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Antimicrobial susceptibility and resistance genes profiles in gram negative isolates from automated teller machines in Cape Coast, Ghana

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Abstract

The prevalence and type of microorganisms present on surfaces is an important factor to consider in infection outbreaks. The study identified bacterial isolates from Automated Teller Machines (ATMs) and screened the isolates against antibiotics of aminoglycosides, penicillins, tetracyclines, and phenicols classes for efficacy. The isolates were further tested for presence of genes that encodes for aminoglycosides, tetracyclines, glycopeptides, penicillins, and beta-lactamases. The bacterial isolates were identified as clinical isolates of Enterobacteriaceae and Pseudomonadaceae and frequently comprised *Escherichia coli* and *Klebsiella* spp. The isolates were more susceptible to gentamicin and kanamycin (95%) and more resistant to flucloxacillin (65%), cloxacillin (65%), and oxytetracycline (60%). Most of the isolates showed multiresistance to the antibiotics and were commonly associated with *Providencia stuartii*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas alcaligenes*. The bla_{TEM} (90%) and *str*A (80%) encoding genes were the most abundant genes within the isolates. *Klebsiella pneumoniae*, *K. aerogenes*, *Enterobacter cloacae*, *Serratia marcescens*, *S. rubidaea*, *Yersinia mollareti*, and *Salmonella* Paratyphi co-habored 33% of carbapenemases, ESBLs, bla_{ampC}, *van*A, and *str*A encoding genes. This is the first study to highlight the emergence of antibiotic-resistant bacteria from ATMs as potential reservoir for emergence and spread of multidrug resistance genes in bacterial pathogens in Cape Coast, Ghana.

Keywords: *Gram negative bacteria, Antimicrobials, Multidrug resistance, Resistance genes, Beta-lactamases*

1. Introduction

Most microbes are harmless to the human body and form part of the human microbiome (Gibbons and Gilbert, 2015). Some microbes are useful in agriculture, industry and food production (Thomashow *et al*., 2019; and

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Zdolec *et al*., 2018). However, relatively few bacteria are classified as pathogens and are known to cause infectious diseases. Bacteria are responsible for some globally relevant human infectious diseases such as tuberculosis (*Mycobacterium tuberculosis*), pneumonia (*Klebsiella pneumoniae*), and cholera (*Vibrio cholerae*) which are associated with significant rates of morbidity and mortality (World Health Organization, 2019).

The discovery of antibiotics saw a rapid decrease in infectious disease mortalities (Jayachandran, 2018). However, the emergence of antibiotic resistance in the late 20th century saw a stall in the decreasing mortality and morbidity rates that came with the discovery of antibiotics in the early 20th century (Frieri *et al*., 2017). The increasing emergence and spread of antibiotic resistance in human bacteria pathogens are significant growing public health concerns and are gradually becoming a threat to development, food production, and ultimately life expectancy (Frieri *et al*., 2017; and von Wintersdorff *et al*., 2016).

Antibiotic resistance in bacteria pathogens results in higher medical costs, prolonged hospital stay, and ultimately increased mortality (Dadgostar, 2019; and World Health Organization, 2020). Tuberculosis was responsible for about 1.5 million deaths in 2018 globally and requires over $10 bn for treatment and management annually (World Health Organization, 2019). The advent of antibiotic resistance in bacteria pathogens threatens the ability to treat common infectious diseases. Consequently, common infections such as pneumonia and gonorrhea are becoming harder and sometimes impossible to treat (Ventola, 2015). Some developed countries have systems that control the advent and spread of antibiotic resistance. However, resource constraints and lack of awareness of the scope of the threat limits the control of the emergence and spread of antibiotic resistance in developing countries (Newman *et al*., 2011).

It may seem strange to focus on antibiotics resistance amidst the COVID-19, a viral pandemic currently. However, the role of secondary infections coupled with antibiotic resistance in patients with COVID-19 cannot be underestimated (Li *et al*., 2020). Bacteria played a major role in deaths in earlier pandemics such as the H1N1 and SARS (Morris *et al*., 2017). With the recent increase in the misuse of antibiotics and the development of antibiotic resistance, bacteria superinfections are inevitable and increase the fatality rate of the pandemic (Ventola, 2015; and World Health Organization, 2020). Besides, nearly all COVID-19 patients receive antibiotics (Zhou *et al*., 2020). This further threatens the global crisis of antibiotic resistance.

Microbes can be transmitted indirectly from one individual to another through contaminated vehicles of transmission such as water and fomites (Stephens *et al*., 2019). This suggests that the development of infectious diseases from indirect contacts can easily be avoided when the intervening agents of transmission are identified. The use of electronic technology such as handheld devices and electronic point-of-care systems has been established as a source of contamination (Saroja *et al*., 2013). Automated Teller Machines (ATMs) are currently an important component of the banking sector as they make transactions easier and faster. For example, the decongestion and practicing of social distancing in banking halls as a result of the COVID-19 pandemic have led to increased reliance on ATMs by customers. This situation presents an increase in the transmission of infectious pathogens since customers are required to make physical hand interactions with the ATMs. Knowledge on the environment as a source and dissemination route for antibiotic-resistant bacteria is fundamental to identify risk scenarios for human health especially in Ghana (Pal *et al*., 2016). However, the pathogen retaining capacity of ATMs is relatively unexplored. Therefore, there is a need to assess the extent to which ATMs harbor and serve as transmission routes for antibiotic-resistant bacteria.

2. Materials and methods

2.1. Study area

The study was focused on the Cape Coast Metropolis (5°06' N, 1°15' W). Cape Coast is the regional capital of the Central Region of Ghana with a population of over 165,000. Owing to the numerous educational institutions in the metropolis, the rich culture, and the history of the town, it sees an influx of individuals, both local and international, who visit the town for educational and tourist purposes. This causes a periodic rise and fall in the population size of the metropolis. The Cape Coast Metropolis has several financial institutions some of which have ATMs located in different areas in the metropolis.

