Original Research Article

Microbial Load, Prevalence and Antibiotic Resistance of Microflora Isolated from the Ghanaian Paper Currency Note: A Potential Health Threat

ABSTRACT

Aims: This study examined the microbial flora contamination of the Ghanaian paper currency notes and antibiotic-resistant in Ejura Municipal, Ashanti Region, Ghana.
Study design: This is a descriptive cross-sectional study designed to assess the profile of microflora contamination of the Ghanaian paper currency notes and antibiotic-resistant in the Ejura Municipality.
Place and Duration of Study: The research was conducted in Ejura, a town in the Ejura Sekyeredumase Municipal District of the Ashanti region of Ghana, from January to May 2019
Methodology: A total of 70 GH¢ notes, 15 each of GH ¢1, GH ¢2, and GH ¢5, 10 each of GH ¢10 and GH ¢20, and 5 of GH ¢50, were randomly sampled from people in various shops, canteens, and commercial drivers. The surfaces of each GH¢ note were gently swabbed, and tenfold serial dilution was inoculated on plate count agar (PCA), MacConkey agar, mannitol salt agar, and deoxycholate citrate agar. For bacterial identification, the study used appropriate laboratory and biochemical tests. The data was analyzed using SPSS-IBM version 20.0.
Results: It was found that 95.2 % of the 70 GH¢ notes tested positive for one or more bacterial isolates. On each GH¢ note, mean counts on PCA ranged from 3.0 cfu/ml $\times 10^5$ to 4.8 cfu/ml $\times 10^5$. Of 124 bacteria isolated. 36 (29.03 %), 32 (25.81%), 16 (12.90 %), 20 (16.13%), 13 (10.48 %), and 7 (5.66 %) were from GH¢1, GH¢2, GH¢10, GH¢5, GH¢20, and GH¢50, respectively. Bacterial isolates were *Escherichia coli* (25.81%), *Staphylococcus aureus* (18.55%), *coagulase-negative Staphylococcus* (15.32%), *Klebsiella species* (12.10%), *Salmonella species* (9.68%), *Shigella species* (8.06%), Pseudomonas aeruginosa (7.26%), and Proteus species (3.23%). Meat shops, commercial drivers, canteens, grocery stores, and vegetable shops contributed 25.81 %, 20.16 %, 19.35 %, 17.74 %, and 16.94 % of GH¢ notes respectively. There was 100% resistance of the isolates to Erythromycin (ERY), and Cotrimoxazole (COT). Amikacin (AMK) was the most effective among the antibiotics as 75% of the isolates were susceptible to it.
Conclusion: This study has demonstrated that the GH¢ notes are heavily contaminated with potentially pathogenic bacteria that are highly resistant to the most widely used antibiotics and are a threat to public health.

1. INTRODUCTION

Paper currency notes are widely exchanged for goods and services in countries all over the world. People frequently contaminate these notes with various microflora such as viruses, fungi, protozoa, and, most notably, bacteria due to unsanitary conditions and habits[1]. Coughing and sneezing on hands before exchanging money, improper washing of hands after using the urinal or toilet and eating, inserting one's hand into one's nasal cavity then touching paper notes,

applying saliva on hands while counting paper notes, and placing or storing paper notes on dirty surfaces are some of these practices [2].

Even during the 'Black Death or the bubonic and pneumonic plague pandemics in England, historical accounts demonstrate that money was thought to contain lethal illnesses [3].
Infections caused by these microflorae are typically by bacteria and are always treated with medicines; however, most of these bacteria have recently developed resistance to antibiotic treatments [4]. Despite the best efforts made to alleviate the situation, this has turned into a canker that has killed millions of lives and consumed a large portion of the government's budget. [5]. such a situation has become a major source of concern for the international community, and it is necessary to investigate the risk of disease transmission. Antibiotic resistance is becoming a serious problem [6].

According to data from the World Health Organization (WHO), antibiotic resistance has become a severe public health problem in about 114 countries, including Ghana. Drug-resistant *Campylobacter*, extended-spectrum *Enterobacteriaceae* (ESBL), drug-resistant *Streptococcus pneumonae*, drug-resistant *Tuberculosis*, vancomycin-resistant *Staphylococcus aureus,* Erythromycin–Resistant group *A Streptococcus*, Clindamycin–Resistant group *B Streptococcus*, drug-resistant *Salmonella serotype Typhi*, multidrug-resistant strains [7]. Antibiotics failed to treat about 63 per cent of infectious diseases, and according to reference [7], no new antibiotic classes have been recorded since 1984. Use one tense when you describe your research, most of the time it is past participle.

