 (12.5) |
| *Enterobacter spp* | 1 | 1 | 1 | 1 | 1 | 5 (8.9) |
| *Salmonella* Typhi | 0 | 1 | 0 | 1 | 1 | 3 (5.4) |
| **Total** | **11** | **12** | **11** | **11** | **11** | **56 (100)** |

Table 3: Antibiotic susceptibility of Gram positive and Gram-negative bacterial isolates of Date fruits in Kaduna, Nigeria

| Antibiotic | Gram positive isolates (n = 37) | | | Gram negative isolates (n = 19) | | |
|---|---|---|---|---|---|---|
| | S | I | R | S | I | R |
| Tetracycline (30µg) | 3 (8.1) | 10 (27.0) | 24 (64.8) | 7 (36.8) | 2 (10.5) | 10 (52.6) |
| Ceftriaxone (30µg) | 14 (37.8) | 9 (24.3) | 14 (37.8) | 9 (47.4) | 4 (21.1) | 6 (31.6) |
| Ciprofloxacin (5µg) | 8 (21.6) | 8 (21.6) | 21 (56.7) | 4 (21.1) | 12 (63.2) | 3 (15.8) |
| Erythromycin (15µg) | 15 (40.5) | 11 (29.7) | 11 (29.7) | 5 (26.3) | 3 (15.8) | 11 (57.9) |
| Chloramphenicol (30µg) | 11 (29.7) | 13 (35.1) | 13 (35.1) | 7 (36.8) | 4 (21.1) | 8 (42.1) |
| Amoxicillin-Clavulanate (30µg) | 10 (27.0) | 4 (11.8) | 23 (62.1) | 4 (21.1) | 3 (15.8) | 12 (63.2) |
| Gentamicin (10µg) | 16 (43.2) | 2 (5.4) | 19 (51.4) | 8 (42.1) | 3 (15.8) | 8 (42.1) |
| Co-trimoxazole (1.25/23.75µg) | 15 (40.5) | 7 (18.9) | 15 (40.5) | 9 (47.4) | 7 (36.8) | 3 (15.8) |
| Cefoxitin (30µg) | 11 (29.7) | 0 | 26 (70.3) | | | |

S = Sensitive; I = Intermediate; R = Resistance

Comparing the antibiotic susceptibility of the Gram-positive and Gram-negative isolates, highest susceptibility was observed to gentamicin (43.2%) and cotrimoxazole (47.4%) by Gram-positive and Gram-negative isolates respectively (Table 3), although *E. coli* was highly susceptible to gentamicin (71.4%) (Table 4). Out of the 20 *S. aureus* isolates, 12 (60%) were phenotypically methicillin-resistant *S. aureus* (MRSA). The result of the antibiotic susceptibility of the MRSA isolates showed that 1 (8.3%) was resistant to all the antibiotics tested (pan-drug resistant). The MRSA isolates were highly resistant to all the beta lactam antibiotics tested

(Table 5). The resistant pattern of the Gram-negative isolates showed that 11 (57.9%) were multidrug resistant, 3 (15.8%) were extensively resistant while 1 (5.3%) was resistant to all the antibiotics tested.

## Discussion:

Bacterial contamination of fruits and vegetables is a potential source of foodborne infection which is of public health concern. In this study all the Date fruits sampled were highly contaminated. The results of the total bacteria viable count show the level of contamination in the sampled fruits to be higher than the allowed limit of not greater than $10^4$ CFU/g (19). The observed bacterial contamination may be from handling, packaging and the environment. The vendors usually use bare hands to pack the fruits into polythene bags before selling. In some places, the fruits are measured directly for sale using milk tins, all of which can contribute to the high total bacteria viable count.

The total bacteria viable count reported in our study agrees with reports of previous studies. Raimi (4) reported similar bacterial load of $4x10^5$ to $2x10^6$ CFU/ml from Date fruits purchased in Owode market Offa, Kwara State, Nigeria, but lower bacterial load was reported by Umar et al., (20) and Aleid et al., (3) from Date fruits sold in Katsina metropolis Nigeria and in Saudi respectively. High coliform count may be an indication of faecal contamination. The values are higher than $10^2$ (CFU/g) coliform limit specified by the Public Health Laboratory Services of Saudi Arabia (19,21). Some of the fruits for sale are kept openly in bowls and wheelbarrows in the open market where they are exposed to possible contamination by houseflies. Housefly can, through its vomits or excrements, transmit *E. coli* that causes diarrhoea or other pathogens that cause shigellosis, typhoid fever and cholera (22). Consuming such contaminated fruits constitute a potential health hazard to the society.

In comparison with other studies on microbiological assessment of Date fruits, lower coliform counts were reported (20,23) and the absence of coliform bacteria and *E. coli* was observed in fresh Dates samples in Dhaka city (24). The highest bacteria and coliform count observed from the samples from Ungwa Rimi market might be an indication of the poor state of hygiene of the vendors in that market compared with other markets.

