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OCCURRENCE AND ANTIBIOGRAM OF *Salmonella enteric serovar enteritides* AND *Proteus mirabilis* IN OFFALS OF SLAUGHTERED CATTLE IN ABIA STATE, NIGERIA

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ABSTRACT

This study was conducted to ascertain the presence of Salmonella entericaserovarenteritidis and Proteus mirabilis in the offal of slaughtered cattle and to determine their in vitro susceptibility to antibiotics. Two hundred and eight samples from the mesenteric lymph nodes (52), rumen (52), jejunum (52) and colon (52) were used in the study. Samples from these tissues/organs were inoculated into Selenite F broth and incubated at 37ºC. Identification was based on cultural characteristics and biochemical tests. Salmonella grouping antisera D was used to confirm the presence of Salmonella entericaserovarenteritidis. Proteus mirabilis was isolated from 105 of the samples; 15 from the mesenteric lymph node, 45 from the colon, 25 from the jejunum and 20 from the rumen. None of the samples yielded Salmonella entericaserovarenteritidis. Proteus mirabilis was susceptible to all the antibiotics used except Ampicillin to which 49% of the isolates were resistant. The isolation of Proteus mirabilis from offal of slaughter cattle is of public health importance and highlights the need for enlightenment campaign for consumers, abattoir and health workers on the potential risk of auto and cross infection to animal and human populations.

Keywords: *Salmonella enterica serovar enteritidis*, *Proteus mirabilis*, offal, cattle, antibiogram.

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INTRODUCTION

Meat and meat products are considered as excellent sources of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron [1]. They are considered as ideal media for the growth of many organisms because of the high moisture, high percentages of nitrogenous compounds of various degrees of complexity, high content of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) and a favorable pH for most of the enteric microorganisms [2]. Contamination of raw meat is one of the main sources of food borne illnesses [3,4]. Unfortunately, the presence of microbial contaminants in meat and edible offal cannot be detected visually [5], which raise both the risks associated with food borne pathogens and the incidence of human diseases [6]. Microbial contamination

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of raw meat starts during slaughter, when the carcass become contaminated with microorganisms residing on external surfaces, the gastrointestinal tract and lymph nodes of the animal, and in the environment [7]. Tissues from healthy animals are sterile. However it has been pointed that during slaughter, dressing and cutting, contamination with microorganisms occur chiefly from the exterior of the animal and its intestinal tract as well as from the knives, clothes, air, carts and equipment in the slaughter environment [7].

The gram negative bacteria account for approximately 69% of the cases of bacterial food borne diseases [8].*Salmonella* species is among the microorganisms most frequently associated with food borne outbreaks of illness [9]. Furthermore, it remains a leading cause of food poisoning in the developed world, resulting in multiple cases of absenteeism from school, work etc., illness, hospitalization and death each year [10]. *Salmonella* species can be frequently found in sewage, sea, and river water and can contaminate a variety of foods [11]. Environmental pathogenic contaminant such as *Proteus mirabilis* is capable of growth in low nutrient conditions [12]. Thus, this bacterium is able to grow in water distribution systems [13], in manure and soil, where it plays an important role in decomposing organic matter of animal origin [14]. *Proteus* was recovered from hides and wool surfaces within the abattoir, from carcasses, butchered meat as well as from environmental samples in meat processing plants [15,16].

In general, knowledge of antibiograms help to guide the clinician and pharmacist in selecting the best empiric antimicrobial treatment in the event of pending microbiology culture and susceptibility results [17].

Given the fact that infections caused by *Salmonella enterica serovar enteritidis*(food-borne poisoning) and *Proteus mirabilis* (urinary tract infection) are not easily detected or diagnosed, there is need for studies to identify the factors associated with their occurrence in abattoirs and the possible intervention practices for their control.