Research conducted by reference [8] confirms that the Ghanaian currency notes are 100% contaminated. The contaminated currency notes go into circulation and contaminate the hands of others transmitting pathogenic organisms in the process [9]. Currency notes are used in Ghana to purchase ready-to-eat food, uncooked meat and vegetables from the market, charcoal, and milk from a local store, drugs, and other goods. Although Ghanaian currency was first put into circulation in July 2007, it has since become filthy and even mutilated. These could be a source of Enteropathogens, which cause food poisoning. This is a significant threat in Ghana because food vendors serve food with their hands while also handling currency notes as they sell.

2. MATERIAL AND METHODS

2.1 Study Area

The research was conducted in Ejura, a town in the Ejura Sekyeredumase Municipal District of Ashanti of Ghana. Ejura Sekyeredumase Municipal, which is one of the Twenty-seven (27) Administrative Districts in the Ashanti Region of Ghana, is located in the northern part of the region [10]. The Municipal is bounded on the North by Nkoranza North and the Atebubu district of the Brong Ahafo region. To the east by the Sekyere central, to the south by Sekyere west and Mampong. The district covers an area of 1782.2qkm which is about 7.3% of the total land area of the Ashanti region. The Municipal capital, Ejura is about 105km from the Regional Capital, Kumasi. Agriculture is the main economic activity within the municipality and employs about 69.5 per cent of the entire labour force [10].

2.2 Study Design

This is a descriptive cross-sectional study designed to assess the profile of microflora contamination of the Ghanaian paper currency notes and antibiotic-resistant in the Ejura Municipality from January to May 2019.

2.3 Sample Size and Sampling

The study includes all the Ghanaian denomination paper notes which include the; GH¢1 note, GH¢2 note, GH¢5 note, GH¢10 note, GH¢20 note and GH¢50 note. A total of 70 Ghanaian currency notes made up of 15 one Ghana cedi notes, 15 two Ghana cedi notes, 15 five Ghana cedi notes, 10 ten Ghana cedi notes, 10 twenty Ghana cedi notes, and 5 fifty Ghana cedi were randomly collected from different sources in Ejura Municipal.

2.3.1 Sample Collection Procedures

Ghanaian cedi notes were aseptically collected randomly from grocery shops, canteens, taxi drivers, meat shops and vegetable shops within a week by either giving them new currency notes and taking theirs or through buying to obtain all the denominations and placed back in plastic envelope bags, sealed and sent to the laboratory for their surface to be swabbed for immediate analysis. The study samples were collected based on the level of usage by a simple random sampling method as follows; 15, 15, 15, 10, 10, and 5 pieces of 1, 2, 5, 10, 20 and 50 Ghana cedi respectively. Only the Ghanaian paper currency introduced by the Bank of Ghana in 2007 was collected otherwise rejected.

2.4 Laboratory Methods and Analysis

2.4.1 Media used for culture

The media used were the products of Oxoid Limited, Basingstoke Hampshire, and England. The study adopted the Oxoid standard protocol for media preparation except for selenite F broth. The media included plate count agar (PCA), MacConkey agar (MCA), mannitol salt agar (MSA) and deoxycholate citrate agar (DCA), Simmons citrate agar, triple sugar iron agar (TSI), selenite broth, and peptone water. Reagents were Kovacs and Remel BactiDrop Oxidase. PCA, MCA, DCA, and MSA were the media used for the total viable count, Gram-negative bacteria, *Salmonella-Shigella*, and *Staphylococcus* isolation in that order. Mueller–Hinton agar (MHA) was the medium used for the antimicrobial susceptibility test.

2.4.2 Sample Processing

Each GH¢ note was given a distinct identifier. Both surfaces of the note were gently swabbed with sterile cotton moistened with sterile buffered peptone water (BPW). The swabs in their respective tubes with 1 ml sterile BPW were then vortexed to get a uniform suspension. The study made tenfold serial dilutions of each suspension for the cultivation and identification of microbial contaminants. The GH¢ notes swabbed for the study were later cleaned with MAK SWAB (alcohol swab).

