Original article

Antibiotic resistance of *Bacillus* species isolated from hawked ‘suya’ meat sold in Kaduna metropolis, North-Western Nigeria

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**Abstract**

**Background:** Food poisoning and antibiotic resistance among bacterial community has become a serious global challenge in the treatment of infections cause by pathogens. This research work was carried out to determine the occurrence and antibiotic resistance of *Bacillus* species in 200 hawked ‘suya’ meat sold in Kaduna metropolis. **Methods:** *Bacillus* species were isolated using a selective medium and biochemical tests. The isolates were further identified using Microgen Bacillus ID identification kit and the antibiotic resistance pattern was determined using single disc diffusion technique on Mueller-Hinton agar. **Results:** The total percentage occurrence of *Bacillus* species in the metropolis was 34.0% (68/200) with *Bacillus cereus* having the highest occurrence of 39.7% (27/68) and *Bacillus lentus* having the lowest occurrence of 1.5% (1/68). The multiple antibiotic resistance (MAR) index of the *Bacillus* species indicates that 72.50% (29/40), 50.0% (29/58), 47.3% (29/58) of *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* respectively had MAR index above 0.20 significant level of MAR of resistant pathogens. **Conclusion:** The presence of *Bacillus* species in the suya gives a warning signal for possible occurrence of foodborne infections and capable of producing outbreak of food poisoning. The multiple antibiotic resistance of *Bacillus* species calls for concern.

**Introduction**

In the globe today, different countries traditionally processed meat into various meat products [1]. In Nigeria, one of the meat products mostly consumed is suya. “Suya” is a traditional meat product gotten from boneless meat usually suya is made from beef, ram and goat meat which is hang on stick and spiced or marinated in groundnut cake, salt, vegetable oil and other flavours followed by roasting around a glowing charcoal fire [2]. Suya originated from the northern parts of Nigeria, it has permeated Nigerian society being affordable for all and available everywhere [3]. It has become very popular as a street delicacy in several countries, in parts of Nigeria and generally in West Africa [3].

However, the preparation process is usually carried out under unhygienic conditions and the risk of contamination is very high [4,2]. Most suya are often displayed in Nigeria markets under poor hygienic conditions and hence contaminated by various microorganisms [1]. Most of the suya are vended by the streets on uncovered trays where dusts containing microbes are raised by vehicles or humans’ activities and contaminate the meat product [3].
Consumption of food that contains *Bacillus* species especially *Bacillus cereus* may result in food poisoning through consumption of food containing pre-formed toxin or toxins produced by these bacteria in the human gut [5,6]. The *B. cereus* groups which are known pathogens or opportunistic pathogens to humans are closely related to *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus mycoides* [6].

Antimicrobial resistance is a growing problem around the world and is associated with increasing mortality and medical costs. This study therefore, sought to assess the occurrence and determination of antibiotic pattern of *Bacillus* species in suya which can be of public health importance [7, 6].

**Materials and Methods**

**Food samples**

A total of 200 suya samples were collected from hawkers and 50 samples were apportioned evenly to the four different markets and were purchased randomly. All the samples were collected during dry season and transported in a sampling box containing ice pack to National Research Institute for Chemical Technology, Zaria for microbial analysis.

**Sample preparation, isolation and phenotypic characterization of *Bacillus* species**

Twenty-five gram (25g) of homogenised food sample was inoculated in 225 ml of buffered bacteriological peptone water and incubated for 24 h at ambient temperature [8]. A loop full of the culture from the enrichment broth was sub-cultured on mannitol egg yolk polymyxin agar plates and incubated at 37°C for 24 h. Typical colonies of *Bacillus* species were identified by biochemical characterization that includes Gram staining, motility, haemolysis, Voges-Prokauer, oxidase, methyl red, nitrate reduction, citrate utilization, indole, coagulase, and urease tests as suggested by Adekanmi et al. [1]. All the isolates were further confirmed using a Microgen® Bacillus ID identification kit (Microgen Bioproducts, U.K.).