MATERIAL AND METHODS

Sample Collection

Samples were collected from randomly selected trade cattle presented for slaughter at the Umuahia slaughter house located at Ubakala, a community in Umuahia South Local Government Area of Abia State. Samples were collected from the rumen, jejunum, colon and mesenteric lymph nodes of randomly selected cattle during slaughter and evisceration for a period of four months (May – August). A grand total of 208 samples were collected; 128 by using a sterile swab stick from the selected organs and 80 by cutting a piece of the organ. In all, 52 samples were collected from each of mesenteric lymph node, rumen, jejunum and colon respectively. Samples were placed in ice packs and transported immediate to the Department of Veterinary Microbiology laboratory for analysis.

Sample Processing

Bacterial Isolation

Each sample was first inoculated into Selenite F broth by direct inoculation of the sterile swab and tissue samples into the sample bottles containing Selenite F broth and incubated at 37^{O} C for 24 hours. After this, a loop-full of the inoculum was plated out on MacConkey agar and *Salmonella-Shigella* agar plates. These plates were then incubated at 37^{O}C for 24hours. Growth that occurred in *Salmonella*-Shigella agar

was examined. Those that showed black colonies due to hydrogen sulphide production were further inoculated into urea agar slopes.

Bacteria Identification

Colonies in the selective media; *Salmonella-Shigella* Agar and Mac Conkey Agar were identified based on cultural characteristics. The biochemical tests carried out on the isolates for their identifications include urease test and indole test. Indole test was carried out to differentiate *Proteus mirabilis* from *Proteus vulgaris*.

Urease Test

The surface of the urea agar slant was streaked with a portion of a well – isolated colony. The cap was loosely covered and the tube incubated at 37^0 C for 48 hours. It was examined for the development of a pink colour.

Indole Test

Colonies positive to the Urease test were further inoculated aseptically by taking the growth from 24 hrs cultures into sterilized test tubes containing 4 ml of tryptophan broth. The tubes were incubated at 37^0 C for 24 hours. Thereafter, 0.5 ml of Kovac's reagent was added to the broth culture. The presence or absence of a pink to red–violet colouring in the surface alcohol layer of the broth was observed to confirm the organisms to be positive (*Proteus vulgaris*) or negative (*Proteus mirabilis*).

Serotyping for *Salmonella*

Salmonella grouping was conducted using group DO *Salmonella* antisera. The colonies suspected to be *Salmonella* organisms were emulsified in a drop of sterile normal saline on a slide. Then a drop of group DO *Salmonella* antisera was added. The mixture was rocked and examined for agglutination. These group D antisera contain the *Salmonella entertidis* antibodies.

Antibiotic Sensitivity Test

The antibiotic susceptibility tests were performed by disc diffusions method as describe by Kirby Bauer techniques [18] in standard antibiotic disk [19]. The multi-discs (oxoid) consists of 10 antibiotics namely: Ampicillin® (PN), 30 μg; Ofoxacine® (OFX), 10 μg; Streptomycine® (S), 30 μg; Ceporex® (CEP), 10 μg; Cotrimoxazole® (SXT), 30 μg; Gentamycin® (CN), 10 μg; Nalidixic acid® (NA) 30 μg; Augumentin® (AU), 30 μg; Ciprofloxacin® (CPX), 10 μg; and Pepflacin (PEF), 10 μg.

Each isolate, 10^5 CFU/ml in 0.1 ml as determined by Kirby-Bauer Disc Diffusion method (CLSI, 2008) was first poured on nutrient agar. Then the disks were placed on the nutrient agar plates and the plates incubated at 37^OC for 24 hours. Zones of inhibition were recorded and zones with more than 10 mm diameter were recorded as sensitive.

RESULTS

Table 1 shows the biochemical and serological properties of the organisms isolated from the various samples that were examined. The results obtained showed that out of 52 samples from the mesenteric lymph nodes, 29 isolates were lactose fermenters, 25 were non-lactose fermenters on Mac Conkey Agar, 22 were hydrogen sulphide producers on *Salmonella*-Shigella Agar which were sub-cultured unto urea

agar slopes, 21 were positive and 1 was negative. 0ut of the 21 positive Urease isolates 15 were Indole negative, however the negative Urease isolates were negative to the *Salmonella* grouping Antisera.