2.4.3 Cultivation and Enumeration

The study used 0.1 ml of each dilution in the study, and each dilution was inoculated into the appropriate media and incubated at 37°C for 12–48 hours. The culture plates were then examined using standard microbiological methods for growth and morphologic characteristics. Discrete colonies were grown on Nutrient Agar for biochemical analysis and Gram's staining. The total viable colonies were then counted using an electronic colony counter, and the average counts were expressed in cfu/ml.

2.4.4 Identification of Isolates

The study identified bacterial isolates using standard microbiological methods. Gram staining, colony morphology, and suitable biochemical tests were the methods used to identify bacterial isolates. For Gram-positive cocci bacteria with purple round shapes, catalase and coagulase tests differentiated *staphylococci* (catalase-positive) from streptococci (catalase-negative). Isolates of Gram-negative rods on MCA were further grouped into lactose and non-lactose fermenters. These isolates were then inoculated into TSI, and indole test, citrate test, etc., were performed to aid identification [11].
(1) *Salmonella* and *Shigella* species. Colonies from samples pre-enriched in Selenite F Broth (DifcoTM) plated onto DCA with pale or colourless colonies with or without a black spot in the middle were suggestive of Salmonella, while colonies with a pink zone indicate *Shigella*[11].

(2) *Staphylococcus species*. Colonies on MSA plates with yellow colonies with halo zone and colonies with pink with reddish-purple zones indicated *Staphylococcus aureus* and *Staphylococcus epidermidis*. Coagulase test differentiates between *Staphylococcus aureus* (coagulase-positive) and *Staphylococcus epidermidis* (coagulase-negative *Staphylococcus* (CNS)) [11].

(3) Other Gram-negative isolates. All Gram-negative bacteria from MCA were subsequently inoculated in TSI and were incubated. Growth with acidic butt, acidic slant, and gas production without hydrogen sulphide (H_2S) indicated either E. coli or *Klebsiella* species. The two isolates were further identified by indole and citrate tests. Red ring formation on the surface of indole indicates E. coli. The blue colour change of citrate after incubation confirms Klebsiella species. An acidic butt, acidic slant, and gas production with H2S indicate Proteus spp. Alkaline butt, alkaline slant, no gas, and H2S production indicate Pseudomonas aeruginosa[11].

2.5 Antibiotic Susceptibility Testing (AST)

Agar diffusion technique on Mueller–Hinton agar (Kirby–Bauer modified disc diffusion technique) according to CLSI guidelines determined the antibiotic susceptibility. The inhibition zone standards for antimicrobial susceptibility were from tables of interpretative zone diameters of the Clinical and Laboratory Standards Institute. The study tested 10 antibiotic discs of the most commonly used drugs to treat human and animal infections caused by bacteria. These include erythromycin (ERY) (5 µg), ciprofloxacin (CIP) (5 µg), gentamycin (GEN) (10 µg), ampicillin (AMP) (10 µg), amoxicillin

(AMX) (5 µg), vancomycin (VAN) (30 µg), tetracycline (TET) (30 µg), chloramphenicol (CHL) (30 µg), amikacin (AMK) (30 µg), and cotrimoxazole (COT) (25 µg) (Hall, 1989).

2.6 Statistical Analysis

The raw data obtained from the microbial analysis was analyzed using Microsoft Excel 2007 spreadsheet. The counts are then transformed into log10 to make it normally distributed. The data obtained from this study were analyzed descriptively with SPSS version 20.0 software. The sources of the currencies were compared using a one-way analysis of variance (ANOVA) to determine the significant difference where the significance was considered at a 95% confidence interval ($p \leq 0.05$) and ($p \geq 0.05$) is not significant. Comparison of means was done using Post hoc analysis with Tukey-Kramer (Tukey's W) multiple comparison analysis.

3. RESULTS AND DISCUSSION

3.1 Results

The results of this study revealed that, out of the 70 Ghanaian paper currency notes studied, 95.2% were contaminated with bacteria. The various bacteria isolated from the currency notes in this research include *Escherichia coli* (25.81%), *Staphylococcus aureus* (18.55%), CNS (15.32%), *Klebsiella* species (12.10%), *Salmonella* species (9.68%), *Shigella* species (8.03%), *Pseudomonas aeruginosa* (7.26%), and *Proteus* species (3.23%). This finding is almost in agreement with the research conducted in Saudi Arabia reference [12], and the United States reference [13].
Table 1, shows the average CFU/ml and log count of bacteria on each culture media. The tables, however, show that the highest count was on PCA and the least on DCA as illustrated in Fig1. The more average count of bacteria came from GH¢1 followed by 10GH¢ and the least came from 20GH¢ and 50GH¢ as illustrated in Fig 1.