**Antibiotic susceptibility test**

The antibiotic susceptibility pattern was determined using Kirby-Bauer-NCCLS modified single disc diffusion technique on Mueller-Hinton agar [6]. Single antibiotic disc such as ampicillin (10µg), vancomycin (30µg), tetracycline (30µg), clindamycin (2µg), erythromycin (15µg), ciprofloxacin (5µg), penicillin G (10µg), gentamycin (10µg), amoxicillin/clavulate (30µg), chloramphenicol (30µg), cefoxitin (30µg) and oxacillin (1µg) Oxoid, England was used to determine the antibiotic susceptibility of the isolates. The zones of inhibitions were measured and the results were interpreted using the guideline from CLSI [9].

**Determination of multiple antibiotics resistance (MAR) index**

Multiple antibiotics resistance determined using the formula MAR = X/Y where X is the number of antibiotics to which the test isolates displayed resistance to and Y is the total number of antibiotics to which the test organism has been evaluated for sensitivity [10].

**Results**

The phenotypic characterization of the *Bacillus* species using microgen identification system as in Table 1 showed that the percentage identification of *Bacillus cereus* group ranged from 97.88 to 99.99%, more of the isolates were identified at 99.88% and 99.99%. *Bacillus megaterium* were mostly identified at 99.99%. *Bacillus subtilis* were identified at 99.93 and 99.68%.

The total occurrence of *Bacillus* species in the four sampling locations was 34.0% (68/200). The total occurrence of *Bacillus subtilis* from the four locations was 45.6% (31/68) and the total percentage occurrence of *Bacillus cereus* was 39.7% (27/68). *Bacillus megaterium* and *Bacillus lentus* had total occurrence of 13.2% (9/68) and 1.5% (1/68) respectively as presented in figure (1). The total number of *Bacillus* species isolated from each of the selected market showed that 48.0% (24/50) were from Kwo Market, 18% (9/50) from Kakuri Market, 24.0% (12/50) from Ungwan Boro Market and 46.0% (23/50) from Tudun wada Market.

The antibiotic susceptibility of *Bacillus* species showed that all the isolates of *Bacillus* species were 100% susceptible to ampicillin, oxacillin, cefoxitin, penicillin and erythromycin (Table 2). One of the isolates (i.e. *Bacillus lentus*) was susceptible to all the antibiotics selected for the research.

The antibiotic resistance pattern of the resistant isolates as presented in table (3) showed that there was much disparity of wide range in...
resistance of Bacillus species to the antibiotics selected for the research. A total of twenty isolates of Bacillus species were resistant to four classes of antibiotics, and a total of sixty-four isolates were resistant to three classes of antibiotics. The total MAR index of the Bacillus species indicates that 72.50% (29/40), 50.0% (29/58), 47.3% (29/58) of Bacillus cereus, Bacillus subtilis and Bacillus megaterium respectively had MAR index above 0.20 significant level of MAR of resistance pathogens (Table 4).

Table 1. Phenotypic characterization of Bacillus species using Microgen identification kit

<table>
<thead>
<tr>
<th>S/N</th>
<th>Organism (No.)</th>
<th>Group(1)</th>
<th>Group(2)</th>
<th>Group(3)</th>
<th>Group(4)</th>
<th>Probability (%)</th>
<th>Inference (Organism)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus cereus</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>71.13</td>
<td>B. cereus Group(8)</td>
</tr>
<tr>
<td>2</td>
<td>B. subtilis</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>69.21</td>
<td>B. cereus Group(8)</td>
</tr>
</tbody>
</table>


Table 2. Antibiotic susceptibility of Bacillus species isolated from the hawked suya (roasted) meat