Out of 52 samples from the colon 1 isolate was lactose fermenter, 40 were non-lactose fermenters on Mac Conkey Agar, 60 isolates were hydrogen sulphide producers on *Salmonella*-Shigella Agar which were sub-cultured unto urea agar slopes, 48 were positive and 12 were negative (Table 2). Out of the 48 positive Urease isolates 45 were Indole negative, however the negative Urease isolates were negative to the *Salmonella* grouping Antisera.

Table 1. Number of isolates showing some biochemical and serological properties in samples collected from the mesenteric lymph nodes, colon, jejunum and rumen.

Test	MLN	Colon	Jejunum	Rumen
Lactose fermenters	29	1	15	17
Non-lactose fermenters	25	40	38	30
Hydrogen sulphide producers	22	60	44	41
Urease positive	15	45	25	20
Urease negative	1	12	9	7
Grouping antisera positive	0	0	0	0
Indole negative	15	45	25	20

* MLN = Mesenteric lymph nodes

Table 2. Numbers of isolates from organs examined.

Number of Isolates	MLN	Colon	Jejunum	Rumen
Proteus mirabilis	15	45	25	20
Salmonella enteritidis	0	0	0	0
Others	6	28	20	12

MLN = Mesenteric lymph nodes

Out of 52 samples gotten from the Jejunum 15 isolates were lactose fermenters, 38 were non-lactose fermenters on Mac Conkey Agar, 44 were hydrogen sulphide producers on *Salmonella*Shigella Agar which were sub-cultured unto urea agar slopes, 35 were positive and 9 was negative (Table 3). Out of the 35 positive Urease isolates 25 were indole negative, however the negative Urease isolates were negative to the *Salmonella* grouping Antisera.

Results from examination of samples collected from the rumen show that out of 52 samples, 17 isolates were lactose fermenters, 30 were non-lactose fermenters on Mac Conkey Agar, 41 were hydrogen sulphide producers on *Salmonella-Shigella* Agar which were sub-cultured unto urea agar slopes, 32 were positive and 7 was negative. Out of the 32 positive Urease isolates, 20 were Indole negative, however the negative Urease isolates were negative to the *Salmonella* grouping Antisera.

The results show that a total of 21 *Proteus mirabilis* were isolated from the mesenteric lymphnodes, 48 *Proteus mirabilis* isolated from the colon, 35 *Proteus mirabilis* isolated from the jejunum and 32 *Proteus mirabilis* isolated from the rumen (Table 2). However there was no confirmed *Salmonella enteritidis* isolate from any of the target organs. The others were not confirmed to be *Proteus mirabilis or Salmonella enteritidis*. Further research was not carried out to identify those isolates to specie level however they were suspected to be *Enterobacteriaceae* organisms.

As shown in Table 1, there were more lactose fermenters in the mesenteric lymphnode than in other organ samples, it however had the lowest number of isolates gotten for the non-lactose fermenters, hydrogen sulphide producers, urea test and indole tests. The colon, however, had a predominantly higher amount of non- lactose fermenters, hydrogen sulphide producers, positive urea isolates and consequently indole negatives. The jejunum and the Rumen isolates numbers were almost the same in all the above tests.

DISCUSSION

Knowledge about the normal microbial populations in different organs has been recognized as important factors in elucidating the pathophysiology of disease in human beings and animals. The resident organisms are normally harmless, but with the presence of predisposing factors such as trauma or concurrent infections, some of these organisms may become potential pathogens, multiplying and causing bacterial diseases [20].

In the present study, out of 208 samples collected from the offal of slaughtered cattle, no *Salmonella enterica* subspecies *enteritidis* was isolated. This result does not indicate absence of *Salmonella enteric* subspecies *enteritidis* but might be due to low sensitivity and specificity to the methods used for isolation since other species of *Salmonella* must have overgrown the *enteritidis* species. These similar results have been published by Duffy *et al.,* [21] from minced meat collected from different localities in Assiuit city. This is also in line with other reports from several European countries which showed that *Salmonella* prevalence in samples from the offal of slaughtered cattle ranged from 0.0% to 3.0% with a mean prevalence of 1.1% [22].