Table1. Average count on culture media

CURRENCY		GH¢1	GH¢2	GH¢5	GH¢10	GH¢20	GH¢50
MCA (cfu/ml	Mean	1.7×10^5	1.2×10^5	1.0×10^5	8.9×10^4	7.8×10^4	4.5×10^4
	Log	5.23	5.08	5.00	4.95	4.89	4.65
MSA (cfu/ml	Mean	1.9×10^5	1.4×10^5	1.2×10^5	1.5×10^5	8.0×10^4	5.8×10^4
	Log	5.27	5.14	5.08	5.18	4.9	4.76
PCA (cfu/ml	Mean	4.8×10^5	4.4×10^5	3.6×10^5	4.0×10^5	3.8×10^5	3.0×10^5
	Log	5.68	5.64	5.56	5.6	5.57	5.48
DCA (cfu/ml	Mean	8.0×10^4	6.5×10^4	2.8×10^4	4.0×10^4	1.5×10^4	1.3×10^4
	Log	4.9	4.81	4.45	4.64	4.18	4.11

Meaning of Abbreviations: PCA, Plate Count Agar; DCA, Desoxycholate Citrate Agar; MSA, Mannitol Salt Agar; MCA, MacConkey Agar.

Figure 1: Graphical representation of average count on culture media

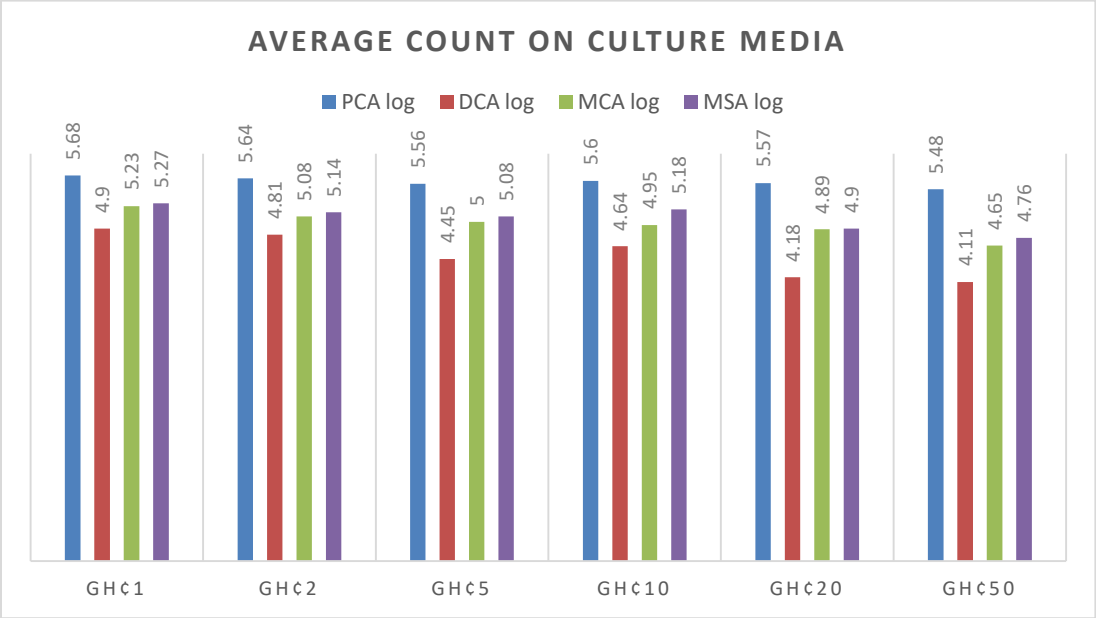


Figure 2: Distribution of bacterial isolates according to sources of currencies

It depicts the distribution of bacterial isolates according to sources of currencies. The numbers of isolates according to the sources of paper currency notes are in the order of meat shops (25.81%), drivers (20.16%), canteens (19.35%), grocery shops (17.74%) and vegetable shops (16.94%).