<table>
<thead>
<tr>
<th>S/N</th>
<th>Antibiotics</th>
<th>Bacillus cereus (n = 27)</th>
<th>Bacillus megaterium (n=9)</th>
<th>Bacillus subtilis (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R(%)</td>
<td>I(%)</td>
<td>S(%)</td>
<td>R(%)</td>
</tr>
<tr>
<td>1</td>
<td>0(0)</td>
<td>0(0)</td>
<td>27(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>0(0)</td>
<td>0(0)</td>
<td>27(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>18(67)</td>
<td>5(19)</td>
<td>4(15)</td>
<td>5(56)</td>
</tr>
<tr>
<td>4</td>
<td>7(26)</td>
<td>-</td>
<td>20(74)</td>
<td>3(33)</td>
</tr>
<tr>
<td>5</td>
<td>16(59)</td>
<td>-</td>
<td>11(41)</td>
<td>7(78)</td>
</tr>
<tr>
<td>6</td>
<td>15(56)</td>
<td>6(22)</td>
<td>6(22)</td>
<td>5(56)</td>
</tr>
<tr>
<td>7</td>
<td>23(85)</td>
<td>-</td>
<td>4(15)</td>
<td>8(89)</td>
</tr>
<tr>
<td>8</td>
<td>24(89)</td>
<td>1(4)</td>
<td>2(7)</td>
<td>5(56)</td>
</tr>
<tr>
<td>9</td>
<td>19(70)</td>
<td>3(11)</td>
<td>5(19)</td>
<td>4(44)</td>
</tr>
<tr>
<td>10</td>
<td>21(78)</td>
<td>-</td>
<td>6(22)</td>
<td>6(67)</td>
</tr>
<tr>
<td>11</td>
<td>15(56)</td>
<td>5(19)</td>
<td>7(26)</td>
<td>4(44)</td>
</tr>
<tr>
<td>12</td>
<td>27(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>8(89)</td>
</tr>
</tbody>
</table>
Table 3. Phenotypic antibiotic resistance patterns of the *Bacillus* species.

<table>
<thead>
<tr>
<th>S/N</th>
<th><em>Bacillus cereus</em> Resistance pattern</th>
<th>Frequency</th>
<th><em>Bacillus megaterium</em> Resistance pattern</th>
<th>Frequency</th>
<th><em>Bacillus subtilis</em> Resistance pattern</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>6</td>
<td>C</td>
<td>3</td>
<td>E</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>TE</td>
<td>4</td>
<td>OX</td>
<td>2</td>
<td>DA</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>P</td>
<td>7</td>
<td>AMP</td>
<td>3</td>
<td>TE</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>AMP</td>
<td>8</td>
<td>P</td>
<td>3</td>
<td>P</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>C, CIP</td>
<td>3</td>
<td>FOX</td>
<td>3</td>
<td>AMP</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>TE, OX</td>
<td>4</td>
<td>E, CN</td>
<td>2</td>
<td>FOX, C</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>FOX, CN</td>
<td>1</td>
<td>C, OX, AMP</td>
<td>1</td>
<td>CN, OX</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>E, TE, P</td>
<td>4</td>
<td>AMC, TE, CIP</td>
<td>3</td>
<td>AMC, E</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>OX, CN, CIP</td>
<td>2</td>
<td>OX, TE, C, CN</td>
<td>1</td>
<td>TE, AMP</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>AMP, FOX, C</td>
<td>3</td>
<td>AMP, E, P, FOX</td>
<td>3</td>
<td>CIP, P, E</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>P, CN, E, CIP</td>
<td>3</td>
<td>CN, OX, P, AMP</td>
<td>1</td>
<td>E, CIP, P</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>AMC, E, TE, P</td>
<td>4</td>
<td>FOX, CIP, P, TE, OX</td>
<td>1</td>
<td>TE, AMC, FOX</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>OX, FOX, CN, CIP</td>
<td>7</td>
<td>C, CIP, P, OX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CN, C, OX, TE, FOX</td>
<td>5</td>
<td>FOX, AMP, C, CN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>OX, CN, AMP, C, E</td>
<td>1</td>
<td>CN, TE, AMC, OX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>AMC, AMP, TE, P, C</td>
<td>3</td>
<td>CIP, OX, TE, C, AMC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KEY: E = Erythromycin, TE = Tetracycline, P = Penicillin, C = Chloramphenicol, OX = Oxacillin, AMP = Ampicillin, FOX = Cefoxitin, CN = Gentamycin, AMC = Ampoxicillin/Clavulate, CIP = Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Multiple antibiotic resistant (MAR) indices of the isolates of *Bacillus* species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of multidrug resistant isolates</th>
<th>Classes of antibiotics to which isolates were resistant to</th>
<th>MAR index</th>
<th>Total MAR index of isolates greater than 0.20 significant value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>11</td>
<td>2</td>
<td>0.17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3</td>
<td>0.25</td>
<td>72.5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>29</td>
<td>2</td>
<td>0.17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>3</td>
<td>0.25</td>
<td>47.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>29</td>
<td>2</td>
<td>0.17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3</td>
<td>0.25</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>
The presence of *Bacillus* species in the suya samples may be due to contamination from long exposure to air during hawking as reported by Adesoji *et al.* [11] and this air might have polluted with different types of microorganisms. The presence of *Bacillus cereus* is of public health importance because it is frequently associated with cases of food poisonings as reported by Agwa *et al.* [12] and Osman *et al.* [6]. This could be as results of toxins produced by the organism. Food poisoning caused by *Bacillus cereus* occurs year-round diffusely over a geographic area [6].