The absence of confirmed *Salmonella enterica* subspecies *enteritidis* organisms in the offal of cattle could also be as a result of suppressed effects of antibiotic treatment or abuse by farmers. Similar reports were confirmed by Amaechi [23] who advocated for farmers to observe appropriate withdrawal periods after treatment with antibiotics.

Proteus mirabilis is one of the most common gram-negative pathogens encountered in clinical specimens and can cause a variety of community illnesses, including urinary tract infections, wound infections, bloodstream infections (BSI) and less commonly lung congestion [24]**.** *Proteus species are said to be* widespread in nature as they can be found in polluted water, soil, sewage, gardens and manure [25]. This is indicative that the colon carries a higher amount of *Proteus mirabilis organisms.*

In this study, the presence of *Proteus mirabilis i*n th*e* offal of slaughtered cattle was confirmed using indole test. *Proteus mirabilis* is indole negative. This is in line with studies conducted by Gus Gonzalez *et al*. [25]. These isolates in this study were predominantly from the colon. This may be due to the physiological composition of the colon as the colon serves as the site for microbial fermentation and

Table 3.Antibiotic sensitivity of *Proteus mirabilis* isolates from the organs.

Antibiotics/Organs	Sensitive (%)	Resistant (%)
Messenteric lymph nodes		
Ampicillin (30 µg)	33	67
Ceporex (10 µg)	86	14
Oxfoxacine (10 µg)	86	14
Nalidixic acid (30 µg)	60	40
Pepflacin (10 µg)	93	7
Gentamycin (10 µg)	93	7
Augmentin (30 µg)	100	0
Ciprofloxacin (10 µg)	100	0
Cotrimoxazole (30 µg)	67	33
Streptomycine (30 µg)	100	0
Colon		
Ampicillin (30 µg)	24	76
Ceporex (10 µg)	56	44
Oxfoxacine (10 µg)	42	58
Nalidixic acid (30 µg)	69	31
Pepflacin (10 µg)	93	7
Gentamycin (10 µg)	71	29
Augmentin (30 µg)	67	33
Ciprofloxacin (10 µg)	87	13
Cotrimoxazole (30 µg)	91	9
Streptomycine (30 µg)	100	0
Jejunum		
Ampicillin (30 µg)	60	40
Ceporex (10 µg)	100	0
Oxfoxacine (10 µg)	100	0
Nalidixic acid (30 µg)	76	24
Pepflacin (10 µg)	100	0
Gentamycin (10 µg)	100	0
Augmentin (30 µg)	88	12
Ciprofloxacin (10 µg)	92	8
Cotrimoxazole (30 µg)	92	8
Streptomycine (30 µg)	100	0
Rumen		
Ampicillin (30 µg)	50	50
Ceporex (10 µg)	85	15
Oxfoxacine (10 µg)	60	40
Nalidixic acid (30 µg)	45	55
Pepflacin (10 µg)	80	20
Gentamycin (10 µg)	85	15
Augmentin (30 µg)	75	25
Ciprofloxacin (10 µg)	90	10
Cotrimoxazole (30 µg)	100	0
Streptomycine (30 µg)	100	0

absorption of the products of microbial fermentation and volatile fatty acids. This may also be attributed to the reason for large amount of Proteus organisms found in feaces and soil [26]. Occurrence of Proteus mirabilis was also confirmed in other parts of the gastrointestinal tracts like the jejunum, rumen and less commonly in the mesenteric lymph node. *Proteus* has been regarded as an undesirable element of intestinal microflora, as the bacteria is opportunistic and can become the causative agent of diarrhoea or bloodstream septicaemia [27], although Ikeobi *et al*. [28] did not notice significant difference in the presence of *Proteus* members in the intestine of healthy individuals and diarrheic patients. Thus, the presence of *Proteus* species in the gastrointestinal tract of cattle although is expected as a normal intestinal flora may be treated as a carrier state because in some conditions it may lead to cross infection or auto infection especially in the urinary tract of humans [29,30,31,32]. Aside from *Proteus mirabilis*, indole positive organisms suspected to be *Proteus vulgaris* was also observed indicating that more than one strain of *Proteus* species was present in the micro flora of the offal of slaughtered cattle. This is in line with the report by Hawkey *et al*. [26] that *Proteus mirabilis*, *P vulgaris*, and other strains of *Proteus* were commonly isolated from bedding contaminated with feaces and urine in cattle farms. The authors concluded that high similarity of the O-stereotype profile of isolated strains from cattle to those seen in human infection is indicative of the fact that food animals may be a source of the *Proteus* strains carried in human guts. The potential risk of acquiring and subsequently spreading of *Proteus* infection is high during food processing and also transmission to human after consumption of the processed food.