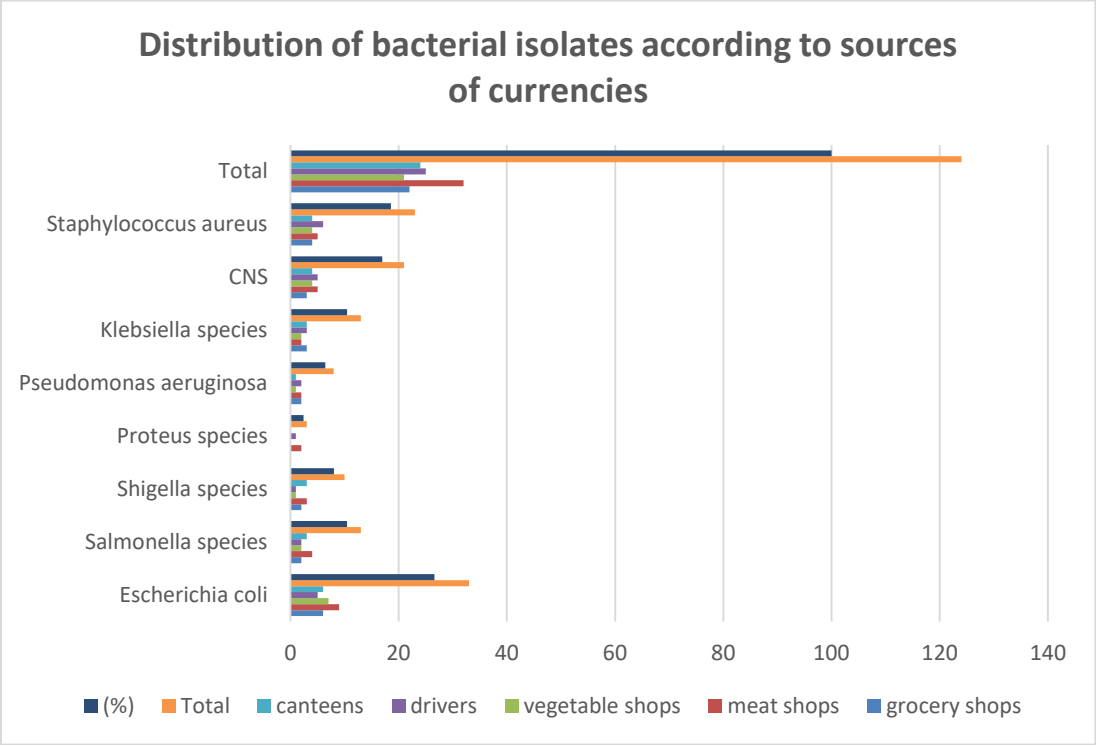
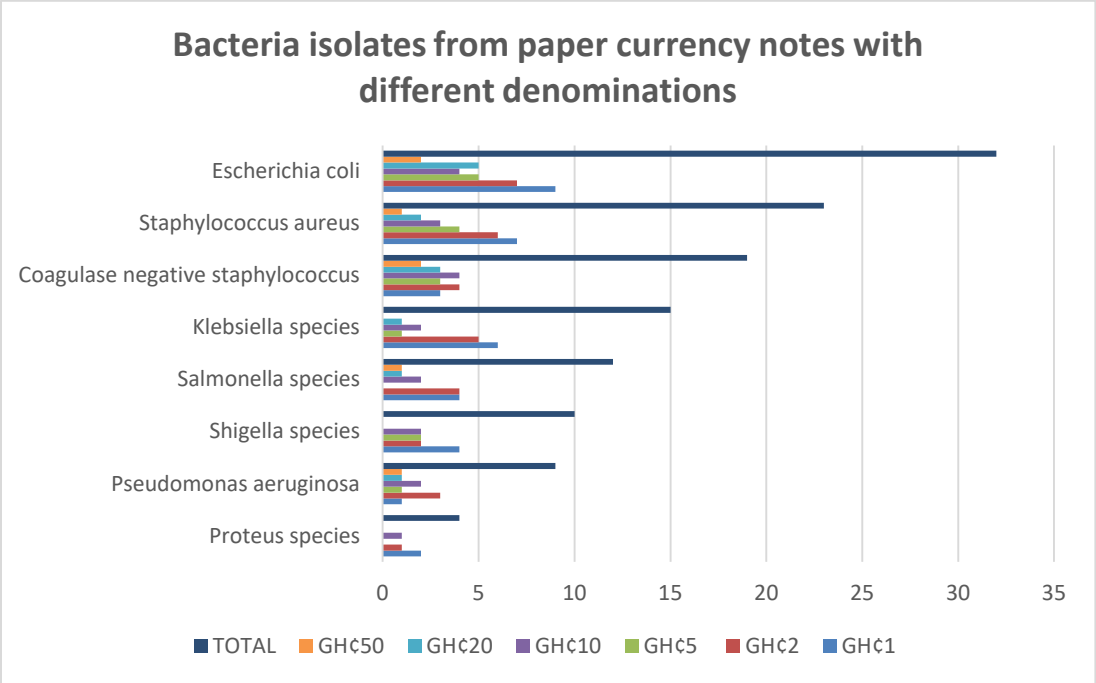


