Antibiotic-resistant *Salmonella* Species in Pork on Display for Sale in Umuahia, Abia State, Nigeria

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**Abstract:** Seventy (70) samples of each of hoof, muscles, intestine, liver and tongue of pigs were collected from different abattoirs in Umuahia, Abia State of Nigeria. The samples were Pre-enriched in Peptone water of pH 7.2 followed by subculture onto solid media, and incubated at 37°C for 1 – 2 days. Suspected colonies were identified based on their morphological and biochemical characteristics. Out of a total of 16, hoof, muscles, intestine, Liver and Tongue samples, 12.50%, 50%, 25%, 37.5% and 50% were positive for *Salmonella* species respectively. *Salmonella* isolates from tongues and muscles were resistant to Nalidixic acid (30 µg/disk), Augumentin (30 µg/disk), and Tetracycline (30µg/disk). These observations are of Public Health Significance, and call for more research attention and public health Campaigns.

**Key words:** Salmonella, Ready-to-eat Porks, Salmonellosis, antibiotics, Resistance

**INTRODUCTION**

Bacterial foodborne diseases are among the most serious health problems affecting public Health and development worldwide. Industrialization, mass food production and human migration have disseminated and increased the incidence and severity of foodborne diseases all over the world[2].

*Salmonella* organisms are among the most common bacterial foodborne pathogens world wide, and have emerged as the second most common cause of bacterial human foodborne illness and a pathogen of Public health Concern[13]. Salmonellosis results from the ingestion of a variety of *Salmonella* serovars, particularly *Salmonella typhimurium*, and *Salmonella enteritidis*. Salmonellosis is characterized by septicaemia, acute and chronic enteritis[15].

In addition, it is profound to note that for an outbreak of Salmonellosis to occur, the food as a vehicle of transmission must be infected with a considerable number of the organisms[10]. *Salmonella* organisms are ubiquitous and gastrointestinal tracts remain its major ecological niche[9]. Although their primary habitat is the intestinal tract, the organism may be found in other parts of the body.

Contamination of parts of the body other than the intestine may be through contact with animals’ spleen, urine, and facees during slaughtering[24]. In United States, Jones and Co workers[15] discovered a *Salmonella*, prevalence rate 34% from Pork.

In Northern America, the tonsils, hoofs, tongues, salivary glands of Pigs have been shown to be infected by *Salmonella* spp[6]. Predisposing factors to *Salmonella* contamination of Pork, beef, Mutton etc have been documented to include poor farm hygiene, poor abattoir hygiene, and carrier status of Butchers and other handlers[1,8].

In relatively recent time, research reports have documented the use of antibacterial drugs to combat various diseases of pigs such as Mastitis.[13] The use of these antibacterial drugs has led to selection of antibiotics resistant strains of bacterial pathogens including *Salmonella* species[11]. In a survey in United Kingdom, 77% of the *Salmonella* species isolated from pigs was resistant to Aminiglycosides including Tetracycline[1].

The aims and objectives of this study were to survey the availability of *Salmonella* pathogens on pork, sold in Umuahia, monitor the susceptibility of the isolated pathogen to commonly used antibiotics in Nigeria, and profer measures towards enlightenment campaign against pork-borne Salmonellosis in Abia State, Nigeria.

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MATERIALS AND METHODS

Study Area and Population Study: The study area was Umuahia, Abia State of Nigeria. Umuahia is the capital city of Abia State. It is located between 4°29’N and 4°38’N (Latitude), and 7°33’E and 7°41’E (Longitude).

Most of the samples were collected from slaughter house in Ubakala in Umuahia south Local Government Area where majority of the meat consumed in Umuahia is slaughtered. Trading, civil service and farming are professions commonly practised in the study Area.

Sample Collection: Seventy (70) pig samples comprising of muscles (16), Hoof (16), Tongues (16), Liver (16) and Intestine (16) were collected for analysis with the aid of sterile blade and sterile containers. The samples were transported within 1-2 hours of collection to the Microbiology laboratory, Abia State University, Uturu- Nigeria.