2.2. Sample size, sampling procedure, and sample collection

Approximately, 20 functional ATMs belonging to nine banks are in the Cape Coast metropolis. Using a combination of functions (Random - RAND, INDEX, and RANK) in Microsoft® Excel 2016, 10 ATMs representing 50% were randomly selected for the study. The 10 ATMs were assigned numbers 1 (ATM_01) to 10 (ATM_10).

The samples were collected by gently swabbing sterile cotton swabs across the keys on the interaction panel of each ATM. The surface areas of the interaction panels averaged 300 cm^2. Each of the 10 ATMs was sampled three times over six months, from October 2019 to March 2020.

2.3. Isolation and identification of bacteria species

The samples were cultured in 10 ml sterile nutrient broth medium and incubated at a temperature of 37 ± 2°C for 24 h. The cultures were serially diluted at ten-fold dilution to the 10^{-6} dilution factor. One-millimeter (1 ml) aliquot from the 10^{-6} dilution factor of each sample was cultured in nutrient agar using the pour plate technique. The number of bacterial colonies in each Petri plate after incubation was recorded with a colony counter (Stuart Sci., UK). The isolates were identified based on colony morphology (form, surface, margin, appearance, and size), cell characteristics, gram staining, and biochemical tests (Triple sugar iron test, motility test, catalase test, indole test, and citrate test). A distinct colony of each identified isolate was sub-cultured for antibiotic susceptibility test and detection of the presence of antibiotic and beta-lactamase genes.

2.4. Phenotypic detection of antibiotic resistance

The antimicrobial susceptibility profile of each bacterial isolate was tested on Mueller-Hinton (MH) agar plates following the Kirby Bauer disk diffusion technique. Twenty microliters (20 µl) aliquot of each isolate was cultured on MH plates. Filter paper discs (0.6 cm) containing antibiotics were placed onto the plates using sterile forceps. The plates were allowed to stand for 1 min and then incubated at 37 ± 2°C for 24 h. The antibiotics used in the study comprised ampicillin (10 µg), cloxacillin (10 µg), and flucloxacillin (10µg) (penicillins class), gentamicin (30 µg) and kanamycin (30 µg), (aminoglycosides class), oxytetracycline (30 µg) (tetracyclines class), and chloramphenicol (10 µg) (phenicols class). These antibiotics are commonly used for the treatment of bacterial infections (Giler-Molina *et al*., 2020; and Rangarajan and Venkataraman, 2020). The zones of inhibition were measured and interpreted according to the guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http: / / www.eucast.org).

2.5. Genomic DNA extraction

The genomic DNA extraction was performed based on a modified version of the method of González-Mendoza *et al*. (2010). An aliquot (4 ml) of each isolate was centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and 300 µl of extraction buffer (3.0% SDS, 1.0 M NaCl, 0.5 mM EDTA, 0.1 mM Tris-HCl, pH 8.0), and glass beads were added in the Eppendorf tubes and vortexed for 2 min. Chloroform-phenol (300 µl) was added to each tube and incubated at 65°C for 10 min. The mixtures were allowed to cool to room temperature (25 ± 2°C) and centrifuged at 14,000 rpm for 5 min. The supernatants were transferred into new tubes. An equal volume of ice-cold isopropanol was added to each tube and mixed gently by inversions. The tubes were then incubated at –20 °C for 30 min and centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and the pellets were washed two times with 70% ethanol. The genomic DNAs were eluted in 30 µl Tris EDTA (TE) buffer and resolved in 1% agarose gel at 80 V for 40 min.

2.6. Detection of genes encoding antibiotic resistance

Beta-lactamases (bla) is the main mechanism of antibiotic resistance in bacteria (Zeng and Lin, 2013). The presence of beta-lactamase genes bla_{OXA-48} and bla_{VIM} (carbapenemases), bla_{CTX-M} and bla_{TEM} (extended-spectrum beta-lactamase) and bla_{ampC} (further beta-lactamase), and a penicillin (*mec*A), aminoglycoside (*str*A), tetracycline (*tet*(A)), and glycopeptide (*van*A) resistance gene in the isolates were detected using primers (Table S1). The polymerase chain reaction (PCR) consisted of 5 µl of *Taq* PCR Master Mix (BBI Life Sci. Corp., China), 1 µl of genomic DNA, 0.5 µl each of forward and reverse primer and 3 µl of molecular grade water. For a negative control reaction mix, sterile distilled was used in place of genomic DNA. The amplification was performed in a thermocycler (BIO-RAD T100™) under the following conditions; Pre-denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55–58°C for 30 s and extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR products were resolved in 2% agarose gel, visualized under ultraviolet (UV) light, and scored for presence or absence of the resistance genes using a 100 bp DNA ladder.

2.7. Data analyses

The qualitative data from the study were presented using descriptive statistics. One-way analysis of variance was used to test for variations in mean bacterial loads at a 5% level of significance using Genstat v21 statistical

software (VSNi, UK). Significance in mean bacterial loads was separated using Duncan's multiple range test at a 5% level of significance.

3. Results

3.1. Estimation of total bacterial loads

The ATMs had significant variations ($p < 0.001$) in total bacterial loads with an average load of 7.89×10^6 CFU ml^{-1} (Figure 1). ATM_04 recorded the highest bacterial load of 18.90×10^6 CFU ml^{-1}. In contrast, the lowest load of 2.70×10^6 CFU ml^{-1} was obtained for bacterial isolates from ATM_02. High bacterial loads were also observed in ATM_03 (13.60×10^6 CFU ml^{-1}) and ATM_09 (12.95×10^6 CFU ml^{-1}). Similar bacterial loads of 4.15×10^6 CFU ml^{-1}, 4.70×10^6 CFU ml^{-1}, 4.80×10^6 CFU ml^{-1} and 4.85×10^6 CFU ml^{-1} were also recorded for isolates from ATMs _10, 08, 05 and 06, respectively. ATM_07 and ATM_01 also gave similar bacterial loads of 6.95×10^6 CFU ml^{-1} and 5.25×10^6 CFU ml^{-1}, respectively. There were no significant differences ($p > 0.05$) in the loads of bacterial isolates from ATMs_01, 05, 06, 08, and 10. Similarly, a significant difference was not observed between the bacterial loads of ATM_03 and ATM_09.