In as much as the occurrence of *Proteus mirabilis* in the gastrointestinal tract of cattle is expected as it constitutes part of the normal flora of the gastrointestinal tract, the presence of *Proteus mirabilis* in the mesenteric lymph nodes as seen in this study, is however not normal as it could be indicative of an acute or chronic infection by *Proteus mirabilis*. This finding is similar to that reported by Tadatsugu *et al*. [33] on necrotizing suppurative nephiritis in a Japanese black feedlot steer due to *Proteus mirabilis* infection with the bacteria isolated from organs and lymph nodes and seen inside macrophages.

The results of the antimicrobial susceptibility test of the isolates obtained from the offal of slaughtered cattle in this study showed thatisolates from 67% of the mesenteric lymph nodes, 76% of the colon, 60% of the jejunum and 50% of the rumen showed resistance to Ampicillin (PN) with an average of 49% resistance. This resistance to Ampicillin by Proteus species has been previously reported [34] and with similar organisms, Escherichia coli [35] and Salmonella species [36]. The cattle slaughtered were trade animals with no known history of antimicrobial treatment, were supposed to be apparently healthy animals such that the edible offal are expected to be safe, wholesome and free from residues. Therefore it is expected that the normal gastrointestinal microflora (Proteus species) being found as a normal habitat of animal intestinal tract should be susceptible to all antibiotics tested.

Drugs that were effective against all isolates includes Ofoxacine (72%), Streptomycine (100%), Ceporex (82%), Cotrimoxazole (88%), Gentamycin (86%), Nalidixic acid (63%), Augumentin (83%), Ciprofloxacin (92%), and Pepflacin (92%). This result was in agreement with the reports from Mordi and Momoh [37]. The high susceptibility to Streptomycine was also observed by Wang et al. [38]. However, contrary to this publication Proteus mirabilis was highly resistant to other antibiotics used by Wang et al. [39].

CONCLUSION

The confirmation of the presence of confirmed *Proteus mirabilis* in the offal of slaughtered cattle in this work is a cause for public awareness to abattoir and health workers. It calls for better antimortem and post mortem inspection, proper guard against cross infection as *Proteus mirabilis* is the cause of 90% of *Proteus* infections and has been implicated in; urinary tract infection, bloodstream infection causing sepsis and systemic inflammatory response syndrome (SIRS), suppurative necrosis, prostitis in men, less commonly pneumonia, meningitis and *otitis media* in children (Sharma and Paul, 2012). The recorded resistance of the isolated *P. mirabilis* is a cause for public health concern as the impact of bacterial resistance is widespread and constitutes a serious threat to humanity. It is considered a public health problem, which includes the medical and social areas. If these bacteria are not controlled in the future they will be even more devastating for humanity, compared to what was experienced in the era pre-antibiotic, since the emergence of new therapeutic resources does not follow the evolution of resistance mechanisms.

RECOMMENDATION

A large percentage of the isolates in this work would appear to be non-pathogenic *Enterobacteriacae* namely; *Proteus mirabilis*. Since the intention of this work, is to screen for *Salmonella enteric* serovar *enteritidis* more work is advocated to screen meat, and edible organs for the occurrence of pathogenic microorganisms like *Salmonella enteritidis* and *Proteus mirabilis.* It is also important that meat and edible offal are screened for the presence of antibiotic residues as their presence can result in the buildup of antibiotic resistance in animals and humans on consumption or contact.

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