Isolation of Salmonella Species: The examination of the Pork samples for detection of Salmonella species was partly based in the procedures of International Standard organization, ISO 6479: 1993 as was reported by Narang.[20]

25 grams of the pork samples were transferred in a glass ware containing 225ml of buffered peptone water (ISO 6579-1993), and thoroughly mixed. 120ml of the above pre-enrichment mixture was added to 100ml of Rappaport – vassilides broth (Fluka), and incubated for 18 – 24 hours at 37°C, for the purpose of selective Enrichment.

A loopful of the pre-enricned mixture in Rappaport-Vassilides medium was transferred to Salmonella - Shigella Agar (SSA) which was prepared using the manufacturer’s instruction. The streaking method was used in inoculating the microbial cells. The plates were incubated at 37°C ± 2°C for 24hrs.

Identification of Bacterial Isolates: Isolates were identified based on colony morphology, Gram reactions, and biochemical reactions. Gram negative rods which are methyl red positive, Voges-Proskauer negative, Hydrogen sulphide production positive, Urease negative, Indole Positive, and ferment glucose with gas production were referred as Salmonella species.[17]

Antimicrobial Susceptibility Testing: The antibiotic susceptibility of the isolates was determined by the disk diffusion method of clinical laboratory standard Institute[6] on Mueller-Hinton Agar (Antec). The following antibiotics (DIFCO) ampicillin (10µg/disk), Ofloxacin(30µg/disk), tarivid(30 µg/disk), Nalidixic acid(30 µg/disk), pefloxaclin(10 µg/disk), gentamycin (10 µg/disk), augumentin (30 µg/disk), ceporex(10 µg/disk), and tetracycline (30 µg/disk) were used.Inoculums of Salmonella species isolated from muscles and tongues were standardized by adjusting its density to equal the turbidity of Barium sulphate(BaSO₄) which is the 0.5 McFarland turbidity standard, and incubated at 37°C ± 2°C for 18hrs. The diameter of zone of clearance (including the diameter of the disk) was measured to the nearest whole number millimeter and interpreted using the specified standard of the CLSI[6].

RESULTS AND DISCUSSION

Findings of this study showed that the prevalence rated Salmonella species from pork studies were 12.50%, 50%, 25%, 37.5% and 50% for the hoof, muscles, intestine, liver, tongue respectively. (Table 1).

Isolates of Salmonella spp from muscles and tongue were used for the susceptibility studies. Isolates from muscles of the pork were 25%, 12.5%, 25%, 87.5%, 37.5% 75%, 50% 12.50% and 57.50% resistant to Ampicillin, Ofloxacin, Tarivid, Nalidixic acid Pefloxaclin, Gentamycin, Augumentin, Ceporex, Tetracycline respectively (Table 2). Isolates from the same muscles were 75%, 75%, 62.5%, 0%, 50%, 12.5% 37.5%, 87.5% and 12.5% susceptible to ampicillin, oflaxacin, tarivid, nalidixic acid, pefloxaclin, gentamycin, augumentin, ceporex and tetracycline respectively (Table 2). Isolates from tongue were 50%, 25%, 25%, 0%, 50%, 75%, 50% and 25% resistant to ampicillin, oflaxacin, nalidixic acid, pefloxaclin, gentamycin, augumentin, ceporex and tetracycline respectively (Table 2). In addition, the isolates from tongue were 50%, 25%, 50%, 0%, 75%, and 37.5% 12.5% 100% and 25% susceptible to the above antibiotics in the same order (Table 2).