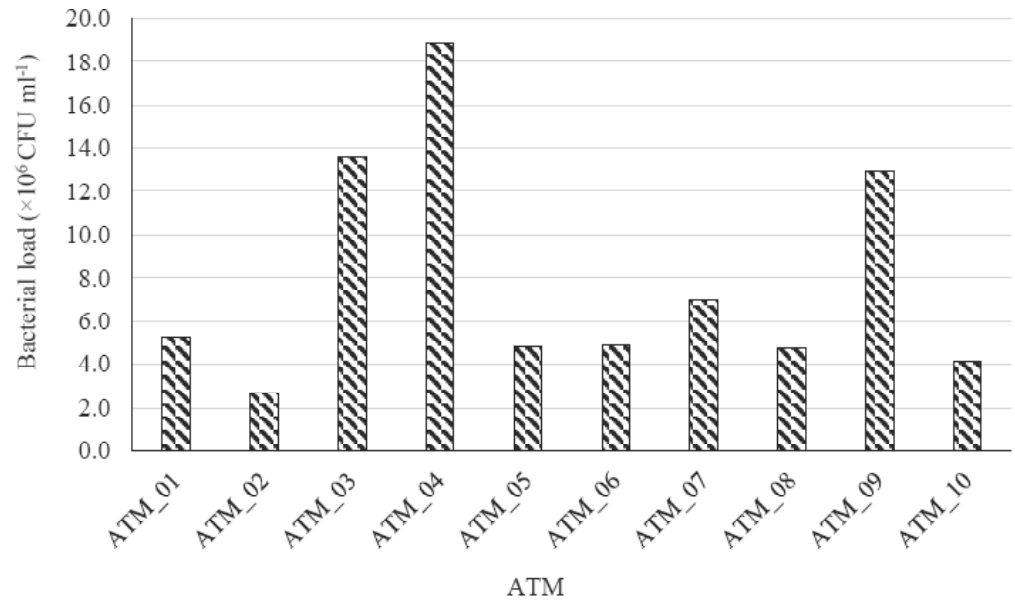

Figure 1: Total bacterial loads of isolates from the ATMs

3.2. Identification of bacterial isolates

A total of 20 bacterial isolates corresponding to 14 distinct species were identified from the ATMs (Figure 2, Table 1). Interestingly, all the bacterial species were gram-negative and rod shaped and belong to the Enterobacteriaceae (93%) and Pseudomonadaceae (93%) families. Two pathogens, *Klebsiella pneumoniae,* and *Enterobacter* spp, belonging to the six bacterial pathogens (ESKAPE) commonly associated with antimicrobial resistance (Ciofu and Tolker-Nielsen, 2019), were identified from ATMs_01 and 06, and ATM_02, respectively. *Escherichia coli* was the most common isolate (20%) and was found in ATMs_01, 02, 07, and 08. *Providencia alcalifaciens* (10%) and *Salmonella* Paratyphi (10%) were common to ATM_02 and ATM_10, ATM_06 and ATM_09, respectively. The remaining nine species (45%) identified were distinct to an ATM. Two species of *Serratia* (*S. rubidaea* and *S. marcescens*) were from ATM_03 and ATM_04. *Klebsiella aerogenes* and *Shigella sonnei* were associated with ATM_03 and ATM_05, respectively. ATM_07 similarly showed presence of *Yersinia mollareti* and *Proteus mirabilis*, respectively. The other species of Enterobacteriaceae that were identified were *Moellerella wisconsensis* (ATM_05) and *Providencia stuartii* (ATM_10). The isolates from ATM_08 also revealed presence of *Pseudomonas alcaligenes* which belongs to the Pseudomonadaceae family.

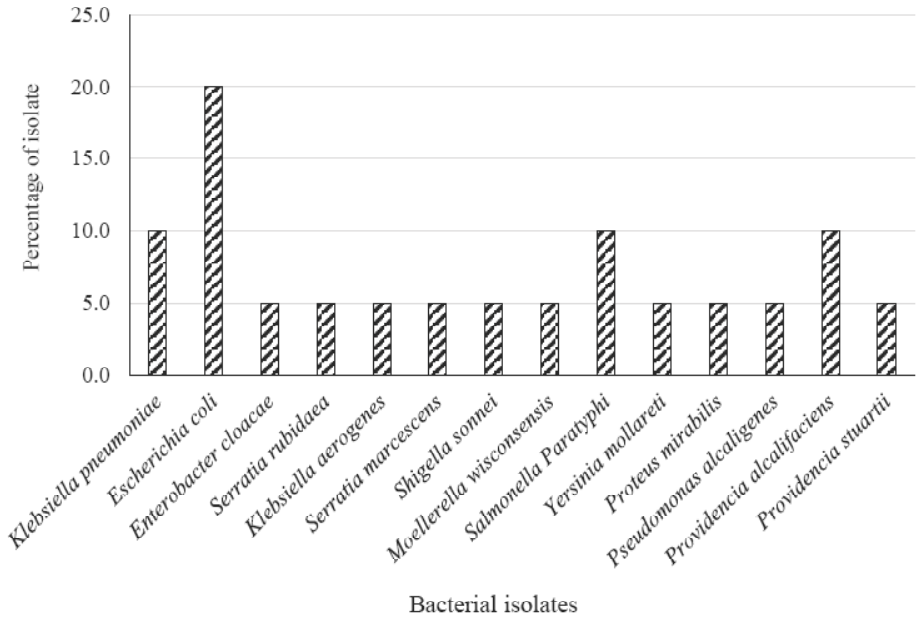

Figure 2: Bacteria isolates from ATMs

Table 1: Resistance and susceptibility patterns of the bacterial isolates to antibiotics