Figure 3: Bacteria isolates from paper currency notes with different denominations

It describes the distribution of bacterial isolates on paper currency notes of different denominations. Out of a total of 124 isolates on the paper currency notes, the contamination level of the different denominations is as follows; GH¢ 1 (26.61%), GH¢ 2 (25%), GH¢ 10 (17.74%), GH¢ 5 (13.71%), GH¢ 20 (10.48%) and GH¢ ¢50 (6.45%). Statistically, there was a significant difference between the denominations and the total plate count at p=0.05. It was observed that smaller denominations were more contaminated than the bigger ones. This implies that the smaller denominations (GH¢ 1and GH¢ 2) are more contaminated because they are often handled. Meanwhile from the study, GH¢ 10 although a bigger denomination was noticed to be more contaminated than GH¢ 5. This could be that GH¢10 is in circulation and handled more than the GH¢5 or probably because there are two different kinds of the GH¢5 paper note accepted and used in Ghana at the same time.



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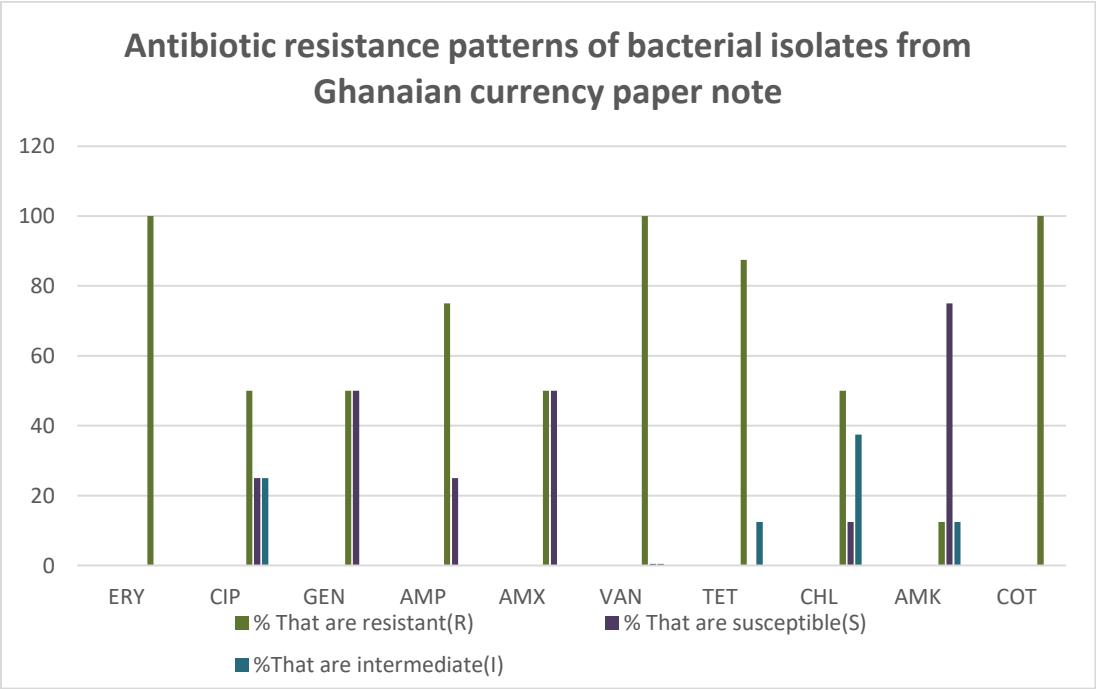
How many times did you collect the notes and re-run the whole experiment? You can add culture plates photos, if you have any. Some molecular experiments will bring different shades in these experiments.