The isolation of Salmonella organisms from all parts of the pork showed that all the parts are contaminated at different levels/prevalence rate. It is profound to acknowledge the observation that isolates from muscles and tongue had the highest prevalence rate of 50% each. The highest prevalence rate on tongue might be as a result of the fact the tongue comes in contact contaminated food more often than other parts. However, as the contaminated food passes through the esophagus down to intestine, the Salmonella antigens triggers off the production of antibodies against the antigens. This might result in considerable reduction of number of cells infecting the intact pig along the digestive tract. This antigen-antibody reaction might explain the reduction of numerical strength of the Salmonella spp down the intestinal tract. Similarly, in Denmark, Stege et al.[23]
Salmonellosis. Experts including medical sociologists should embark on public health campaigns against pork-associated infectious diseases. In addition, the emergence of tetracycline-resistant Salmonella spp is a big challenge. It is now clear that biosecurity measures, painstaking hygiene should be taken throughout the pig production and distribution channels. Hazard analyses include Critical Control Points (HACCP) procedures should be observed in the abattoir during the slaughter of the pigs and sales of pork.

Proper cooking of the pork by consumers is hereby recommended as measures to control the spread of pork-associated infectious diseases. Furthermore, Government, Non-governmental agencies, Organized private sectors, Public health experts including medical sociologists should embark on public health campaigns against pork-associated salmonellosis.

Isolates from both muscles and tongues showed considerable susceptibility to commonly used antibiotics. Meanwhile, it was observed that the resistance of the muscles isolates to nalidixic acid (87.5%), gentamycin (75%), and tetracycline (87%) was grossly high. The indiscriminate and widespread use of these antibiotics in veterinary medicine, coupled with the long period of time these drugs were available in Nigeria could account for the high resistance observed.

Isolates from tongue were also highly resistant to nalidixic acid (75%), augumentin (62.5%) and tetracycline (62.5%). The widespread and indiscriminate use of these antibiotics in the chemotherapy of pigs is responsible for the high resistance.

In addition, the emergence of tetracycline-resistant microbial pathogens from food products in Nigeria is in very high rates, and of considerable medical significance. The control of antibiotics resistance to Salmonella spp isolated from pork in Umuahia, Abia State and sales of pork.

Table 1: Prevalence rates of Salmonella spp in pork sold in Umuahia Abia State-Nigeria.

<table>
<thead>
<tr>
<th>Organ analysed</th>
<th>No of samples</th>
<th>No of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoof</td>
<td>16</td>
<td>2(12.50)</td>
</tr>
<tr>
<td>Muscles</td>
<td>16</td>
<td>8(50.00)</td>
</tr>
<tr>
<td>Intestines</td>
<td>16</td>
<td>4(25.00)</td>
</tr>
<tr>
<td>Liver</td>
<td>16</td>
<td>6(37.50)</td>
</tr>
<tr>
<td>Tongue</td>
<td>16</td>
<td>8(50.00)</td>
</tr>
</tbody>
</table>

Numbers in brackets are in percentages.

Table 2: Antibiotic resistance rates of Salmonella spp isolated from pork in Umuahia, Abia State.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Amount/disk</th>
<th>RESISTANCE</th>
<th>SUSCEPTIBILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MUSCLES N = 8</td>
<td>TONGUE N = 8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10µg</td>
<td>2(25)</td>
<td>4(50)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>30µg</td>
<td>1(12.5)</td>
<td>4(50)</td>
</tr>
<tr>
<td>Tarivid</td>
<td>30µg</td>
<td>2(25)</td>
<td>3(37.5)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30µg</td>
<td>7(87.5)</td>
<td>6(75)</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>10µg</td>
<td>3(37.5)</td>
<td>-0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10µg</td>
<td>6(75)</td>
<td>4(50)</td>
</tr>
<tr>
<td>Augumentin</td>
<td>30µg</td>
<td>4(50)</td>
<td>5(62.5)</td>
</tr>
<tr>
<td>Ceporex</td>
<td>10µg</td>
<td>1(12.5)</td>
<td>-0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30µg</td>
<td>7(87.5)</td>
<td>5(62.5)</td>
</tr>
</tbody>
</table>

Number in brackets are in percentages.
Government agencies such as veterinary organizations of Nigeria should establish well-equipped laboratories and deploy veterinary, Public Health Personnel in all abattoirs in Nigeria.

REFERENCES

6. CLSI., 2005. Performance standards for antimicrobial susceptibility testing; fifteenth Informational supplement, m100- S15, 25(1). Clinical Laboratory Standards Institute, Wayne PA, USA.