ATM	Bacterial isolate	Amp	Chl	Clo	Flu	Oxytet	Gen	Kan
ATM_01	*Klebsiella pneumoniae*	S	S	R	R	S	S	S
ATM_01	*Escherichia coli*	S	S	S	S	R	S	S
ATM_02	*Escherichia coli*	S	S	R	R	R	S	S
ATM_02	*Enterobacter cloacae*	S	S	R	R	R	S	S
ATM_03	*Serratia rubidaea*	S	S	R	R	S	S	S
ATM_03	*Klebsiella aerogenes*	S	R	R	R	S	S	S
ATM_04	*Serratia marcescens*	S	R	R	R	R	S	S
ATM_05	*Shigella sonnei*	S	S	S	S	R	S	S
ATM_05	*Moellerella wisconsensis*	S	S	S	S	R	S	S
ATM_06	*Salmonella* Paratyphi	S	S	R	S	S	R	R
ATM_06	*Klebsiella pneumoniae*	S	R	R	R	R	S	S
ATM_07	*Yersinia mollareti*	S	S	R	R	S	S	S
ATM_07	*Proteus mirabilis*	S	S	R	R	S	S	S
ATM_07	*Escherichia coli*	S	S	S	S	R	S	S
ATM_08	*Escherichia coli*	S	S	R	R	R	S	S
ATM_08	*Pseudomonas alcaligenes*	R	S	R	R	R	S	S
ATM_09	*Providencia alcalifaciens*	S	S	S	S	S	S	S

Table 1 (cont.)

ATM	Bacterial isolate	Amp	Chl	Clo	Flu	Oxytet	Gen	Kan
ATM_09	*Salmonella* Paratyphi	S	S	S	R	S	S	S
ATM_10	*Providencia stuartii*	R	R	R	R	R	S	S
ATM_10	*Providencia alcalifaciens*	S	S	S	S	R	S	S
	Percentage	$10^{R}/90^{S}$	$20^{R}/80^{S}$	$65^{R}/35^{S}$	$65^{R}/35^{S}$	$60^{R}/40^{S}$	$5^{R}/95^{S}$	$5^{R}/95^{S}$

Note: Amp: Ampicillin, Chl: Chloramphenicol, Clo: Cloxacillin, Flo: Flucloxacillin, Oxytet: Oxytetracycline, and Gen: Gentamicin, Kan: Kanamycin.

3.3. Resistance and susceptibility patterns of bacterial isolates

Based on the responses to the antibiotics, the bacterial isolates were classified as either resistant or susceptible to an antibiotic according to the guideline of EUCAST (Table 1). The bacterial species were more susceptible (67%) than resistant (33%) to antibiotics. The resistance and susceptibility patterns were independent of the antibiotics' classes and species' type. The isolates were more resistant to cloxacillin and flucloxacillin (65%), followed by oxytetracycline (60%). Resistance to chloramphenicol and ampicillin was recorded for 20% and 10% of the isolates and was evident in *50 Klebsiella aerogenes*, *Klebsiella pneumoniae* (ATM_06), *Serratia marcescens*, and *P. stuartii* , and *Pseudomonas alcaligenes* and *P. stuartii* , respectively. All the bacterial isolates were susceptible to gentamicin and kanamycin except *Salmonella* Paratyphi isolated from ATM_06. *P. stuartii* showed the most resistance (71%) to all the antibiotics. In contrast, *Providencia alcalifaciens* was susceptible to all the antibiotics. Thirty percent of the isolates (*Moellerella wisconsensis*, *Shigella sonnei*, *Escherichia coli* (ATM_01 and 07), *Providencia alcalifaciens* (ATM_09), and *Salmonella* Paratyphi (ATM_09)) also showed single resistances to oxytetracycline (83%) and flucloxacillin (17%). High resistances of 57% were recorded for *Klebsiella pneumoniae* from ATM_06, *Pseudomonas alcaligenes*, and *Serratia marcescens* and were commonly observed in cloxacillin, flucloxacillin, oxytetracycline, and chloramphenicol.

3.4. Detection of genes that encode antibiotic resistance

The presence or absence of beta-lactamase genes bla_{OXA-48} and bla_{VIM} (carbapenemases), bla_{TEM}, and bla_{CTX-M} (EBSLs), bla_{ampC}, and *mec*A, *str*A, *tet*(A), and *van*A genes in the isolates were determined using primers specific to the genes (Table 2). The bla_{TEM} and *str*A genes were the most predominant and were found in 90% and 80%

Table 2: Antibiotic resistance gene (ARG) profile of bacterial isolates

ATM	Bacterial isolate	bla_{ampC}	bla_{CTX-M}	bla_{OXA-48}	bla_{VIM}	bla_{TEM}	*mec*A	*str*A	*tet*(A)	*van*A
ATM_01	*Klebsiella pneumoniae*	–	–	–	+	+	–	+	–	–
ATM_01	*Escherichia coli*	–	–	–	–	+	–	+	–	–
ATM_02	*Escherichia coli*	–	–	–	–	+	–	+	–	–
ATM_02	*Enterobacter cloacae*	+	–	+	–	+	–	–	–	–
ATM_03	*Serratia rubidaea*	–	–	–	+	+	–	+	–	–
ATM_03	*Klebsiella aerogenes*	+	–	–	–	+	–	+	–	–
ATM_04	*Serratia marcescens*	+	–	+	–	–	–	+	–	–
ATM_05	*Shigella sonnei*	–	–	–	–	+	–	+	–	–
ATM_05	*Moellerella wisconsensis*	–	–	–	–	+	–	+	–	–
ATM_06	*Salmonella* Paratyphi	–	–	–	–	+	–	+	–	+

Table 2 (cont.)