Suya may serve as a bed for multiplication of *Bacillus* species because of poor roasting and can result to be a major threat to human populations or public health as reported by Orogu and Oshilim [7]. Suya can serve as potential bed for multiplication of *Bacillus* species because it is rich in nitrogenous compounds (amino acids, peptides, proteins), minerals and other growth factors as reported by El-Hersh *et al.* [13]. In addition, they have some fermentable carbohydrates, usually glycogen and keep favourable pH for growth of most microorganisms [14].

These constituents promote the growth and multiplication of various organisms in meat borne pathogens such as aerobic spore formers (e.g., *Bacillus* species) that may constitute public health hazards [15,16]. It is not surprising that *Bacillus* species were isolated from suya because these bacteria can survive heat during roasting; this is because *Bacillus* species can form spores to resist high temperature [8,1]. The spores can grow to vegetative form once there are favourable environmental conditions [15].

The presence of antibiotic-resistant *Bacillus* species in the food samples has an important health implication, since there is frequent and uncontrolled use of antibiotics in both veterinary and human medicine [17]. Antibiotic-resistance among pathogens usually interfere with effective treatment measures of diseases cause by the infectious agents [18].

Detection of multidrug resistant pathogenic bacteria in foods is therefore considered as a public health threat [19]. Excessive application of antibiotics in both human and veterinary medicine may lead to distribution of antibiotic-resistant pathogens in foods and environment. Multiple antibiotic resistances in *Bacillus* species might be attributed to antimicrobial selective pressure and gene transfer mechanisms between and among *Bacillus* species [20].

Key: U/Boro = Ungwan Boro, T/Wada = Tudun Wada

**Figure 1.** Occurrence of *Bacillus* species in hawked suya from the selected market.
Multiple antibiotic resistance index value lower than 0.20 indicates that the Bacillus species might have originated from a lower risk source in which the antibiotics are seldom or never used [21]. Multiple antibiotic resistance index value higher than 0.20 indicates that the isolates of Bacillus species might have originated from the environment where there is indiscriminate use of the antibiotics. This calls for concern because it is an alert that the antibiotics might have been abused in environment where Bacillus species were isolated.

**Conclusion**

The overall occurrence of the Bacillus species in the food samples is 34.0% (68/200), Bacillus subtilis had highest occurrence of 45.6% (31/68) and Bacillus lentus had the least occurrence of 1.5% (1/68). Twenty (20) isolates of Bacillus species were resistant to four classes of antibiotics, and sixty-four (64) isolates were resistant to three classes of antibiotics. It is therefore, recommended that hawkers should ensure that their suya is well roasted and protected from contamination to avoid foodborne infections associated with Bacillus species.

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**Competing interests:** No competing interest declared

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