ORIGINAL ARTICLE

PUBLIC HEALTH IMPLICATIONS OF *SALMONELLA* SPECIES CONTAMINATION OF NAIRA NOTES OBTAINED FROM BUTCHERS IN ABAKALIKI MEAT MARKET, EBONYI STATE

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ABSTRACT

Daily transactions have made paper currencies to pass through many hands, and pathogens become imposed on them before they are finally deposited in banks. This study evaluates the public health implications of *Salmonella* species contamination of naira notes obtained from butchers in Abakaliki meat market, Ebonyi State. A total of 95 samples of naira notes in different denominations (₦5 to ₦1000) were randomly collected from butchers within Abakaliki metropolis between the months of March and May, 2016. All the naira note samples were analysed using standard microbiological procedures. A total of nine (9.5 %) *Salmonella* spp isolates were obtained from the 95 naira note samples. All the *Salmonella* spp isolates were completely resistant (100 %) to ceftazidime. The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, meropenem, imipenem, and ofloxacin with values ranging from 88.9 % - 77.8 %. There was a statistically significant difference in the mean percentage resistance and susceptibility of the *Salmonella* spp isolates (P < 0.05). Most of the *Salmonella* spp isolates exhibited multi-drug resistant traits as they were resistant to at least two different classes of antibiotics. The average multiple antibiotic resistance indices (MARI) of the *Salmonella* spp isolates was 0.35. This study shows that ciprofloxacin, gentamicin, meropenem, imipenem, and ofloxacin are still effective in the treatment of bacterial infections caused by *Salmonella* spp. Proper and hygienic handling of paper currencies is of public health importance so as to avert health challenges associated with microbial contamination of bank notes especially *Salmonella* spp.

Keywords: *Salmonella*, Naira notes, butchers, antibiotics, public health

CONSÉQUENCES SUR LA SANTÉ PUBLIQUE DE L'ESPÈCE SALMONELLA CONTAMINATION DE NAIRA NOTE OBTENUE À PARTIR DE LA VIANDE DE BOUCHERIE À ABAKALIKI, MARCHÉ DE L'ETAT D'EBONYI

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Résumé

Transactions quotidiennes ont fait des monnaies papier à passer entre de nombreuses mains, et d'agents pathogènes deviennent imposées sur eux avant qu'ils ne sont finalement déposés dans des banques. Cette étude évalue les conséquences sur la santé publique de l'espèce Salmonella contamination de naira note obtenue à partir de la viande de boucherie à Abakaliki marché, l'état d'Ebonyi. Un total de 95 échantillons de naira notes dans différentes dénominations (N5 à N1000) ont été prélevés au hasard à partir de bouchers au sein de Metropolis Abakaliki entre les mois de mars et mai 2016. Tous les échantillons ont été analysés note naira en utilisant les procédures microbiologiques standard. Un total de neuf (9,5 %) des isolats de Salmonella spp ont été obtenues à partir de la naira 95 remarque d'échantillons. Tous les isolats de Salmonella spp, étaient totalement résistants (100 %) de la ceftazidime. La plus haute fréquence de sensibilité a été observée pour la ciprofloxacine, gentamicine, méropénem, imipenem, et de l'ofloxacine avec des valeurs allant de 88,9 % - 77,8 %. Il y avait une différence statistiquement significative dans le pourcentage moyen de résistance et de sensibilité des isolats de Salmonella spp, le < (P 0,05). La plupart des isolats de Salmonella spp, présentait des caractéristiques multi-résistante car ils étaient résistants à au moins deux classes différentes d'antibiotiques.

La moyenne des indices de résistance multiple aux antibiotiques (MARI) de la isolats de Salmonella spp, était de 0,35. Cette étude montre que la ciprofloxacine, gentamicine, méropénem, imipenem, et l'ofloxacine sont toujours efficaces pour le traitement des infections bactériennes causées par Salmonella spp. Bon et la manipulation hygiénique des monnaies de papier est de l'importance de la santé publique, afin d'éviter les défis de santé liés à la contamination microbienne des billets de banque en particulier Salmonella spp.