ATM	Bacterial isolate	bla_{ampC}	bla_{CTX-M}	bla_{OXA-48}	bla_{VIM}	bla_{TEM}	*mec*A	*str*A	*tet*(A)	*van*A
ATM_06	*Klebsiella pneumoniae*	+	–	–	–	+	–	+	–	–
ATM_07	*Yersinia mollareti*	+	–	–	–	+	–	–	–	+
ATM_07	*Proteus mirabilis*	–	–	–	–	+	–	+	–	–
ATM_07	*Escherichia coli*	–	–	–	–	+	–	+	–	–
ATM_08	*Escherichia coli*	–	–	–	–	+	–	+	–	–
ATM_08	*Pseudomonas alcaligenes*	–	–	–	–	+	–	+	–	–
ATM_09	*Providencia alcalifaciens*	+	–	–	–	–	–	+	–	–
ATM_09	*Salmonella* Paratyphi	+	–	–	–	+	–	+	–	–
ATM_10	*Providencia stuartii*	–	–	–	–	+	–	–	–	–
ATM_10	*Providencia alcalifaciens*	–	–	–	–	+	–	–	–	–
	Percentage	$35^{+}/65^{-}$	100^{-}	$10^{+}/90^{-}$	$10^{+}/90^{-}$	$90^{+}/10^{-}$	100^{-}	$80^{+}/20^{-}$	100^{-}	$10^{+}/90^{-}$

Note: – : Absence of gene; and + : Presence of gene.

of the isolates. However, none of the isolates showed the presence of bla_{CTX-M}, *tet*(A), and *mec*A genes. The presence of bla_{ampC} was high (35%) within the isolates. The genes bla_{OXA-48}, bla_{VIM}, and *van*A were present in *Serratia marcescens* and *Enterobacter cloacae* (10%), *Serratia rubidaea* and *Klebsiella pneumoniae* from ATM_01 (10%), and *Yersinia mollareti* and *Salmonella* Paratyphi from ATM_06, respectively. The *Klebsiella*, *Enterobacter*, *Serratia*, *Salmonella,* and *Yersinia* species had the highest number of genes (33%) among the isolates. In contrast, the *Providencia* species (*P. stuartii* and *P. alcalifaciens*) had the least number of genes. About 45% of the isolates, predominantly *Escherichia coli*, were positive for 22% of the genes. The isolates (45%) similarly exhibited co-occurrence of the resistance genes.

4. Discussion

The physical surfaces in built environment play a significant role in the spread of infectious diseases (Edemekong *et al*., 2020). In this study, we investigated the antibiotic resistance profile of bacteria species isolated from the interaction panels of ATMs. The highest bacterial load of 18.90×10^6 CFU ml^{-1} from ATM_04 may be due to a high number of people patronizing this ATM. The ATM is situated at the regional head office of one of the largest banks in Ghana. Moreover, the area is closer to one of the most popular tourist sites in the region and is found within the commercial zone of the Cape Coast metropolis. Even though the ATM_02 is located on a commercial street, it had the lowest bacterial load of 2.70×10^6 CFU ml^{-1}. This observation may be due to low patronage by customers. The finding is similar to the study by Saroja *et al*. (2013) who reported the presence of bacteria contaminations in ATMs in Chennai, India with a relatively low range of 40.0 CFU ml^{-1} to 9.7×10^6 CFU ml^{-1}.

The ATM_03 and ATM_09 are also located in densely populated zones of the metropolis and recorded similar and relatively higher bacterial loads. Similar amounts of bacterial loads were found in 50% of the ATMs (ATMs_01, 05, 06, 08, and 10), indicating that these ATMs, as well as ATMs_03 and 09, pose similar levels of infection, respectively, when customers use them. The findings may suggest that there exists a relationship between the level of bacterial contamination on an ATM and the frequency of usage of the ATM. Earlier studies have reported that the diversity and number of customers who use ATMs influence the microbial loads on ATMs (Nworie *et al*., 2012). The low level of awareness of the capacity of contact surfaces to retain and serve as a source of medium for transfer of microbes coupled with a rudimentary hand hygiene practice in Ghana could be associated with the high level of bacterial contamination observed on the ATMs (Roberts *et al*., 2013).

Escherichia coli was the most abundant Enterobacteriaceae followed by *Klebsiella* species (*K. pneumoniae* and *K. aerogenes*), *Providencia* species (*P. alcalifaciens* and *P. staurtii*), *Serratia* species (*S. marcescens* and *S. rubidaea*), and *Salmonella* Paratyphi. The other species of the group were *Shigella sonnei*, *Enterobacter cloacae*, *Moellerella wisconsensis*, *Yersinia mollareti,* and *Proteus mirabilis*. *Pseudomonas alcaligenes* was the only species of Pseudomonadaceae found, suggesting a low prevalence of the group in isolates from ATMs. Previous studies have reported variations in the number and types of bacteria species isolated from ATMs (Odeyemi *et al*., 2018; and Okoro *et al*., 2018). Mahmoudi *et al*. (2017) reported an average of seven isolates in 96 ATMs studied in western Iran. However, a study conducted on four ATMs within the Calabar metropolis of Nigeria identified 79 bacteria isolates (Agu *et al*., 2018).

Even though ATM_04 had the highest bacterial load, only one species (*S. marcescens*) was found from the ATM compared with the remaining ATMs which had low to high bacterial loads and at least two bacterial species each. This indicates that microbial populations compete and contra-inhibit their exponential growth and survival rates. The study of Allen and Waclaw (2018) indicated that bacteria interactions cause competition for space between lineages. There is an increasing prevalence of all the bacterial species found from this study as pathogens and frequently associated with healthcare outbreaks (such as urinary tract infections, lung diseases, acute diarrhea, and neonatal sepsis) and increased morbidity and mortality (Ahmad *et al*., 2020; and Sands *et al*., 2021).