Figure 4: Antibiotic resistance patterns of bacterial isolates from Ghanaian currency paper note

It shows the antimicrobial susceptibility testing of the bacterial isolates. The figure shows that all 8 isolated bacterial species showed 67.5% resistance and 50.00% susceptibility to Ciprofloxacin, Gentamycin, Amoxil and Chloramphenicol, 100% resistance to Erythromycin and Vancomycin, 85% resistance to Tetracycline and 75% resistance to Ampicillin. Amikacin was effective with just resistance of 12.50% by the isolates, 12.50% intermediate and 75% susceptible



Abbreviations: ERY, erythromycin; CIP, ciprofloxacin; GEN, gentamicin; AMP, ampicillin; AMX, amoxil ; VAN, vancomycin; TET, tetracycline; CHL, chloramphenicol ; AMK, amikacin ; COT, cotrimoxazole.

3.2 Discussion

Money is one of the common substances that circulate readily among the general public. This implies that once money is contaminated with pathogens, it can spread these disease-causing organisms from one person to another even all over the globe. The paper currency may serve as a vehicle to spread pathogenic infections which could be resistant to antibiotics. The findings from this study reveal that the Ghanaian paper currency notes were 95.2% of which the smaller denominations (GH¢1, GH¢2, GH¢5 and GH¢10) were more contaminated than the larger notes (GH¢20 and GH¢50) p=.05. The data reflects that the highest denominations is the bank and compared to smaller denominations which get circulated more often.

Similar findings of bacterial contamination on the Iraqi currency were 96% [14], 100% contamination on Ghanaian Cedi note[8], 96.25% contamination of Palestine note 96% contamination of South African banknote[15], 75% contamination of Nepal banknote[16] and 95% contamination of Nigerian banknote [17]. It was observed that *Escherichia* is the most common contaminant of the currency in circulation. This observation agrees with research conducted by reference [18]. It signals that the contamination of the Ghanaian paper currency by faeces. The presence of *Klebsiella* spp, *Staphylococcus aureus*, *Pseudomonas spp* and *Proteus spp* is a clear indication of unhygienic practices and also a serious health problem.

Our study differs from other research which found Coagulase Negative Staphylococcus as the most commonest contaminant of the circulation currency paper. This stems from the fact that hygiene practices vary from people to person,

town to town, country to country etc. it was also found that there wasn't any significant difference between the type of denomination and source of currencies in terms of microbial contamination (*P*-value of 0.138 and 0.945) for the type of denomination and source of currency respectively, which means almost all the denominations have similar log cfu/ml. This is in agreement with research conducted in Cameroon on money. Our research revealed that bacterial contamination was highest in meat shops and least in the vegetable source. This is probably a result of the fact that blood and animal parts are all found on their benches which makes that environment suitable for bacteria to be harboured.
We again reported that most of the isolates were resistant to antibiotics. The health implications are that many multidrug strains of different isolates were prevalent on the Ghanaian currency emphasising the public health significance of the notes and vividly indicating a massive resistance to the commonly used antibiotics in Ghana. Because the isolates offered 100% resistance to Erythromycin and Vancomycin, 50% resistance to Ciprofloxacin, Amoxil, Gentamycin and Chloramphenicol, 75% resistance to Ampicillin and 87.50% resistance to Tetracycline, give an awareness of the prevalence of antibiotic-resistant bacteria on the Ghanaian currency note. 12.50% resistance to Amikacin means; it is very effective against a wide range of bacteria. This result agrees with that of the reference [19], that the presence of antibiotic-resistant bacteria is a great threat to the public. Studies conducted by other research reveal that the increasing nature of antibiotic resistance is due to the excessive usage of antibiotics without prescriptions from a pharmacist.
In a nutshell, although the characterization of isolates was done with media and simple biochemical tests, this study found valuable data to be used for immediate intervention rather than only using it for studies.

CONCLUSION

This study has demonstrated that the GH¢ notes are heavily contaminated with potentially pathogenic bacteria that are highly resistant to the most widely used antibiotics and are a threat to public health. The use of E-commerce and M-money is commendable to use for goods and services to minimize the abuse and deterioration of the GH¢ notes.

Acknowledge the Institution or the company who gave you money to run these experiments.

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