Mots-clés: Salmonella, Naira notes, des bouchers, des antibiotiques, de la santé publique

INTRODUCTION

Paper currency notes could be one of the most potential vehicles of transmitting diseases amongst the people. Daily transactions have made the paper currency to pass through many hands, and pathogens become imposed on them before they are finally deposited in banks. Modern scientific studies have confirmed the presence of various pathogenic bacteria on paper currencies. In most day-to-day cash transactions, money, in form of notes and coins, pass through the hands of many people; as against exchange dependent on double coincidence of wants [1]. Various denomination of the naira notes have been minted by the Central Bank of Nigeria (CBN). They are released to the public, through the commercial banks. Currently, there are eight denominations of the naira notes: N5, N10, N20, N50, N100, N200, N500 and N1000 notes. The N5, N10, N20, N50, N100 and N200 naira notes are the most common and are more involved in daily cash transactions. They are common especially among the populace while the N500 and N1000 notes are commonly used among the wealthy and in corporate transactions [2]. Individuals handling the notes shed some of their body flora on the notes; leading to the spread of the microorganisms among the handlers. This has been implicated in serious health hazard such as impairment of lungs function [3]. The contamination of the notes can be traced to dust, soil, water, microflora of the body of handlers (hand, skin, etc.), and the saliva often used when counting the notes and wounds. Some money handling habits such as: keeping naira notes in brassiere, socks, pockets, under the carpet or rugs, and squeezing in the hand frequently introduce microbes to the notes. *Citrobacter* spp, *Salmonella* spp, *Shigella* spp, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been isolated from naira notes [4]. Most of them are normal flora of the human skin; however, some examples are opportunistic pathogens. This suggests that the notes could serve as fomites for some infectious agents [2]. *Salmonella* is one of the most frequent pathogen associated with food borne disease outbreaks. Cooked food products and raw milk were most commonly contaminated with food borne pathogens and many of them were resistant to different antibiotics. Meat and milk products are often contaminated with bacterial pathogens. It is currently not possible to effectively and consistently exclude such multi-drug resistant strains from the human food chain, which means that they continue to pose a significant clinical threat to consumers and concomitant economic threats to food production and processing industry. The Presence of enterotoxigenic and antimicrobial resistant strains of *Salmonella* has become remarkably widespread in foods. This requires a better control of food contamination sources and distribution of antimicrobial-resistance organisms [5]. This study evaluates the public health implications of *Salmonella* species contamination of Nigerian naira notes obtained from butchers in Abakaliki meat market, Ebonyi State.

MATERIALS AND METHODS

Study Area: Ebonyi State is popularly known as the 'Salt of the Nation,' apparently because of the large deposits of salt water in the state. The State capital is Abakaliki. It lies approximately within longitude 7^0 30' and 8^0 30' E and 5^0 40' and 6^0 45'N. According to data from the 2006 Population and Housing Census, Ebonyi State has an estimated population of 2.3 million and a land mass of 5,935 km².

Collection of Samples

A total of 95 samples of naira notes in different denominations (₦5 - ₦ 1000) were randomly collected from butchers within Abakaliki metropolis between the months of March and May, 2016. The naira notes were collected with hands covered with sterile gloves. The notes were then immediately placed inside sterile polythene bags and labeled accordingly. In some instances, the naira notes were obtained during exchange for bigger denominations; others were obtained after buying items with bigger denominations. The naira note samples were then immediately transported to the department of Applied Microbiology laboratory, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis. The currencies were observed to have been in circulation for about 2 - 4 years (2011-2014). Mint paper currencies were used as controls.

Culturing, isolation, phenotypic characterization and identification of the isolates

A sterile swab stick moistened with sterile physiological saline (0.85 % NaCl) was used to swab

both sides of each note. Each of the swabs was then aseptically transferred into test tubes containing 5 ml of peptone water and incubated overnight at 37 °C. After incubation, the swabs were then streaked onto already prepared Salmonella-Shigella (SS) agar (Oxoid, UK). The inoculated plates were then incubated at 37 °C for 24 hours. Colonies displaying typical *Salmonella* characteristics (smooth, small and colourless colonies with dark centre) were picked and sub-cultured on freshly prepared Salmonella-Shigella agar plates so as to obtain pure colonies. Pure colonies of *Salmonella* isolates were picked from the Salmonella-Shigella agar plates and inoculated on prepared nutrient agar slants and preserved in the refrigerator at 4 °C for further tests. The suspected *Salmonella* isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test, and other biochemical tests which include oxidase test, indole test, citrate utilization test, H_2S production test, Voges-Proskauer test, methyl red test, urease test, nitrate reduction test, and sugar fermentation test (Cheesbrough, 2004).

Antibiotic susceptibility test

Antibiotic susceptibility of the *Salmonella* isolates was done using the Kirby Bauer disc diffusion method and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2007) formerly known as National Committee for Clinical Laboratory Standards (NCCLS). Mueller-Hinton agar was prepared according to the manufacturer's instructions. The medium was cooled to 45-50 °C and poured into plates. Plates were allowed to set on a level surface to a depth of approximately 4 mm. When the agar has solidified, plates were allowed to dry before use. An 18-24 hour old broth culture of the *Salmonella* isolate was standardized by diluting to 0.5 Mcfarland's standard. A sterile swab stick was inserted into the standardized *Salmonella* inoculum, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. After this, the inoculated Mueller-Hinton agar plate was allowed to dry for a few minutes at room temperature with the lid closed. After the agar surface has dried for few minutes, antibiotic impregnated discs (Oxoid, UK) of known concentrations; gentamycin (30 µg), aztreonam (30 µg), ofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), and imipenem (10 µg) were carefully applied on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates were then incubated at 37 °C for 18-24 hours. After incubation, the diameters of the zones of inhibition were measured with a ruler to the nearest millimeter and recorded. The results were recorded as resistant, intermediate and susceptible according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2007).