Table 4: Antibiotic susceptibility of Gram-negative bacterial isolates of Date fruits in Kaduna, Nigeria

| **Antibiotics** | ***Proteus mirabilis* (n=4)** | | | ***Escherichia coli* (n=7)** | | | ***Enterobacter* spp (n=5)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **% S** | **% I** | **% R** | **% S** | **% I** | **% R** | **% S** | **% I** | **% R** |
| Tetracycline (30µg) | 50 | 0 | 50 | 28.6 | 14.3 | 57.1 | 60 | 20 | 20 |
| Ceftriaxone (30µg) | 50 | 0 | 50 | 57.1 | 0 | 42.9 | 20 | 40 | 40 |
| Ciprofloxacin (5µg) | 25 | 25 | 50 | 14.3 | 85.7 | 0 | 0 | 80 | 20 |
| Chloramphenicol (30µg) | 25 | 25 | 50 | 28.6 | 14.3 | 57.1 | 80 | 0 | 20 |
| Amoxicillin-Clavulanate (30µg) | 25 | 25 | 50 | 28.6 | 0 | 71.4 | 20 | 40 | 40 |
| Gentamicin (30µg) | 25 | 0 | 75 | 71.4 | 0 | 28.6 | 20 | 20 | 60 |
| Co-trimoxazole (1.25/23.75µg) | 50 | 25 | 25 | 57.1 | 42.9 | 0 | 60 | 20 | 20 |

S = Sensitive; I = Intermediate; R = Resistance

Table 5: Antibiotic resistance of MRSA isolates from Date fruits in Kaduna, Nigeria

| **Antibiotics** | **Resistant n (%)** | **Sensitive n (%)** |
|---|---|---|
| Tetracycline (30µg) | 11 (91.7) | 1 (8.3) |
| Ceftriaxone (30µg) | 8 (66.7) | 4 (33.3) |
| Ciprofloxacin (5µg) | 11 (91.7) | 1 (8.3) |
| Erythromycin (15µg) | 9 (75.0) | 3 (25.0) |
| Chloramphenicol (10µg) | 8 (66.7) | 4 (33.3) |
| Amoxicillin-Clavulanate (30µg) | 11 (91.7) | 1 (8.3) |
| Gentamicin (30µg) | 6 (50.0) | 6 (50.0) |
| Cotrimoxazole (1.25/23.75µg) | 7 (58.3) | 5 (41.7) |

The pathogenic organisms isolated from the Date fruits in this study included *S. aureus, E. coli, Bacillus subtilis, Streptococcus* spp*, Enterobacter* spp*, S.* Typhi and *P. mirabilis*. *S. aureus* was the most frequent isolate, which is usually carried transiently in the anterior nares of about 30% healthy individuals and about 20% individuals have it on their skin. Through direct tissue invasion, *S. aureus* can cause skin infections, pneumonia, endocarditis, osteomyelitis and infectious arthritis. In a related study (4), similar microorganisms were isolated from Date fruits purchased from Owode market in Offa, Nigeria while *S. aureus, Klebsiella* and *Bacillus* spp were isolated from Date fruits in Ado Ekiti and Akure, Nigeria (25). Each of these isolated organisms are potentially hazardous to human health. Although *E. coli* may be a harmless commensal flora of the gastrointestinal tract, it can cause relatively mild diarrhoea but some *E. coli* pathotypes can cause severe stomach cramps, bloody diarrhoea and vomiting, and extra-intestinal infections such as urinary tract infection, blood stream infections and neonatal meningitis. *Salmonella* Typhi is the causative organism of typhoid fever, which is usually acquired from contaminated food and water (26). Bacteremia, endocarditis, pneumonia and septicemia have also been attributed to *B. subtilis.*

The samples of the Date fruits used in this study were obtained from open market, implying the possibility of cross-transmission of multi-drug resistant bacterial pathogens, with gentamicin and co-trimoxazole being the only tested antibiotics, demonstrating some *in vitro* anti-microbial activity. The issue of antimicrobial resistance has become a great concern globally. Self-medication, purchase of antibiotics over the counter without prescription, proliferation of un-registered patent medicine stores and unrestricted sales of drugs including antibiotics in the open market are all possible factors responsible for increased rate of antimicrobial resistance.

## Conclusion:

The Date palm fruits sampled in this study were all contaminated by bacteria pathogens above the official level considered fit for human consumption. High level of antibiotic resistance was also observed among the bacterial isolates, with gentamicin and co-trimoxazole being the only antibiotics demonstrating *in vitro* anti-bacterial actions against the pathogens. It is recommended that there should be increased awareness among the fruit vendors on good personal hygiene, the dangers of consuming contaminated fruits among the general public. Also, increased advocacies about the implications of antibacterial resistance and the methods of prevention in the society are highly recommended.

## Contributions of authors:

AFO conceived the idea, designed the study, and wrote the initial manuscript; DSY was involved in sample collection, and MTD was involved in data analysis. All the authors approved the final manuscript.

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## Conflict of interest:

Authors declared no conflict of interest.

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