To ascertain the antibiotic resistance pattern of the bacterial isolates, a combined approach of phenotypic and genotypic assaying was employed. The isolates responded differently to antibiotics of the aminoglycosides (gentamicin and kanamycin), tetracycline (oxytetracycline), phenicols (chloramphenicol), and penicillins (flucloxacillin, ampicillin, and cloxacillin) classes. The high resistance of the bacterial isolates to oxytetracycline, flucloxacillin, and cloxacillin suggests that flucloxacillin, cloxacillin, and oxytetracycline may be less effective at controlling these bacterial isolates. The presence of low resistances of the bacterial isolates to chloramphenicol, gentamicin, and kanamycin has been reported (Şimşek, 2019). The resistance of *Salmonella* Paratyphi from ATM_06 to both kanamycin and gentamicin indicates cross-resistance between these aminoglycoside antibiotics. Cross-resistance among aminoglycoside antibiotics including gentamicin, kanamycin, and streptomycin has similarly been reported by Zhang *et al*. (2015).

In contrast, gentamicin and kanamycin were the most potent against the isolates with 95% of isolates being susceptible. This observation contradicts the previous reports that indicated that aminoglycoside antibiotics such as kanamycin and gentamycin have become less effective against bacteria (Krause *et al*., 2016). Cross-resistances to the antibiotics were also observed for most of the bacterial isolates. The high resistance of *P. stuartii* to the antibiotics indicates an increasing antibiotic resistance in *P. stuartii*. Warda *et al*. (2018) has similarly reported multidrug resistance (MDR) in *P. stuartii*. This corroborates the current increasing prevalence of *P. stuartii* infections (Liu *et al*., 2020). The susceptibility of all the *Salmonella* Paratyphi isolates to chloramphenicol has similarly been observed by Maharjan *et al*. (2021).

The level of diversity in Antibiotic Resistance Genes (ARGs), particularly in human pathogens, does not depend on gene abundance and the number of samples (Bengtsson-Palme, 2018). Moreover, a small set of ARGs could provide an insight into the total diversity of resistance genes (Bengtsson-Palme, 2018). The most common genes detected in the isolates were the bla_{TEM} and *str*A genes. The study by Sheikh *et al*. (2012) concluded that bla_{TEM} genes are highly associated with *str*A genes. These genes are responsible for transferable ampicillin and penicillin (bla_{TEM}), and streptomycin (*str*A) resistances (Alcock *et al*., 2020). The observed recurrence of the bla_{TEM} and *str*A genes in the bacterial isolates aligns with previous study in a tertiary care center in India (Grover *et al*., 2013). Bacteria species that harbored bla_{ampC} (bla class C) were high compared with the carbapenemase genes bla_{OXA-48} (bla class D) and bla_{VIM} (bla class B), and *van*A genes which were present in few species. These genes have catalytic efficiency against cephalothin, cefazolin, and most penicillins cefoxitin (bla_{ampC}), carbapenems comprising imipenem, meropenem, doripenem, and ertapenem (bla_{OXA-48} and bla_{VIM}), and vancomycin (*van*A), (Arredondo-Alonso *et al*., 2021; and Sakkas *et al*., 2019).

Even though 60% of the bacterial isolates were resistant to oxytetracycline, none of the isolates had the *tet*(A) gene, as well as the bla_{CTX-M} and *mec*A genes, present. Besides, bla_{TEM} and bla_{CTX-M} belong to the same class (A) of beta-lactamases. This suggests that antibiotics belonging to the same class may exhibit different actions against bacteria species (Wang and Lipsitch, 2006). The *tet*(A), *mec*A, and bla_{CTX-M} genes code for the ability of bacteria to hydrolyze tetracycline, methicillin, and beta-lactam antibiotics such as cefotaxime, ceftazidime, and aztreonam, respectively (Liu *et al*., 2020). *Escherichia coli* was the most common isolate and

harbored only bla_{TEM} and *str*A genes. This suggests the presence of an association between bla_{TEM} and *str*A genes in the pathogen and confirms the findings of Sheikh *et al*. (2012) who concluded that bla_{TEM} genes are highly linked to *str*A genes in *Escherichia coli*. *Enterobacter cloacae* exhibit bla_{OXA-48} carbapenemase encoding genes (Solgi *et al*., 2020).

Despite the MDR of *P. stuartii* , the pathogen had the presence of only the ESBL bla_{TEM} gene. *P. stuartii* showed the presence of only bla_{OXA-48} and bla_{NDM} genes when genotyped for four carbapenemase genes including bla_{VIM} (Warda *et al*., 2018). The coexistence of the carbapenemase genes bla_{OXA-48} and bla_{VIM}, ESBL bla_{TEM} gene, and bla_{ampC}, *str*A, and *van*A genes in all the isolates, except *P. alcalifaciens* from ATM_10 and *P. stuartii*, is threatening to public health. The *Klebsiella pneumoniae* isolates commonly exhibited bla_{TEM} and *str*A and either bla_{VIM} or bla_{ampC}. *Klebsiella pneumoniae* isolates similarly tested positive for bla_{TEM} and bla_{OXA-48} but negative for bla_{VIM} genes (Ferreira *et al*., 2019; and Solgi *et al*., 2020). The absence of bla_{OXA-48} in *K. pneumoniae* as observed in the study has been documented (Ferreira *et al*., 2019). This suggests that bla_{TEM} may be a common ESBL-encoding gene in *K. pneumoniae*. *Proteus mirabilis* showed bla_{TEM} and *str*A genes and phenotypic resistance to flucloxacillin and cloxacillin. Current reports have highlighted the emergence and spread of MDR and antimicrobial resistance genes including beta-lactamases in *P. mirabilis* (Girlich *et al*., 2020). The presence of the bla_{OXA-48} gene in *Serratia marcescens* concords with the outbreak of OXA-48-producing *S. marcescens* reported in Iran (Solgi *et al*., 2020). *Shigella sonnei* similarly tested positive for bla_{TEM} and *str*A genes and negative for bla_{VIM} genes in an earlier study in China (Wang *et al*., 2019).