Determination of Multiple Antibiotics Resistance Index (MARI)

Multiple antibiotic resistance indices (MARI) of the bacterial isolates were calculated using the technique described by Christopher *et al.* (2013) and Subramani *et al.* (2012). This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

STATISTICAL ANALYSIS

Statistical analysis was performed using T-test and Pearson correlation index at $P < 0.05$ with statistical software SPSS Data Editor Version 16.

RESULTS

TABLE 1: CURRENCY DESCRIPTION POSITIVE *SALMONELLA* SPP

S/N	Currency Type(₦)	Smooth and Neat	Rough and Dirty	*Salmonella* spp growth after 24 hours
1	500	+	-	+
2	50	-	+	+
3	200	-	+	+
4	50	-	+	+
5	100	-	+	+
6	1000	-	+	+
7	10	-	+	+
8	20	-	+	+
9	5	-	+	+
1	Control (mint)	-	-	-

Key: + = Positive; - =Negative

All the naira notes were rough and dirty except one (₦ 500) which was smooth and neat. All the naira notes were positive for *Salmonella* species except the control (mint)

TABLE 2: PERCENTAGE OCCURRENCE OF RESPONSES OF THE *SALMONELLA* ISOLATES TO DIFFERENT ANTIBIOTICS

Antibiotics Used	Resistance (%)	Susceptible (%)
Ciprofloxacin	1 (11.1 %)	8 (88.9 %)
Cefepime	4 (44.4 %)	5 (55.6 %)
Gentamicin	1 (11.1 %)	8 (88.9 %)
Aztreonem	4 (44.4 %)	5 (55.6 %)
Meropenem	1 (11.1 %)	8 (88.9 %)
Imipenem	2 (22.2 %)	7 (77.8 %)
Ofloxacin	2 (22.2 %)	7 (77.8 %)
Ceftazidime	9 (100 %)	0 (0 %)

All the *Salmonella* spp isolates were completely resistance (100 %) to ceftazidime. This was closely followed by aztreonam (44.4 %) and cefepime (44.4 %). The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, meropenem with values of 88.9 % each. This was closely followed by imipenem and ofloxacin with values of 77.8 % each.

TABLE 3: ANTIBIOTIC RESISTANCE PATTERNS OF THE *SALMONELLA* ISOLATES

S/N	Isolate Code	Resistance Patterns
1	Sal 14	CAZ^R
2	Sal 16	FEP^R MEM^R IPM^R CAZ^R
3	Sal 18	FEP^R ATM^R IPM^R OFX^R CAZ^R
4	Sal 19	CAZ^R
5	Sal 20	FEP^R ATM^R CAZ^R
6	Sal 27	CAZ^R
7	Sal 38	ATM^R CAZ^R
8	Sal 17	CAZ^R
9	Sal 46	CIP^R FEP^R CN^R ATM^R OFX^R CAZ^R

Key: R= Resistant, CIP = Ciprofloxacin, FEP = Cefepime, CN = Gentamincin, ATM = Aztreonam, MEM = Meropenem, IPM = Imipenem, OFX = Ofloxacin, CAZ = Ceftazidime

Five (Sal 2, Sal 3, Sal 5, Sal 7, and Sal 9) out of the nine (9) isolates were multi-drug resistant as they were resistant to at least two different classes of antibiotics.

TABLE 4: MULTIPLE ANTIBIOTIC RESISTANCE INDEX (MARI) OF THE *SALMONALLA* ISOLATES

Isolates	Sal 14	Sal 16	Sal 18	Sal 19	Sal 20	Sal 37	Sal 38	Sal 17	Sal 46	Total	Average
MARI value	0.13	0.50	0.63	0.13	0.38	0.13	0.3	0.13	0.8	3.13	**0.35**

The multiple antibiotic resistance indices (MARI) of the *Salmonella* spp isolates ranged from 0.13 to 0.8. This high MARI might be an indication of abuse and misuse of antibiotics.

DISCUSSION

Paper currencies have been used for transaction purposes both within Nigeria and beyond. However, this essential item have often times been predisposed to microbial contamination through unhygienic practices by traders and pedestrians alike. *Salmonella*, an enteric Gram-negative rod-shaped bacterium has been frequently implicated in paper currency contamination.