5. Conclusion

Bacterial isolates from ATMs in the Cape Coast metropolis comprised Enterobacteriaceae and Pseudomonadaceae species that belonged to 11 genera. Most of the isolates exhibited multiple antibiotic resistance. Gentamicin and kanamycin were the most effective antibiotics against the bacterial isolates. The isolates revealed the presence of beta-lactamases and *mec*A genes and demonstrated a high potential for the acquisition of bla_{TEM} and *str*A genes. This is the first study on antibiotic susceptibility and resistance gene pool of bacterial species from ATMs in the Cape Coast metropolis and further recommend proper cleaning of ATMs in the metropolis as well as hand sanitization by customers.

Conflicts of Interest

The authors declare no conflict of interest.

References

Agu, R.C., Osondu-Anyanwu, C. and Nwachukwu, A.A. (2018). Isolation and identification of microorganisms associated with automated teller machines in calabar metropolis. *J Adv. Biol Biotechnol*., 18(3), 1-7.

Ahmad, N., Ali, S.M. and Khan, A.U. (2020). Co-existence of bla_{NDM-1} and bla_{VIM-1} producing *Moellerella wisconsensis* in NICU of North Indian Hospital. *J Infect Dev Ctries*, 14(02), 228-231.

Alcock, B.P., Raphenya, A.R., Lau, T.T., Tsang, K.K., Bouchard, M., Edalatmand, A. and Liu, S. (2020). CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res*., 48(D1), 517-525.

Allen, R.J. and Waclaw, B. (2018). Bacterial growth: A statistical physicist's guide. *Rep Prog Phys.,* 82(1), 016601.

Arredondo-Alonso, S., Top, J., Corander, J., Willems, R.J.L. and Schürch, A.C. (2021). Mode and dynamics of *vanA*-type vancomycin resistance dissemination in Dutch hospitals. *Genome Med*., 13(1), 1-18.

Bengtsson-Palme, J. (2018). The diversity of uncharacterized antibiotic resistance genes can be predicted from known gene variants—but not always. *Microbiome*., 6(1), 125.

Ciofu, O. and Tolker-Nielsen, T. (2019). Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents—How *P. aeruginosa* can escape antibiotics. *Front Microbiol*., 10, 913.

Dadgostar, P. (2019). Antimicrobial resistance: Implications and costs. *Infect Drug Resist*, 12, 3903–3910.

Edemekong, P. F., Kopparapu, A.K. and Huang, B. (2020). Epidemiology of prevention of communicable diseases. Retrieved from https: / / www.ncbi.nlm.nih.gov / books / NBK470303 /

Ferreira, R.L., da Silva, B., Rezende, G.S., Nakamura-Silva, R., Pitondo-Silva, A., Campanini, E.B. and Cunha, A.F.D. (2019). High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. *Front Microbiol*., 9, 3198.

Frieri, M., Kumar, K. and Boutin, A. (2017). Antibiotic resistance. *J Infect Public Health*, 10(4), 369–378.

Gibbons, S.M. and Gilbert, J.A. (2015). Microbial diversity—exploration of natural ecosystems and microbiomes. *Curr Opin Genet Dev*., 35, 66–72.

Giler-Molina, J.M., Zambrano-Intriago, L.A., Quiroz-Fernández, L.S., Napoleão, D.C., dos Santos Vieira, J., Simões Oliveira, N. and Rodríguez-Díaz, J.M. (2020). Degradation of oxytetracycline in aqueous solutions: Application of homogeneous and heterogeneous advanced oxidative processes. *Sustainability*, 12(21), 8807.

Girlich, D., Bonnin, R.A., Dortet, L. and Naas, T. (2020). Genetics of acquired antibiotic resistance genes in *Proteus* spp. *Front Microbiol*., 11, 256.

González-Mendoza, D., Argumedo-Delira, R., Morales-Trejo, A., Pulido-Herrera, A., Cervantes-Díaz, L., Grimaldo-Juarez, O. and Alarcón, A. (2010). A rapid method for isolation of total DNA from pathogenic filamentous plant fungi. *Genet Mol Res*., 9(1), 162-166.

Grover, N., Sahni, A. and Retd, S.B. (2013). Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. *Med J Armed Forces India*., 69(1), 4-10.

Jayachandran, S. (2018). Pre-antibiotics era to post-antibiotic era. *J Indian Acad Oral Med Radiol*., 30(2), 100.

Krause, K.M., Serio, A.W., Kane, T.R. and Connolly, L.E. (2016). Aminoglycosides: an overview. *Cold Spring Harb Perspect Med*., 6(6), a027029.

Li, H., Liu, S.M., Yu, X.H., Tang, S.L. and Tang, C.K. (2020). Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents*, 55(5), 105951.

Liu, J., Wang, R. and Fang, M. (2020). Clinical and drug resistance characteristics of *Providencia stuartii* infections in 76 patients. *J Int Med Res*., 48(10), 0300060520962296.

Maharjan, A., Dhungel, B., Bastola, A., Thapa Shrestha, U., Adhikari, N., Banjara, M.R and Rijal, K.R. (2021). Antimicrobial susceptibility pattern of *Salmonella* spp. Isolated from enteric fever patients in Nepal. *Infect Dis Rep*., 13(2), 388-400.

Mahmoudi, H., Arabestani, M.R., Alikhani, M.Y., Sedighi, I., Kohan, H.F. and Molavi, M. (2017). Antibiogram of bacteria isolated from automated teller machines in Hamadan, West Iran. *GMS Hyg Infect Control*., 12, 1–6.

Morris, D.E., Cleary, D.W. and Clarke, S.C. (2017). Secondary bacterial infections associated with influenza pandemics. *Front Microbiol*., 8, 1041.