This study evaluates the role of *Salmonella* spp. in Nigeria's paper currency (Naira notes) contamination and their antibiotic susceptibility patterns. A total of ninety five (95) naira notes ranging from ₦ 5 to ₦1000 were collected from butchers in Abakaliki meat market while a naira note in mint condition obtained from a commercial bank was used as a control. All the 95 naira notes obtained from butchers at meat market, Abakaliki showed bacterial growth after 24 hours.

Most of the naira notes that had bacterial growths were rough and dirty in appearance. The control (mint) obtained from a commercial bank in the same study area did not show any bacterial growth. Out of the 95 naira notes obtained for this study, 9 were positive for *Salmonella* spp. growth after 24 hours of incubation at 37 °C (Table 1). This result is in agreement with the work of Ayandele and Adeniyi [10] but differs from a separate report by Omar and Bassan [11] that isolated only one (1) *Salmonella* species from paper currency in Saudi Arabia.

All the *Salmonella* spp. isolates were completely resistant (100 %) to ceftazidime. This was closely followed by aztreonam (44.4 %), and cefepime (44.4 %) (Table 2). This observation is similar to report by Alemu [12] in Ethiopia where he recorded 87.5 % resistance among *Salmonella* isolates recovered from paper currency. The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, and meropenem with susceptibility frequency value of 88.9 % each. This was closely followed by imipenem and ofloxacin with susceptibility frequency value of 77.8 % each (Table 2). The results of the one sample t-

test conducted showed that there was a statistically significant difference in the mean percentage resistance of the *Salmonella* isolates (mean = 33.3125, SD = 30.28495), t = 3.111, P = 0.017 (P < 0.05). One sample t-test also showed that there was a statistically significant difference in the mean percentage susceptibilities of the *Salmonella* isolates (mean = 66.6875, SD = 30.28495), t = 6.228, P = 0.000 (P < 0.05). Results of the independent samples t-test conducted to compare the percentage resistances and percentage susceptibilities of the *Salmonella* isolates to different classes of antibiotics showed that there was a statistically significant difference in the mean percentage resistance (mean = 33.3125, SD = 30.28495) and mean percentage susceptibility (mean = 66.687, SD = 30.28495); t = -2.204, P = 0.045 (P < 0.05). The result of this work shows that ciprofloxacin, gentamicin, meropenem, imipenem and ofloxacin are still active against *Salmonella* spp. isolates in Abakaliki, Ebonyi state. Results also showed that ceftazidime, a third generation cephalosporin, is no longer effective in treating bacterial infections caused by *Salmonella* spp. as all the 9 *Salmonella* isolates obtained in this study were resistant to this particular antibiotic. Four (44.4 %) out of the 9 *Salmonella* isolates did not exhibit multi-drug resistance traits as they were resistant to only one class of antibiotic; ceftazidime, a third generation cephalosporin (Table 3). However, the remaining 5 (55.6 %) *Salmonella* spp. isolates were multi-drug resistant as they exhibited resistance to at least two classes of antibiotics (Table 3). The average multiple antibiotic resistance index (MAR1) value of the *Salmonella* spp. isolates was 0.35 and this signifies their high antibiotic resistance frequency values to commonly used antibiotics for treating bacterial infections (Table 4). The presence of microorganisms on paper currencies suggests that it can be a favourable environment for their growth. The presence of *Salmonella* in paper currencies could possibly arise from faecal contamination. It is a common practice among traders (butchers and meat sellers inclusive) to handle paper currencies improperly without maintaining good handling hygienic practices especially washing of hands properly after butchering of animals or displaying meats for sale on dirty tables in open places where flies are always perching on the meat being sold. These unhygienic practices predisposes the traders to *Salmonella* infections especially *Salmonella* food poisoning, typhoid fever, paratyphoid fever among others. Consequently, proper handling of bank notes is of public health importance considering the fact that some communicable diseases can spread by means of fomites including money.

CONCLUSION

Salmonella contamination of paper currencies could be a potential source of community-acquired infection. This research has shown the presence of *Salmonella* species on Nigeria's paper currencies collected from butchers in Abakaliki meat market, Ebonyi State. The study also revealed that *Salmonella* isolates were susceptible to most of the antibiotics except ceftazidime. This study has also revealed that ciprofloxacin, gentamicin, meropenem, imipenem and ofloxacin are still effective in treating bacterial infections caused by *Salmonella* spp. *Salmonella* species can cause serious community acquired infections. Hence, appropriate hygienic measures should be adopted while handling naira notes.

CONFLICT OF INTEREST STATEMENT: The authors declare that there are no conflicts of interest.

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