Newman, M.J., Frimpong, E., Donkor, E.S., Opintan, J.A. and Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana. *Infect Drug Resist*., 4, 215-220.

Nworie, O., Mercy, M., Chukwudi, A., Oko, I., Chukwudum, S.O., Agah, V.M. and Ekuma, U.O. (2012). Antibiogram of bacteria isolated from automated teller machines within Abakaliki metropolis. *Am J Infect Dis*. 8(4), 168.

Odeyemi, A.T., Sulaimon, A.M., Odunmbaku, E. and Afolabi, I.E. (2018). Bacteriological contamination of user interface of automated teller machines (ATM) of banks in Ekiti State University, Ado-Ekiti. *Intern J Res Stud Microbiol Biotechnol*., 4(3), 18-25.

Okoro, J., Olonine fa, S.D., Ojonigu, A.F. and Sani, M. (2018). Assessment of some selected automated teller machines in Kaduna metropolis for pathogenic bacteria contamination. *Br. J Environ Sci*., 6(1), 19–35.

Pal, C., Bengtsson-Palme, J., Kristiansson, E. and Larsson, D.G.J. (2016). The structure and diversity of human, animal and environmental resistomes. *Microbiome*., 4(1), 1-15.

Rangarajan, R. and Venkataraman, R. (2020). Antibiotics targeting Gram-negative bacteria. In P. Kesharwani, S. Chopra and A. Dasgupta (Eds.), *Drug Discovery Targeting Drug-Resistant Bacteria* (pp. 39-70). Cambridge: Academic Press.

Roberts, M.C., Soge, O.O. and No, D. (2013). Comparison of multi-drug resistant environmental methicillin-resistant Staphylococcus aureus isolated from recreational beaches and high touch surfaces in built environments. *Front Microbiol*., 4, 74.

Sakkas, H., Bozidis, P., Ilia, A., Mpekoulis, G. and Papadopoulou, C. (2019). Antimicrobial resistance in bacterial pathogens and detection of carbapenemases in *Klebsiella pneumoniae* isolates from hospital wastewater. *Antibiotics*., 8(3), 85.

Sands, K., Carvalho, M. J., Portal, E., Thomson, K., Dyer, C., Akpulu, C. . . . and Hender, T. (2021). Characterization of antimicrobial-resistant gram-negative bacteria that cause neonatal sepsis in seven low-and middle-income countries. *Nature Microbiology*, 6(4), 512-523.

Saroja, V., Kamatchiammal, S., Brinda, K. and Anbazhagi, S. (2013). Enumeration and characterisation of coliforms from Automated Teller Machine (ATM) centers in urban areas. *J Mod Biotechnol*., 2(1), 14–22.

Sheikh, A.A., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Boerlin, P and Aslam, M. (2012). Antimicrobial resistance and resistance genes in *Escherichia coli* isolated from retail meat purchased in Alberta, Canada. *Foodborne Pathogen and Disease*, 9(7), 625-631.

Şimşek, M. (2019). Determination of the antibiotic resistance rates of *Serratia marcescens* isolates obtained from various clinical specimens. *Niger J Clin Pract*., 22(1).

Solgi, H., Nematzadeh, S., Giske, C.G., Badmasti, F., Westerlund, F., Lin, Y.-L. and Shahcheraghi, F. (2020). Molecular epidemiology of OXA-48 and NDM-1 producing *Enterobacterales* species at a University Hospital in Tehran, Iran, between 2015 and 2016. *Front Microbiol.,* 11, 936.

Stephens, B., Azimi, P., Thoemmes, M. S., Heidarinejad, M., Allen, J. G. and Gilbert, J. A. (2019). Microbial exchange via fomites and implications for human health. *Curr Pollut Rep*., 5, 198-213.

Thomashow, L. S., Kwak, Y. S. and Weller, D. M. (2019). Rootassociated microbes in sustainable agriculture: models, metabolites and mechanisms. *Pest Manage Sci*., 75(9), 2360-2367.

Ventola, C.L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharm Ther*., 40(4), 277.

von Wintersdorff, C.J.H., Penders, J., Van Niekerk, J.M., Mills, N.D., Majumder, S., Van Alphen, L. B and Wolffs, P.F.G. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol*., 7, 173.

Wang, Y., Ma, Q., Hao, R., Zhang, Q., Yao, S., Han, J. . . . and Xu, X. (2019). Antimicrobial resistance and genetic characterization of *Shigella* spp. in Shanxi Province, China, during 2006–2016. *BMC Microbiol*., 19(1), 1-11.

Wang, Y.C. and Lipsitch, M. (2006). Upgrading antibiotic use within a class: tradeoff between resistance and treatment success. *Proc Natl Acad Sci USA*., 103(25), 9655–9660.

Warda, K., Said, L.A., Zerouli, K., Katfy, K. and Zahlane, K. (2018). First report of a *providencia stuartii* strain coproducing a Beta-metalloenzyme NDM-1 type and an oxacillinase type OXA-48. *International Journal of Collaborative Research on Internal Medicine & Public Health*, 10(1), 0-0.

World Health Organization. (2019). *Global tuberculosis report 2019*. Geneva: World Health Organization.

World Health Organization. (2020). Antibiotic resistance. Retrieved from https: www.who.int / news-room / fact-sheets / detail / antibiotic-resistance

Zdolec, N., Lorenzo, J.M. and Ray, R.C. (2018). Use of microbes for improving food safety and quality. *BioMed Res Int*., 1-2.

Zeng, X. and Lin, J. (2013). Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. *Front Microbiol*., 4, 128.

Zhang, D.F., Li, H., Lin, X.M. and Peng, X.X. (2015). Outer membrane proteomics of kanamycin-resistant *Escherichia coli* identified MipA as a novel antibiotic resistance-related protein. *FEMS Microbiology Letters*, 362(11).

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z. and Gu, X. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*., 395(10229), 1054-1062.

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